ANNEX XV RESTRICTION REPORT

PROPOSAL FOR A RESTRICTION

SUBSTANCE NAME(S): Substances in tattoo inks and permanent make up

IUPAC NAME(S): not applicable

EC NUMBER(S): not applicable

CAS NUMBER(S): not applicable

CONTACT DETAILS OF THE DOSSIER SUBMITTER:

ECHA with Denmark, Italy and Norway[1]

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[1] In addition, Germany has significantly contributed to the development of the dossier.

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A.1. Manufacture, import and export

A.1.1. Manufacture of tattoo inks and PMU

Tattoo ink manufacturers formulate tattoo and PMU inks using different chemical substances, but they usually do not synthesize them. One important problem highlighted by several authors in the literature (e.g., (Blume, et al., 2015), (Hauri, 2016), (Jacobsen & Clause, 2015), (Petersen & Lewe, 2015)) and by many stakeholders, such as TIME (Tattoo Ink Manufacturers' of Europe, a manufacturers' association), is that the colourants used in the formulation of tattoo and PMU inks are not produced for the purpose of being injected sub-cutaneously. Therefore, they have not undergone any risk assessment that takes into account their injection into and long-term presence in the human body.

The colourants incorporated in tattoo and PMU inks are usually produced by the chemical industry for outdoor applications in products such as textiles, paints for cars and plastics, because they show good light fastness properties (resistance to fading when exposed to light) (JRC, 2015b). According to Petersen & Lewe, the development of new colourants for the special demands of several industrial applications (e.g., for growing industries in applications such as automotive coatings and interior or exterior paints) is the reason for the current presence of a huge variety of different chemical structures and modifications. Manufacturers of colourants sometimes offer the same type of colourant for more than one application (plastics, coatings, printing, textiles). Some colourants approved for use in cosmetics (in accordance with the EU Cosmetic Products Regulation, i.e., Regulation (EC) N° 1223/2009 or the CPR) are also available. However, because the tattoo business is small and not profitable compared to other industries, such as cosmetics or industrial coatings, colourants for tattoo inks and PMU are not specifically developed, produced and assessed for their function. Results from analytical test of colourants show the presence of impurities such as chromium VI in chromium oxides, nickel, copper and cobalt in iron oxides, aromatic amines in azo colourants and polycyclic aromatic hydrocarbons (PAHs) in carbon black. (JRC, 2015b)

Colourants can comprise up to 60% (typically around 25% as stated in Annex B) of the final formulation of tattoo inks and PMU. They are responsible for the colour, brilliance and light fastness of the tattoo or PMU. (JRC, 2015b) Because many pigments used in the formulation of tattoo inks and PMU are produced for other applications, where higher contents of impurities are unproblematic, their purity is not very high: it has been reported to be between 70 and maximum 90% (JRC, 2015b). In general, cosmetic or medical grade pigments have the highest purity, however, their costs can be higher in comparison to industrial grades for the same colour. Therefore, the selection of pigments is one of the critical aspects of the formulation process. Many companies manufacturing tattoo inks and PMU are micro, small or medium sized and do not have the capacity to conduct extensive risk assessment of their inputs or final products, although it has been reported that analytical testing of the chemical composition is common.

More than 40 100 litres of tattoo inks and 11 000 litres of PMU inks were formulated within the EU in 2016. (See Table 1.) The main EU manufacturers of tattoo inks are based in the UK and Germany, while Germany dominates the EU-based manufacturing of PMU according to survey by (JRC, 2015b). Other Member States mentioned in the JRC report where EU manufacturers of tattoo inks are located include Italy, Spain, Sweden, and Poland, while PMU is also produced in Italy, Spain, France, Austria, Switzerland and the Netherlands,

although there is uncertainty of the exact place of origin of some products. The JRC study notes that the EU and global market is complex and "it is not easy to understand who is producing what", as one manufacturer may produce more than one brand (own or for private label). In total, the study suggests that there are about 90 EU-based and international manufacturers of tattoo inks on the EEA31 market and about 55 PMU manufacturers (JRC, 2015b).

Many of the tattoo inks and PMU manufacturers are often specialised in the manufacture of tattoo inks and PMU only and some are further specialised in formulating one of the two. Some are downstream integrated with tattoo parlours or with the manufacture of specialised instruments.

Many tattoo inks and PMU formulators are micro and small businesses. The largest company in the industry is the US based Starlight Enterprises which built its business on the reputation of its founders and owner Mario Barth who following the success of its tattoo parlours, introduced in 2002 the Intenze line of tattoo inks. The company is privately owned, therefore, public financial information is not available. It is estimated to have multi-million dollar revenue (IBISWorld, 2016), although it is uncertain what contribution tattoo ink formulation has to the company revenue and profit stream.

Table 1 Tattoo inks and PMU on the EEA31 market – 2016 estimates (litres)

	Tattoo ink	**PMU**	**Total**
EU31 manufactured	40 100	11 300	51 400
Exported	2 100	2 100	4 200
Imported to EU31	114 000	1 600	115 600
Total on EU31 market	**152 000**	**10 800**	**162 800**

Notes: Estimates based on interviews with selected manufacturers and JRC data (JRC, 2015b). See Annex C: Baseline for further information.

A.1.2. Import and export of tattoo inks and PMU

As shown in Table 1, a substantial share of the tattoo inks on the EU market is imported (about 114 000 litres in 2016). Between 70% and 80% of the tattoo inks on the EU market are manufactured outside the EU, with US products mainly being used by professional tattooists, while Chinese products are typically used by amateur tattooists. (Michel, 2015) (JRC, 2015b) Other imported tattoo inks originate from Japan, Brazil, or Mexico. For example, in the UK 32% of tattoo inks appear to be domestic, 40% imported from the US, 10% from Asian and 4% is of EU origin (other than UK). (NVWA, 2017) (JRC, 2015b).

According to reports, about 20% of PMU on the EU market is imported, primarily from the US or China. (Michel, 2015), (JRC, 2015b)

As shown in Table 1, a small percentage of the EU produced tattoo inks is exported (about 5%), while close to 20% of EU formulated PMU is sold internationally (primarily in North America).

Prices of tattoo inks have been reported by stakeholder between €6 and €25 per 30 ml bottle, while for PMU inks between €15 and €120 per 15 ml bottle.

For further information regarding the tattoo inks and PMU on the EU market, see Annex C: Baseline and the report by (JRC, 2015b).

A.2. Uses

Tattoos and the tattoo process

According to CoE ResAP(2008)1, "tattooing is a practice whereby a permanent skin marking or design (a "tattoo") is administered by intradermal injection of product consisting of colourants and auxiliary ingredients."

Types of tattoos

Tattoos can be decorative, traditional, medical, traumatic or temporary:

Decorative tattoos

Decorative tattoos represent the majority of tattoos today. They are designs, drawings, or inscriptions using modern tattoo inks and electric tattoo machines. Although fading over time, they are considered permanent in the sense that they are long-lasting, often for several decades. Studies show the application of decorative tattoos everywhere on the human body with trunk and arms being the most preferred areas. (Høgsberg, et al., 2013) Tattoos on mucous membranes (e.g., in the mouth, genital areas or the sclera of the eye) are rare but do occur.

Traditional tattoos

Traditional tattoos differ from decorative tattoos in terms of the purpose for the tattoo (e.g., tribal tattoos signifying rite of passage or status and position) and the materials used (e.g., traditional ink solution is injected into the skin using a sharp object).

Medical tattoos

Tattooing is also employed as a therapeutic modality or a diagnostic method. Examples of such medical applications include to camouflage pathological skin conditions (e.g., alopecia (loss of hair from the body), vitiligo[2], birthmarks), to mask scars (accidental or surgical), to complete the aesthetic results of plastic and reconstructive and craniomaxillofacial surgeries (e.g., nipple-areola complex reconstruction, cleft lip or palate), marking of implantation devices (e.g., pacemakers), etc. Medical tattoos are usually made by medical professionals.

In radiation therapy, tattoo markings (a set of dark pigment tattoos along the treatment axes) assist with target localisation to ensure precise beam alignments, as reproducible and accurate positioning of the patient is imperative during the course of the radiotherapy. The gastrointestinal tract may be marked via endoscopic tattooing by an intramural injection of a staining agent (India ink is most used but other also have application) for future surgical or endoscopic surveillance, e.g., to mark tumours or areas of acute gastrointestinal haemorrhage (bleeding) preoperatively. Permanent tattooing of the cornea can be performed for both cosmetic and optical reasons, although it has been decreasing in popularity. The technique is similar to other tattoo applications: insoluble pigments (India ink, iron oxide, titanium dioxide) and imbedded into the cornea stroma by means of multiple punctures (Vassileva & Hristakieva, 2007).

For the medical application of tattoos in European literature the term dermatography has emerged to designate the art of tattooing applied to permanently correct various

[2] Vitiligo is a loss of skin melanocytes that causes areas of skin depigmentation of varying sizes. Cause is unknown, but genetic and autoimmune factors are likely. (MSD, 2017)

cosmetically disabling disorders. This is in contrast with the term micropigmentation to convey the use of tattooing for cosmetic reasons and PMU (Vassileva & Hristakieva, 2007).

Another emerging area of tattoo application is for medical alert purposes. Although these tattoos are not sanctioned by the medical community, patients (e.g., with diabetes who may be found unconscious due to hypoglycaemia or with allergy to specific medication) on their own initiative are replacing medical jewellery with tattoos as a pragmatic, permanent tool to alert physicians, paramedics or anyone in public places in case of emergency (Kluger & Aldasouqi, 2013).

Traumatic tattoos

Traumatic tattoos are the result of accidents in which pigmented particles get embedded in the skin. These can arise form abrasive damage, e.g., asphalt from a bicycle accident, or a penetrating force, such as graphite pencil or explosion from fireworks and gun powder (Eklund & Troilius Rubin, 2015). This type of tattoo is not covered by the restriction proposal.

Temporary tattoos

Decal tattoos[3] and henna (mehndi)[4] are placed on the surface of the skin and have a temporary effect, intended to last from a day up to several weeks.

The intentional tattoos with permanent tattoo inks (i.e., decorative, traditional or medical) are included in the scope of the restriction dossier, while non-intentional (traumatic tattoos) and temporary tattoos (henna and decal) are outside the its scope.

Tattoo process

Tattoo needles inject tattoo ink into the dermis by puncturing the epidermis at a rate of 50 to 5 000 times per minute, depending on the type of machine used. Capillary action acts to draw ink further into the dermis. The tattoo becomes permanent when the person's immune system begins the wound healing process due to the breaking of the skin barrier and the injection of foreign bodies into the skin. Because the immune system sees the pigment particles as a foreign invader, the macrophages engulf the particles in order to eliminate them from the body. Only pigment particles introduced through the skin surface, below the dermal-epidermal junction, are retained by the dermal macrophages and fibroblasts where they reside permanently, producing an indelible change of the skin colour under the form of recognisable patter or design. (Vassileva & Hristakieva, 2007) Only a small portion of the originally injected pigment, between 1-13% (Lehner, et al., 2011), remains at the tattoo site permanently, as some of these macrophages are trapped in the gel-like matrix of the dermis. The majority of the ink, with the macrophages or otherwise, is transported to local

[3] Decal temporary tattoos are used to decorate any part of the body, including areas of the face and around the eyes, and may last for a day or up to a week or more. They are especially popular with children and at Halloween. There are two kinds of decal tattoos: images attached to a removable backing (the decal image is removed from the backing by wetting, and the image is then applied directly to the skin) or images with backing that adheres to the skin, creating a partial or complete barrier between the skin and the dyes used in the image. https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/HomeHealthandConsumer/ConsumerProducts/ContactLenses/ucm108569.htm

[4] Henna is a think mixture of dried and powdered plant Lawsonia inermis. It is used to dye hair, nails and skin. Natural henna gives a red colour. Coffee, black tea, and recently other colouring agents are used to create a larger variety of colours. An example of the latter is paraphenylenediamine (PPD), sometimes in concentrations of up to 15%. For comparison, the limit of PPD in cosmetics is 6% according to the CPR. (De Cuyper & D'hollander, 2010)

lymph nodes and via the lymph system to other organs of the body to be eliminated or stored. The tattoo ink is diffused from the tattoo site via the following modes: a) elimination during the tattoo process as a result of the bleeding, b) elimination during the healing process, c) natural epidermal replacement as epidermal cells have a life span of two to three weeks and the ink in the epidermis is removed as the epidermal cells are replaced with new cells, d) soluble substances are transported almost immediately throughout the body, while insoluble substances are eliminated via the macrophages, are broken down over time to decomposition products that can be more easily eliminated or stored by the body or remain at the tattoo site in the dermis.

PMU and the PMU process

According to CoE ResAP(2008)1, "a PMU consists of colourant and auxiliary ingredients which are injected intradermally for the purpose of enhancing the contours of the face." PMU, also referred to as cosmetic tattooing or micropigmentation, is the application of PMU inks in the superficial layer of the dermis to enhance natural beauty and increase physical appeal. The majority of people choose cosmetic tattooing because it offers an easy alternative to conventional make-up be that due to convenience, aesthetics, or medical conditions impeding conventional make-up application, e.g., allergies to conventional make-up, disability (arthrosis, arthritis), trembling hands (Parkinson disease), poor vision or hay fever (De Cuyper, 2015).

De Cuyper describes the PMU procedure as usually performed with an electrical tattoo device or a tattoo pen with a rotating or oscillating disposable needle. Small droplets of specific PMU ink are implanted (often) in the superficial layer of the dermis in contrast with decorative tattooing, in which the deposition of pigment occurs deeper within the dermis. Some PMU devices are developed for specific procedures. The elimination of pigment can begin during the first days of healing. After healing, the remaining pigment particles are stored in dermal macrophages and fibroblasts. The nature of the material used and the level of implantation influence the quality and stability of the results. Because the level of application of PMU can be more superficial than in decorative tattooing, spontaneous elimination of the colourant (with skin regeneration) and fading may occur within a few years. Short-term side effects include mild swelling and crusting, which are usually dealt with by the clients and tattoo artists and not reported (De Cuyper, 2015).

PMU are often made in laser surgery clinics, spas and wellness centres by beauticians and other wellness and sometimes medical professionals. As the procedure is very similar to a tattoo, they can also be made by professional or amateur tattooist. The inks are very similar (although PMU can be more viscous and the colours used are often less vibrant and resemble more the natural tones of the skin) as well as the instruments (although more specialised equipment has been developed for PMUs and the needles tend to be thinner and fewer).

Professional use of tattoo inks/PMU

The profession of tattoo artist or tattooist is not well-defined as there is currently no official education or training in any EU Member State that qualifies someone to become a tattoo artist. Traditionally, tattooing is learnt by years of apprenticeship in a tattoo parlour in contact with experienced tattooists. (Kluger, 2015a) In most Member States there are no specific requirements to receive certification or licencing or membership in a professional association (JRC, 2015a).

Virtually anyone can become a tattooist by buying tattoo materials. Opening a tattoo shop also often does not require specific certification, although such obligations vary according to country. For instance, in France, since 2009, tattooists have been required to register and undergo training on sepsis and hygiene (Kluger, 2015a). Some countries require license for, e.g., hair stylists (Finland), to open a tattoo studio. (JRC, 2015a) In some countries, tattooists have organised into unions/syndicates to promote the tattoo profession. (See Table 3)

In literature, professional tattoo artists or tattooists are considered only those who are licenced. As seen in *Table 2*, the non-licensed or amateur or home tattooists (also referred to as "backyard tattooists" or "scratchers") comprise substantial share of practicing tattoo artists. (JRC, 2015b) The development of an informal market has been facilitated by the internet, which allows anyone to buy tattoo kits and inks. Registered tattoo artists consider them unfair competition because unlicensed tattooists do not pay taxes or expenses related to parlour management. They can usually be found on the internet, providing inexpensive tattoos at home. In addition, some professional tattoo artist claim that home tattooing increases the risk of low-quality tattoos, which may lead to an increased number of tattoo removal procedures and in increased risk of infections because the sessions are not performed under adequate conditions of asepsis. However, the real impact of this market is difficult to assess in terms of public health. Lastly, the proportion of tattoo allergic reactions that could be attributed to non-professional tattooing and the use of unauthorised inks is unknown. (Kluger, 2015a) Two studies of self-reported tattoo reactions show that the majority of tattoos that lead to a reaction were performed by professional tattoo artists (93.8% (Høgsberg, et al., 2013) and 96.3% (Klügl, et al., 2010)); however, it is not reported whether the frequency of reactions is higher for tattoos made by non-professional versus professional tattooists.

As licencing is not required in all EU member states and as a large share of tattooist practice legally without such licences, for the purpose of this dossier, anyone receiving payment for permanent tattoo procedures is considered a professional tattoo artist or tattooist (the two terms are used interchangeably).

Table 2 presents the number of professional and non-professional tattoo artists by country. It can be seen from the table that on average, the ratio between the number of professional and non-professional tattooist is more than 1:2.5.The number of non-registered tattooists should be interpreted with caution as the methodology of their estimation may vary by country. The number may be substantially overestimated if this is based on the sold tattoo starter kits[5] on the internet, as those kits may be purchased for limited use only.

[5] Tattoo starter kit is a kit containing essentials for tattooing: needles, inks and a collection of designs.

Table 2 Number of professional and non-registered tattooists by country

Country	Professional tattooists	# Professional tattooists/inhabitants	Non-registered tattooists	Reference
Germany	6000	1/13000	6000-20000	*, ‡
Denmark	500	1/11200	1000-1200	*, ‡
Spain	3000-3500		2000-5000	*, ‡
France	2000-4000	1/22600		*, †
Iceland	8-10	1/30000	16-70	*, †
Italy	1200-10000	1/20000	4000-30000	*, †, ‡
Norway	400-650	1/10000	3000-5000	*, ‡
Sweden	2000-3000	1/3200	3000-20000	*, ‡
Switzerland	550-900	1/13000	1000	*, ‡

Extracted from: (JRC, 2015b)

Sources: * (Kluger, 2015a), † Questionnaire of Member States (JRC, 2015b), ‡ Questionnaire of Tattooist Associations (JRC, 2015b)

Table 3 presents the number of associations by country of tattoo artists and PMU practitioners. Although at least half the countries in the EU have associations, only a small percentage of tattooist and PMU practitioners are members (less than 20% of all tattooists). (JRC, 2015b)

There is no information about the training and certification requirements of persons making PMU. Interviews with PMU manufacturers have revealed that private PMU labels provide training on the best application of their colours. As PMU professionals are employed in laser surgery clinics, spas and other wellness centres, their primary training and certification is likely in the area of beauty, wellness or health (medical occupation). Their membership in professional organisations is likely related to their primary training and occupation.

Table 3 List of tattoo/PMU professional associations by country

EEA Country	**Professional Association and its abbreviation**
Austria	Association within the Chamber of Commerce WKO
Switzerland	Swiss Association of Tattoo Artists VST
Germany	German Tattoo Organisation DOT German Federal Association for tattooing BVT Pro Tattoo e.V. PT United European Tattoo Artists UETA
Denmark	Dansk Tatovør Laug DTL Danish Professional Tattoo Organisation DPTA
Spain	Spanish National Union of Professional Tattooists UNTAP
Finland	Finnish Tattoo Artist Association FTAA
France	Association Tatouage et Partage ATP Syndicat National des Artistes Tatoueurs SNAT
Hungary	Association of Hungarian Tattoo Artists MTSZ Professional and Interest Association of Tattoo Artists TSZEE
Italy	Associazione.it ART Italian Association of Professional Piercers and Tattooists APTPI Association of Corrective Aesthetic Tattoos ATEC Association of united tattoo artists ATIR National Artwork Confederation CNA
Netherlands	Advocacy for Tattoo artists and Piercers BVTP
Norway	Norwegian Tattoo Union NTU
Romania	Roman Tattoo union UTR Asociatia Tattoo & Piercing Romania ATPR
Sweden	SRT Swedish Registered Tattoo Artists Association SRT
United Kingdom	Tattoo and Piercing Union TPU Tattoo Club of Great Britain TCGB Tattooing and Piercing Industry Union TPIU British Tattoo Artists Federation BTAF

Source: (JRC, 2015b)

A.3. Uses advised against by the registrants

Table 4 contains information on the colourants used in tattoo inks and PMU that are registered under REACH. As shown in the table, the intended use of the colourants is primarily in industrial applications such as automotive or textiles. Some registrants explicitly advise against the use in tattoo inks, e.g., carbon black. Therefore, if these pigments are used in tattoos, then the formulator should have carried out their own downstream user chemical safety assessment if they use the substance in a quantity of above 1 tonne per year. No notifications of use in tattoo inks have been received for downstream users under Article 38 of REACH.

Table 4 Pigments used in tattoo inks and PMU registered under REACH

Substance Name	Description	EC #	CAS #	Uses advised against	Tattoo/ PMU uses advised against	CPR Annex II entry #	CPR Annex IV entry #	Tonnage band displayed
Chromium trioxide		215-607-8	1333-82-0	Professional: All uses apart from as a laboratory chemical Consumer: All	yes			10000 - 100000 tonnes per annum; Intermediate Use Only
Copper oxide		215-269-1	1317-38-0	Industrial: unknown				10000 - 100000 tonnes per annum
Zinc oxide	CI 77947/ White	215-222-5	1314-13-2, 7440-66-6	*"none" stated*			145	100000 - 1000000 tonnes per annum; Intermediate Use Only
6,15-dihydroanthrazine-5,9,14,18-tetrone	CI 69800/ Blue	201-375-5	81-77-6	*"none" stated*			95	100 - 1000 tonnes per annum
29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32 copper	CI 74160 / PIGMENT BLUE 15	205-685-1	147-14-8	*"none" stated*		1367	105	10000 - 100000 tonnes per annum
5,12-dihydro-2,9-dimethylquino[2,3-b]acridine-7,14-dione	CI 73915/Red	213-561-3	980-26-7	*"none" stated*			103	1000 - 10000 tonnes per annum
5,12-dihydroquino[2,3-b]acridine-7,14-dione	CI 73900 / PIGMENT VIOLET 19	213-879-2	1047-16-1	Industrial: Coatings and paints, thinners, paint removes; Use at industrial site leading to inclusion into/onto article		1366	102	1000 - 10000 tonnes per annum

Diiron trioxide	CI 77015/Red, CI 77491/ CI 77499; Diiron trioxide; Ferric oxide/ Red, CI 77499/Black	215-168-2	1309-37-1, 7439-89-6	Industrial: no uses advised against are identified				100000 - 1000000 tonnes per annum
Aluminatesilicate	CI 77004 / ALUMINUM SILICATE/ White	215-475-1	1327-36-2	*"none" stated*			119	1000 - 10000 tonnes per annum; 100 - 1000 tonnes per annum
Polychloro copper phthalocyanine	CI 74260 / Pigment Green 7	215-524-7	1328-53-6	*"none" stated*		1369	107	1000 - 10000 tonnes per annum
Carbon black	CI 77266 / CARBON BLACK	215-609-9	1333-86-4	Industrial, professional, consumer: Use as pigment in tattoo colours for humans	yes		126a, 126	1000000 - 10000000 tonnes per annum; 10000 - 100000 tonnes per annum; 1000 - 10000 tonnes per annum
Trisodium 5-hydroxy-1-(4-sulphophenyl)-4-(4-sulphophenylazo)pyrazole-3-carboxylate	CI 19140 / ACID YELLOW 23	217-699-5	1934-21-0	Professional, consumer: PC 12: Fertilisers PC 24: Lubricants, greases, release products PC 27: Plant protection products PC 28: Perfumes, fragrances PC 35: Washing and cleaning products (including			44	0 - 10 tonnes per annum

				solvent based products) PC 39: Cosmetics, personal care products				
1-(4-methyl-2-nitrophenylazo)-2-naphthol	CI 12120/Red	219-372-2	2425-85-6	*"none" stated*			10	100 - 1000 tonnes per annum
2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide	CI 11680/Yellow	219-730-8	2512-29-0	*"none" stated*			4	100 - 1000 tonnes per annum
Trisodium 1-(1-naphthylazo)-2-hydroxynaphthalene-4',6,8-trisulphonate	CI 16255 / ACID RED 18	220-036-2	2611-82-7	Professional, consumer: PC 12: Fertilisers PC 24: Lubricants, greases, release products PC 27: Plant protection products PC 28: Perfumes, fragrances PC 35: Washing and cleaning products (including solvent based products) PC 39: Cosmetics, personal care			35, 31	10 - 100 tonnes per annum

				products				
Disodium 2-amino-5-[(4-sulphonatophenyl)azo]benzenesulphonate	CI 13015/Yellow	220-293-0	2706-28-7	*"none" stated*			16	0 - 10 tonnes per annum
Disodium 6-hydroxy-5-[(4-sulphonatophenyl)azo]naphthalene-2-sulphonate	CI 15985/Yellow	220-491-7	2783-94-0	*"none" stated*			31	10 - 100 tonnes per annum
1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol	CI 12085/Red	220-562-2	2814-77-9	*"none" stated*		1345	9	100 - 1000 tonnes per annum
Disodium 4-hydroxy-3-[(4-sulphonatonaphthyl)azo]naphthalenesulphonate	CI 14720 / ACID RED 14	222-657-4	3567-69-9	*"none" stated*			19	10 - 100 tonnes per annum
Disodium 2,2'-(9,10-dioxoanthracene-1,4-diyldiimino)bis(5-methylsulphonate)	CI 61570/Green	224-546-6	4403-90-1	*"none" stated*			92	0 - 10 tonnes per annum
Bisbenzimidazo[2,1-b:2',1'-i]benzo[lmn][3,8]phenanthroline-8,17-dione	CI 71105/ Orange	224-597-4	4424-06-0	*"none" stated*			97	10 - 100 tonnes per annum
Calcium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	CI 15850/ PIGMENT RED 57:1	226-109-5	5281-04-9	*"none" stated*			27	10000 - 100000 tonnes per annum
2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-chloro-2,5-	CI 21108/Yellow	226-939-8	5567-15-7	Industrial, professional, consumer: other uses except			48	1000 - 10000 tonnes per annum

dimethoxyphenyl)-3-oxobutyramide]				pigment				
2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-N-phenylbutyramide]	CI 21090	228-787-8	6358-85-6	*"none" stated*		1263		10000 - 100000 tonnes per annum
Calcium 3-hydroxy-4-[(1-sulphonato-2-naphthyl)azo]-2-naphthoate	CI 15880/Red	229-142-3	6417-83-0	*"none" stated*		1349	29	0 - 10 tonnes per annum
2-[(4-chloro-2-nitrophenyl)azo]-N-(2-chlorophenyl)-3-oxobutyramide	CI 11710/Yellow	229-355-1	6486-23-3	*"none" stated*			5	100 - 1000 tonnes per annum
3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide	CI 12370 / PIGMENT RED 112	229-440-3	6535-46-2	*"none" stated*		1346	11	1000 - 10000 tonnes per annum
Barium sulfate	CI 77120/ Barium Sulfate/White	231-784-4	7727-43-7	*"none" stated*			122	10000 - 100000 tonnes per annum
Ammonium manganese(3+) diphosphate	CI 77742/ Violet	233-257-4	10101-66-3	*"none" stated*			140	10 - 100 tonnes per annum
Aluminium, 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)azo]-1H-pyrazole-3-carboxylic acid complex	CI 19140/ ACID YELLOW 23 ALUMINUM LAKE	235-428-9	12225-21-7	*"none" stated*			44	0 - 10 tonnes per annum
Sodium aluminosilicate violet	CI 77007 / Ultramarines/ Blue	235-811-0	12769-96-9	*"none" stated*			120	100 - 1000 tonnes per annum
Titanium dioxide	CI 77891, Titanium	236-675-5	13463-67-7	*"none" stated*			143	10000 - 100000 tonnes per annum

	dioxide/ White							
Iron hydroxide oxide yellow	CI 77492/ Iron hydroxide oxide yellow; CI Pigment Yellow 42 /Yellow	257-098-5	51274-00-1	Industrial: not applicable			136	100000 - 1000000 tonnes per annum
1,4-dihydroxyanthraquinone		201-368-7	81-64-1	*"none" stated*				Intermediate Use Only
Barium bis[2-[(2-hydroxynaphthyl)azo]naphthalenesulphonate]		214-160-6	1103-38-4	*"none" stated*				10 - 100 tonnes per annum
1-[(2-methoxyphenyl)azo]-2-naphthol		214-968-9	1229-55-6	*"none" stated*		1231		0 - 10 tonnes per annum
4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide		220-509-3	2786-76-7	*"none" stated*				100 - 1000 tonnes per annum
2,9-dichloro-5,12-dihydroquino[2,3-b]acridine-7,14-dione		221-424-4	3089-17-6	*"none" stated*				100 - 1000 tonnes per annum
1-[(2,4-dinitrophenyl)azo]-2-naphthol		222-429-4	3468-63-1	*"none" stated*		397		100 - 1000 tonnes per annum
4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one]		222-530-3	3520-72-7	*"none" stated*				100 - 1000 tonnes per annum
4,4'-diamino[1,1'-bianthracene]-9,9',10,10'-tetraone		223-754-4	4051-63-2	*"none" stated*				100 - 1000 tonnes per annum

Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]		225-935-3	5160-02-1	*"none" stated*		401		1000 - 10000 tonnes per annum
N-(4-chloro-2,5-dimethoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide		226-103-2	5280-68-2	*"none" stated*				100 - 1000 tonnes per annum
2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(2-methylphenyl)-3-oxobutyramide]		226-789-3	5468-75-7	*"none" stated*				1000 - 10000 tonnes per annum
2,9-dimethylanthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone		226-866-1	5521-31-3	*"none" stated*				100 - 1000 tonnes per annum
3,3'-[(2-chloro-5-methyl-p-phenylene)bis[imino(1-acetyl-2-oxoethylene)azo]]bis[4-chloro-N-(3-chloro-o-tolyl)benzamide]		226-970-7	5580-57-4	*"none" stated*				100 - 1000 tonnes per annum
3,3'-(1,4-phenylenediimino)bis[4,5,6,7-tetrachloro-1H-isoindol-1-one]		226-999-5	5590-18-1	*"none" stated*				0 - 10 tonnes per annum
4-[(2,5-dichlorophenyl)azo]-3-hydroxy-N-phenylnaphthalene-2-carboxamide		227-930-1	6041-94-7	*"none" stated*				100 - 1000 tonnes per annum

2-[(2-methoxy-4-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide		228-768-4	6358-31-2	*"none" stated*				1000 - 10000 tonnes per annum
2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-methylphenyl)-3-oxobutyramide]		228-771-0	6358-37-8	*"none" stated*				0 - 10 tonnes per annum
3-hydroxy-4-[(2-methyl-5-nitrophenyl)azo]-N-phenylnaphthalene-2-carboxamide		229-245-3	6448-95-9	*"none" stated*				0 - 10 tonnes per annum
2,2'-[(3,3'-dimethoxy[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-N-phenylbutyramide]		229-388-1	6505-28-8	*"none" stated*				0 - 10 tonnes per annum
2-[(4-methoxy-2-nitrophenyl)azo]-N-(2-methoxyphenyl)-3-oxobutyramide		229-419-9	6528-34-3	*"none" stated*				100 - 1000 tonnes per annum
Barium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate		231-494-8	7585-41-3	*"none" stated*				100 - 1000 tonnes per annum
2-[(4-chloro-2-nitrophenyl)azo]-N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxobutyramide		235-462-4	12236-62-3	*"none" stated*				100 - 1000 tonnes per annum
[1,3,8,16,18,24-hexabromo-2,4,9,10,11,15,17,22,23,25-decachloro-29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]copper		238-238-4	14302-13-7	*"none" stated*				100 - 1000 tonnes per annum

4,4'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[2,4-dihydro-5-methyl-2-(p-tolyl)-3H-pyrazol-3-one]		239-898-6	15793-73-4	*"none" stated*				1000 - 10000 tonnes per annum
N,N'-[6,13-diacetamido-2,9-diethoxy-3,10-triphenodioxazinediyl]bis(benzamide)		241-734-3	17741-63-8	*"none" stated*				10 - 100 tonnes per annum
3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide		250-063-5	30125-47-4	*"none" stated*				100 - 1000 tonnes per annum
2-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]benzoic acid		250-830-4	31837-42-0	*"none" stated*				100 - 1000 tonnes per annum
5,5'-(1H-isoindole-1,3(2H)-diylidene)dibarbituric acid		253-256-2	36888-99-0	*"none" stated*				1000 - 10000 tonnes per annum
N-(5-chloro-2-methoxyphenyl)-3-hydroxy-4-[[2-methoxy-5-[(phenylamino)carbonyl]phenyl]azo]naphthalene-2-carboxamide		268-028-8	67990-05-0	*"none" stated*				10 - 100 tonnes per annum
N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxo-2-[[2-(trifluoromethyl)phenyl]azo]butyramide		268-734-6	68134-22-5	*"none" stated*				100 - 1000 tonnes per annum
Zinc ferrite brown spinel		269-103-8	68187-51-9	Industrial: not applicable				10000 - 100000 tonnes per annum

Tetramethyl 2,2'-[1,4-phenylenebis[imino(1-acetyl-2-oxoethane-1,2-diyl)azo]]bisterephthalate		271-176-6	68516-73-4	*"none" stated*				100 - 1000 tonnes per annum
Aluminium, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid complex		271-524-7	68583-95-9	*"none" stated*				0 - 10 tonnes per annum
2,2'-[ethylenebis(oxyphenyl-2,1-eneazo)]bis[N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxobutyramide		278-770-4	77804-81-0	*"none" stated*				100 - 1000 tonnes per annum
Silicic acid, aluminum sodium salt, sulfurized		309-928-3	101357-30-6	*"none" stated*			120	10000 - 100000 tonnes per annum
3,6-bis(4-chlorophenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione		401-540-3	-	*"none" stated*				100+ tonnes per annum; 10 - 100 tonnes per annum; 100 - 1000 tonnes per annum; 0 - 10 tonnes per annum
3,6-diphenyl-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione		402-400-4	-	*"none" stated*				Tonnage Data Confidential; 10 - 100 tonnes per annum; 100 - 1000 tonnes per annum
3,6-bis(4-tert-butylphenyl)-1H,2H,4H,5H-pyrrolo[3,4-c]pyrrole-1,4-dione		416-250-2	-	*"none" stated*				100+ tonnes per annum; 0 - 10 tonnes per annum; Tonnage Data Confidential
6-chloro-2-(6-chloro-4-methyl-3-oxobenzo[b]thien-2(3H)-ylidene)-4-methylbenzo[b]thiophene-	CI 73360 / VAT RED 1	219-163-6	2379-74-0			1365	100	0 - 10 tonnes per annum

3(2H)-one								
N-(5-chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulphonyl]-2-methoxyphenyl]azo]-3-hydroxynaphthalene-2-carboxamide	CI 12490 / PIGMENT RED 5	229-107-2	6410-41-9	*"none" stated*		1347	14	10 - 100 tonnes per annum
3-hydroxy-4-[(2-methyl-4-nitrophenyl)azo]-N-(o-tolyl)naphthalene-2-carboxamide		229-102-5	6410-32-8	*"none" stated*				10 - 100 tonnes per annum
4-[(4-chloro-2-nitrophenyl)azo]-3-hydroxy-N-(2-methylphenyl)naphthalene-2-carboxamide		229-314-8	6471-50-7					0 - 10 tonnes per annum
4-[(2,5-dichlorophenyl)azo]-N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-hydroxynaphthalene-2-carboxamide		230-258-1	6992-11-6	*"none" stated*				10 - 100 tonnes per annum
N-(4-chloro-2,5-dimethoxyphenyl)-2-[[2,5-dimethoxy-4-[(phenylamino)sulphonyl]phenyl]azo]-3-oxobutyramide		235-427-3	12225-18-2	*"none" stated*				100 - 1000 tonnes per annum
N,N'-(2,5-dichloro-1,4-phenylene)bis[4-[[2-chloro-5-(trifluoromethyl)phenyl]azo]-3-hydroxynaphthalene-2-carboxamide]		257-776-0	52238-92-3	*"none" stated*				10 - 100 tonnes per annum

N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-2-[(2-methoxyphenyl)azo]-3-oxobutyramide		279-914-9	82199-12-0					100 - 1000 tonnes per annum
A mixture of: N-(4-chlorophenyl)-4-(2,5-dichloro-4-(dimethylsulfamoyl)phenylazo)-3-hydroxy-2-naphthalenecarboxamide; N-(4-chlorophenyl)-4-(2,5-dichloro-4-(methylsulfamoyl)phenylazo)-3-hydroxy-2-naphthalenecarboxamide		412-550-2	-					10 - 100 tonnes per annum; Tonnage Data Confidential
Reaction mass of 4-[[4-(aminocarbonyl)phenyl]azo]-N-(2-ethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide and 4-[[4-(aminocarbonyl)phenyl]azo]-3-hydroxy-N-(2-methoxyphenyl)naphthalene-2-carboxamide		911-436-4	-					10 - 100 tonnes per annum
Barium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate		241-806-4	17852-98-1	*"none" stated*				

Annex B. Information on hazard and risk

B.1. Identity of the substance(s) and physical and chemical properties

B.1.1. Name and other identifiers of the substance(s)

In excess of four thousand substances fall within the scope of the restriction proposal (in the categories described in section 1.1.4 of the report). Table 5 gives a breakdown of the number of these substances by category:

Table 5 Breakdown of substances in the restriction proposal

Total number of substances in the scope:	Approximately **4 130**
1. Substances with harmonised classification in the Classification, Labelling and Packaging Regulation (EC) No 1272/2008 as:	Approximately **2 390**
a. carcinogenic and mutagenic Cat. 1A, 1B, and 2	Only classified as Cat 1A and 1B: **862** Classified as Cat. 1A, 1B, and 2 (with other relevant classifications): **1287**
b. reproductive toxicant Cat. 1A,1B, and 2	Only classified as Cat 1A and 1B: **74** Only classified as Cat 2: **36** Classified as Cat 1A, 1B and Cat 2 (with other relevant classifications): **368**
c. skin sensitisers Cat. 1, Cat. 1A, Cat. 1B	Only classified as skin sensitiser Cat 1, 1A and 1B: **415** Classified as skin sensitiser Cat 1, 1A and 1B (with other relevant classifications): **1 159**
d. skin irritant (Cat. 2), skin corrosive (Cat. 1, Cat. 1A, 1B, 1C), eye irritant (Cat. 2) or eye damaging (Cat. 1) Irritation, corrosive.	Only classified as skin irritant (Cat. 2), skin corrosive (Cat. 1, Cat. 1A, 1B, 1C), eye irritant (Cat. 2) or eye damaging (Cat. 1) Irritation, corrosive: **895** Classified as skin irritant (Cat. 2), skin corrosive (Cat. 1, Cat. 1A, 1B, 1C), eye irritant (Cat. 2) or eye damaging (Cat. 1) Irritation, corrosive (with other relevant classifications): **1 577**
2. Substances on CPR Annex II:	Total: **1 490**

	Classified as CMR Cat 1A, 1B and 2: **795** Classified as skin sensitiser Cat 1, 1A and 1B: **103**
3. Substances on CPR Annex IV: a. restricted due to conditions on use (in column g of Annex IV) allowed in tattoo inks under specific conditions (columns h-i of Annex IV):	Total on Annex IV: **260** Restricted due to conditions on use: **74** Allowed under specific conditions: **119** Classified as CMR or skin sensitiser/irritant/corrosive or eye irritant/damaging: **1**
4. Substances on the Council of Europe Resolution on requirements and criteria for the safety of tattoos and permanent make-up (CoE, 2008)	Approximately in total: **4 130** Excluding those in points 1-3: **36**

Appendix B.1. List of substances in the scope of the restriction gives a detailed list of all substances included in the restriction proposal at the time of preparing the dossier (additional substances may have been added though changes to CLP or CPR after the submission of the dossier).

B.1.2. Composition

See Annex A.1 and D.2.1 for description of the composition of tattoo inks and PMU. The list of substances covered by this restriction (Appendix B.1. List of substances in the scope of the restriction) indicates where the substances have been found in tattoo inks but the use of other substances on the list cannot be excluded.

B.1.3. Physicochemical properties

Not included in this report due to the number of substances within scope (some specific parameters may be included for the substances assessed on a case-by-case basis).

B.1.4. Justification for targeting

The justification for targeting the substances in this restriction is explained under 1.1 introduction and 1.1.4 scope.

B.2. Manufacture and uses (summary)

More than 40 100 litres of tattoo inks and 11 000 litres of PMU inks were formulated within the EU in 2016. A substantial share of the tattoo inks on the EU market is imported (about 114 000 litres in 2016). Between 70% and 80% of the tattoo inks on the EU market are manufactured outside the EU (Michel, 2015), while about 20% of PMU on the EU market is imported, primarily from the US or China. (Michel, 2015), (JRC, 2015b). A small percentage of the EU produced tattoo inks is exported (about 5%), while close to 20% of EU formulated PMU is sold internationally (primarily in North America). For further information regarding the tattoo inks and PMU on the EU market, see Annex A.

B.3. Classification and labelling

B.3.1. Classification and labelling in Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation)

The classifications of the substances in the scope are included in Appendix B.1. List of substances in the scope of the restriction

B.3.2. Classification and labelling in classification and labelling inventory/ Industry's self-classification(s) and labelling

Due to the large number of substances in the scope of the restriction dossier the notified classification and labelling in the classification and labelling inventory (Industry's self-classification(s) and labelling is not included in Appendix B.1. and is only included in a few of the specific substance assessments when it is deemed to be relevant.

B.4. Environmental fate properties

B.4.1. Degradation

Not relevant for this Dossier.

B.4.2. Environmental distribution

Not relevant for this Dossier.

B.4.3. Bioaccumulation

Not relevant for this Dossier.

B.4.4. Secondary poisoning

Not relevant for this Dossier.

B.5. Human health hazard assessment

To efficiently and effectively deal with all the substances included in the scope of the restriction (see sections B.1.1 of this Annex and 1.1.4 of the Report), the Dossier Submitter has addressed a number of substances through a qualitative approach and the remaining, in a (semi-)quantitative manner.

According to REACH Annex I para 1.1.2. and ECHA Guidance R.8 (ECHA, 2012), when no reliable dose descriptor can be set for a given endpoint, a qualitative approach (analysis) has to be taken. The relevant endpoints/hazard categories where a qualitative analysis is appropriate are: irritation/corrosion, sensitisation, acute toxicity, carcinogenicity and mutagenicity. For most of these, a threshold cannot be identified.

In the case of this restriction, the Dossier Submitter has therefore included the following groups of substances based solely on their intrinsic hazardous properties:

- All substances with inherent properties that may cause an effect with no threshold. This is the case for most substances with C and M classifications (annex /8. Carcinogenicity and mutagenicity), as well as for lead (EFSA CONTAM Panel, 2013) (annex Toxicity for reproduction).
- All substances classified as skin sensitisers, based on the observation that when allergens are deposited into the dermis via an injection, stronger sensitisation/elicitation reactions may occur and with lower doses than when deposited on the skin (annex Sensitisation).

In theory skin sensitisers have thresholds, but data is very seldom available to set the threshold, and concentration limits established based on epidermal exposure cannot be used to set limit values for tattoo inks.

- All substances classified as skin irritants/skin corrosive and eye irritants/eye damaging, based on the assumption that the effects will be more severe when these substances are injected into the skin rather than applied on the skin (annex /4. Irritation and corrosivity).

Other substances are included in the scope of this restriction based on their intrinsic properties and evaluated quantitatively since a DNEL can be derived:

- Substances with R-classifications 1A and 1B, and 2 (annex Toxicity for reproduction).
- Certain substances listed on table 3 of the CoE ResAP and which are considered to be impurities found in tattoo ink (such as zinc, copper, barium) (annex Council of Europe Resolution ResAP(2008)1, Table 3).
- Methanol, due to its classification as STOT SE and that it has been found to be present in tattoo ink (annex Acute toxicity and Specific target organ toxicity – single exposure (STOT SE)).

For substances that are prohibited from use according to the Cosmetics regulation the Dossier Submitter also adopts a qualitative approach. Therefore, based on the assumption that substances not allowed to be used in cosmetic products on the surface of the skin should also not be allowed to be injected into the skin, the following substances are included in the scope:

- Substances on Annex II to the Cosmetics regulation (annex CPR Annex II, list of substances prohibited in cosmetic products).
- Substances on Annex IV to the Cosmetics regulation that are not allowed to be used in contact with mucous membranes, eyes or in prolonged contact with the skin (column "g") or subject to other conditions specified in columns "h" to "i" of the Annex (e.g., purity requirements) (annex CPR Annex IV, colourants in cosmetic products).

B.5.1. Toxicokinetics (absorption, metabolism, distribution and elimination)

Very limited information is available on the toxicokinetics of substances contained in tattoo ink and permanent make-up (PMU) after entry into the body. The uniqueness of this exposure route (intradermal injections) matches no other exposure situation for consumer chemicals regulated under REACH.

B.5.1.1. Absorption

Absorption describes the potential for a substance to diffuse across biological membranes. In the case of tattoo ink and PMU it is more relevant to discuss bioavailability than absorption, as the colourant is injected into the dermis (1-2 mm) to make a tattoo permanent. During tattooing there may be loss of a minor part of the ink due to subsequent bleeding of the injured epidermis. However, since tattooing is an injury of the skin barrier, the ink could be considered similar to substances instantly absorbed by the human body. The Dossier Submitter therefore assumes 100% bioavailability (proxy for absorption) of all the constituents in the risk assessment.

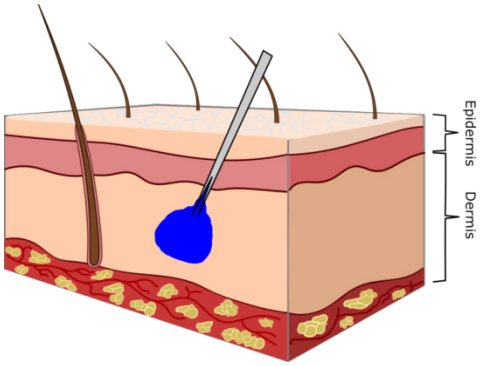


Figure 1. Diagram of the skin showing deposition of the tattoo ink (blue colour) in the dermis. From the report "Allergy and tattoos" by the National Allergy Research Centre, University of Copenhagen, Denmark (DEPA, 2017a).

The assumption of a 100% uptake of pigments is justified due to the fact that:

- Only a few percent of the initially injected pigment is required to keep the tattoo visible even after 20 – 30 years.
- Engel et al. and Lehner et al. (Engel, et al., 2010) (Lehner, et al., 2011) demonstrated that the amount of pigment in the skin of mice was reduced to a great extent during the weeks and months following the tattooing.

This assumption is considered conservative and will be further described in the uncertainty and sensitivity analysis.

Because the immune system sees the pigment particles as a foreign invader, the macrophages engulf the particles in order to eliminate them from the body. Only pigment particles introduced through the skin surface, below the dermal-epidermal junction, are retained by the dermal macrophages and fibroblasts where they reside permanently, producing an indelible change of the skin colour under the form of recognisable pattern or design (Vassileva & Hristakieva, 2007).

B.5.1.2. Distribution

The distribution of substances in tattoo inks in the body is not well documented. There is insufficient data available to derive any temporal distribution pattern to conclude on systemic availability for the separate chemical substances in tattoo ink. Soluble substances are probably distributed quickly in the body, e.g. during one day, and the insoluble colourants are considered to (partially) remain in the skin, as tattoos often remain visible even after 20 – 30 years (see Appendix F.1 Questionnaire on the tattoo process).

Cui et al. (Cui, et al., 2005) suggested that the mechanisms for tattoo fading may include: 1) the dispersion of pigments through the skin; 2) their phagocytosis and consequent removal; 3) the occurrence of their metabolism in the skin; 4) the photochemical degradation of pigments. In line with this Bäumler (Bäumler, 2015) states that tattoos are known to fade over time which in part can be explained by photolytic decomposition and in part by slow distribution from the skin (e.g. "photo stable" carbon black) to body fluids or other organs. Furthermore, other types of decomposition cannot be excluded.

The majority of the ink, with the macrophages or otherwise, is transported to local lymph nodes and via the lymph system to other organs of the body to be eliminated or stored. The tattoo ink is diffused from the tattoo site via the following modes: a) elimination during the tattoo process as a result of the bleeding, b) elimination during the healing process, c) natural

epidermal replacement as epidermal cells have a life span of two to three weeks and the ink in the epidermis is removed as the epidermal cells are replaced with new cells, d) soluble substances are transported almost immediately throughout the body, while insoluble substances are eliminated via the macrophages, are broken down over time to decomposition products that can be more easily eliminated or stored by the body or remain at the tattoo site in the dermis (Lehner, et al., 2011).

In a recently published paper, Schreiver et al. (Schreiver, et al., 2017) reported translocation of tattoo particles in the nano- and micrometre range from skin to lymph nodes. Transportation was assumed to have taken place either passively transported via blood and lymph fluids or phagocytized by immune cells and subsequently deposited in regional lymph nodes. Coloured tattoos in skin from human corpses were analyzed and compared with analyses of content in lymph nodes from the same corpses (Element analysis via ICP-MS: 20 skin and 25 lymph node samples; identification of organic pigments via LDI-ToF: 8 skin and 8 lymph node samples, μ-XRF: 3 skin and 3 lymph node samples; v-XRF: 1 skin and 1 lymph node sample). Also there were two control corpses. Analysis showed that the concentration of elements like Al, Fe, Cr, Cu, Ni and Cd were higher in lymph nodes than in the skin with the tattoos in some of the corpses and mainly explained by the pigments in the tattoos. The element Ti probably derived from TiO2 in tattoos was found in the skin and lymph nodes. The average particle size of TiO2 in both skin and lymph nodes was 180 nm. It is assumed that transport of smaller particles is preferential, e.g. particles from phtalocyanine green 36 containing Br were polydisperse with the smallest particles as low as the resolution of 50 nm. In the skin the tattoo pigment particles of phthalocyanine green 36 were up to several micrometers in size. In addition to translocation, biomolecular change was observed indicating a reaction to the tattoos even though chronic inflammation was not observed.

Insoluble substances

Since pigments are gathered in particles to be visible, they may not be absorbed and transported away from the tattoo site in the same degree as the liquid matrix in which the pigment is suspended. To be visible, some of the pigment has to remain in the skin – otherwise, the tattoo would not be visible - while the matrix containing potential impurities and degradation products to a much higher degree will be available for distribution via the lymphatic system and blood. In a study by Engel et al. (Engel, et al., 2010) mice were tattooed using an ink with pigment red 22 (PR 22). The amount of pigment in the skin was reduced by 32% over 6 weeks and by 60% after exposure to a sunlight simulator. Therefore, it was considered that during those 6 weeks, the pigment is transported and distributed elsewhere in the organism either as pigment or degradation products. According to (Lehner, et al., 2011) only a small portion of the originally injected pigment, between 1-13% remains at the tattoo site permanently. In an investigation of pigment content (using the same pigment) in human donor skin and assuming an amount of 2.53 mg pigment per cm^2 based on the initial study by Engel et al. and Lehner et al. (Engel, et al., 2010), (Lehner, et al., 2011), it has been estimated that a reduction of 87-99% of red pigment occurs at the injection site over a period of months to years. However, the authors point out that as the initial amount of 2.53 mg pigment/cm^2 is most likely too high the reduction is probably less than 87-99%. It is nevertheless concluded that only a few percent of the initially injected pigment is required to keep the tattoo visible.

An indicator of the slow distribution of insoluble pigments is the observation of tattoos fading very slowly with time. An argument for a quicker distribution is the colouring of the lymph node, which indicates that some of the pigments may be transferred relatively quickly to the

lymphatic system. Danish EPA (DEPA, 2012) estimated that about 25% of the pigment could be retrieved in the lymph nodes. Deposition of pigment in the lymph nodes gives further evidence that the substance is systemically distributed.

It is well established that a part of the pigment can be found in macrophages and is also transported to neighbouring lymph nodes (Dominguez, et al., 2008); (Engel, et al., 2010); (Lehner, et al., 2011). In addition, the pigments are also known to distribute in the body and has been found in diffecombinedrent organs such as the liver (Sepehri, et al., 2017a)[6].

According to Bäumler (Bäumler, 2015), the concentration of pigments initially deposited into the dermis may be reduced by three major mechanisms. 1) the bleeding occurring during or immediately after the tattooing process; 2) the transportation through the lymphatic or blood vessel systems; 3) the photodecomposition of colourants due to sunlight, UV or laser radiation.

Independent of the local deposition and/or (partial or complete) translocation of the pigment or degradation products, the starting assumption is that the substance is systemically available. Therefore, total bioavailability of the insoluble substance will probably be close to 100% over the recipient's lifetime.

Soluble substances and impurities

It is probable that soluble particles are transferred relatively quickly to the lymphatic system. For all substances that are readily water soluble like ethanol, glycerine etc., it is assumed that there will be an initial high systemic bioavailability followed by slower distribution from remaining parts of the ink deposited in the skin.

Tattoo inks are known for containing impurities (JRC, 2015b). The same impurities may have different origin (see e.g. the justification for PAA). First, impurities may result from the production of the pigments. Further, as the pigments decompose impurities may be formed. These may also be referred to as break down products. However, often the origin of the impurities is unknown and could be both.

Beside the pigments the rest of the ink consists of a solution, which will be adsorbed and distributed in the body. The solution may contain soluble substances including impurities from the pigment or other ingredients. Soluble constituents of the ink are considered to be distributed within hours or days; thus being systemically available almost immediately. Thus in the calculation a 100 % uptake is assumed.

However, solid ink particles may continue to release soluble chemical components (impurities) as the ink particles decompose with time. This decomposition may be enhanced by exposure to sunlight. Additionally, substances might be attached to the pigment surface (e.g. PAH to carbon black particles) and will be released over time. In theory, this is comparable with a small reservoir of impurities being placed in the skin. Due to lack of data it is not possible to assess this release and the kinetics of the ink and the release of impurities in the body. However, the release of impurities may thus possibly give rise to further and continuous exposure.

Soluble substances (released from pigment particles) may thus show a time-dependent pattern of systemic bioavailability. This pattern will be variable depending on the solubility of the

[6] See also (The YouTube Channel of the American Chemical Society - https://www.youtube.com/watch?v=Fs9rR4W0EeA).

individual substance/compound and as distribution from the skin to other parts of the body can be different (Serup, et al., 2015).

In this assessment it is assumed that the impurities released from pigments are excreted - i.e. removed from the body - and that the sustained contribution from new release of impurities does not exceed the initial concentration of the impurities in the ink when injected into the body. This assumption is described in the uncertainty and sensitivity analysis.

Conclusion

The substance at the injection site (sub dermis) is considered to be systemically available due to the blood supply and tissue reactions. There is no robust kinetic data on the fate of individual pigments and other insoluble compounds after deposition in the skin. For that reason, the Dossier Submitter assumes that following injection and distribution 100% of the injected substances are systemically available over time, which is the sum of the amount of substances at the injection site (dermis and sub dermis), at the local lymph nodes, in lymph, blood, and other tissue

B.5.1.3. Metabolism

According to the review from Laux et al. (Laux, et al., 2016), evidence indicates that tattoo colourants are subject to metabolism.

Azo colourants which may be cleaved into carcinogenic primary aromatic amines and are especially of high concern as constituents in tattoo ink and PMU (DEPA, 2017c). It has not been possible to describe the stability of the azo colourants quantitatively. However, some general remarks can be made based on the different investigation of decomposition of the azo colourants. In general, azo colourants with simple structures and low molecular weight exhibit higher rates of degradation and decomposition than high molecular weight compounds. Further, mono-azo colourants are less stable than di-azo colourants. Electron withdrawing groups such as SO_3H or SO_2NH_2 attached to the phenyl ring also increases the stability of the azo bond and azo colourants with hydroxyl groups is less stable compared to methyl, methoxy, sulpho or nitro groups attached to the phenyl ring (Environment Canada, 2012).

B.5.1.4. Excretion

Very limited information is available about the excretion of substances in tattoo ink. In general it is assumed that non-soluble particles in tattoo colourants are excreted to a low degree, while more soluble compounds may be transported, metabolised and excreted via the liver (into bile) or kidney (into urine). This is supported by coloured lymph nodes that have been observed (Serup, et al., 2015) and the fact that tattoos remain visible for many years. Because the level of application of PMU is more superficial than in decorative tattooing, spontaneous elimination of the colourant and fading may occur within months or a few years (De Cuyper, 2015).

The assumptions and the consequences have been described in the uncertainty and sensitivity analysis.

Removal of tattoos by laser treatment

It is relatively common to remove tattoos. According to the Joint Research Centre in the US and Canada between 14-17% of the tattooed individuals regret having a tattoo and consider removing it (JRC, 2015b). In Europe, this percentage is in line with data reported for Denmark, Belgium, Bulgaria, Italy and Poland, while Germany, France, Hungary and Iceland indicate a percentage lower than 10%. Among tattooed people, only a small part undertakes the process

of removal: in Denmark and the Unites States for instance only 5% and 11% of removals are reported, respectively.

Q-switched laser treatment for removing tattoos is probably the most common method for removing tattoos (JRC, 2015b). Such treatment might perhaps lead to a large transport of more or less soluble substances from the tattooed skin within very short period of time.

According to Engel et al. (Engel, et al., 2008), about 51% of the (remaining 68%) pigment was removed during laser treatments. Since the efficiency of the laser depends on the depth of the localisation of the pigment in the tissue, during initial treatments more (superficial) pigment will be removed than during later treatments. On average, at least five to six sessions would be needed, but frequently a lot more. Even so, complete removal of the tattoo is most of the time not achievable (pers. comm. W. Bäumler).

The critical aspect concerning laser treatment is the decomposition and the substances formed during laser treatment. In particular, this is relevant for azo pigments that due to their chemical structure may form primary aromatic amines during laser treatment. See the section on metabolism above and also the description of primary aromatic amines (PAAs) and azo dyes for more information (Appendix B.2. PAAs and azo colourants). Currently, the long-term safety of laser treatments used for tattoo removal is unclear, particularly due to the laser-induced photodecomposition products of inks (JRC, 2015b). According to Laux et al. (Laux, et al., 2016), the possible production and release of toxic or carcinogenic compounds following a laser removal needs to be investigated.

For the PMU laser treatment is also relevant, even if the PMU should fade over time (within months to a few years). There are also serious problems observed with laser removal when the ink contains TiO_2 and iron oxides, as the laser treatment can lead to paradoxical darkening e.g. black lip liner (De Cuyper, 2015).

B.5.2. Acute toxicity and Specific target organ toxicity – single exposure (STOT SE)

The Dossier Submitter considers that it will be difficult to group all the substances with harmonised acute toxicity and STOT SE classifications as they give rise to very diverse health risks. Therefore, it was decided to include in the scope only those substances with STOT SE classifications (not covered by other group or individual assessments) present in tattoo inks or PMU. The only such substance was methanol, which has been identified to be present in tattoo inks and PMU (JRC, 2015b) and is classified with STOT SE.

Methanol

Methanol is classified for STOT SE 1 based on the effects on the optic nerve (nervus opticus) and central nervous system seen after a single exposure. Commission Directive 2006/15/EC of 7 February 2006 establishing a second list of indicative occupational exposure limit values, contains an OEL for methanol of 260 mg/m^3 or 200 ppm for an 8 hour exposure. This OEL is considered to be, in the majority of cases, also protective from very slight, sub-clinical CNS effects of methanol inhalation, which are reported to start to appearing at 270 mg/m^3 (FIOH, 2008).

The Dossier Submitter proposes that the DNEL should be based on the OEL value according to Appendix R.8-13 (Deriving DNELs when community/national Occupational Exposure Limit (OEL) is available) to ECHA Guidance R.8 (ECHA, 2012) (Characterization of dose [concentration]-response for human health of Guidance on information requirements and chemical safety assessment (ECHA).

B.5.3. /4. Irritation and corrosivity

The Dossier Submitter proposes that all substances classified with skin corrosion/irritation or eye damage/eye irritation (harmonised classification in Regulation 1272/2008 (CLP)) should be restricted in tattoo inks and PMU. This is based on the assumption that substances with these classifications, have intrinsic properties that will give at least the same, if not more severe effects when they are injected into the skin than applied on the skin. This assumption also applies to the eyes.

Substances causing skin corrosion/irritation or eye damage/eye irritation are classified according to effects in animals or humans according to the criteria in Annex I of regulation 1272/2008 (CLP).

As written in the introduction to the chapter on skin irritation/skin corrosion in Chapter R.7a: Endpoint specific guidance (ECHA, 2016e) substances causing local effects after single or multiple exposure can be distinguished as irritant or corrosive substances, depending on the severity, reversibility or irreversibility of the effects observed. Corrosive substances destroy living tissues with which they come into contact, whereas irritant substances are non-corrosive substances that, through immediate contact with the tissue under consideration may cause inflammation (see Section R.7.2.1.1 of the guidance for complete definitions). These tissues are in the present context the intradermal tissue.

The information available for skin corrosion/irritation and eye damage/eye irritation (ECHA Guidance R.8 (ECHA, 2012)) is usually the available in vitro and in vivo studies which tend to provide only qualitative (yes or no) or semi-quantitative/potency information. For example, corrosive after 3 minutes or 4 hours exposure; higher or lower scores for erythema, oedema and other irritative effects, as explained in Appendix R.8-9. Therefore, it is usually not possible to derive a DNEL for these substances and a qualitative risk assessment is therefore warranted.

In this restriction, it is assumed that all substances that have a harmonised classification as irritant/corrosive/eye damaging also will exert this effect when injected intradermally.

In a 2010 survey in German-speaking countries (Klugl, et al., 2010), about 68% of tattooed people reported skin problems after tattooing. As reported in (JRC, 2016a) the relative frequencies of the various pathologic effects have so far hardly been estimated due to lack of epidemiologic studies. According to Serup's data on 405 sick tattooed patients treated in a specialised dermatologic clinic between October 2008 and June 2015 (Serup, 2015b) the bulk of the non-infectious reactions (88%) is mainly (65%) of inflammatory nature (allergic or not).

It is difficult to distinguish between allergic and non-allergic reactions in the skin (DEPA, 2017a). Likewise it is often not possible to determine the origin of the effects, whether they are due to the chemicals in the tattoo ink, or just the skins reaction to the tattoo procedure. However, some reactions seen and described in tattooed individuals are more likely due to irritation of the skin rather than an allergic response. According to (Høgsberg, et al., 2013) complaints up to 3 months after tattooing were reported in 15% (23/154) of participants. Some of the recalled symptoms that may be due to irritation were major itching (6/342), ulceration (3/342), redness and swelling (9/342) and delayed healing (1/342). Of the tattoo complaints beyond 3 months after tattooing, skin elevation and itching were the most frequent complaints. Itching was mainly mild and spontaneously reported as comparable to a gnat/mosquito bite but one person consulted his physician three times due to major itching.

For more details of some of the dermatological effects seen in tattooed individuals, see section D.6.

B.5.5. Sensitisation

The Dossier Submitter proposes that all substances classified as skin sensitisers (SS) Category 1, 1A or 1B (harmonised classification in Regulation 1272/2008 (CLP)) should be restricted in tattoo inks and PMU. This is based on the intrinsic hazardous properties of the substances. Skin sensitising substances, depending on the concentration of the substances in the tattoo ink, may cause allergic contact dermatitis when applied to the epidermis or injected into the dermis.

Of the substances classified as skin sensitiser categories 1, 1A or 1B (see Table 5 in section B 1.1), which thus are in the scope of this restriction, 22 are substances present in tattoo inks according to the Joint Research Centre (JRC, 2015b). However, as any skin sensitisers may be present in tattoo inks, the Dossier Submitter proposes it is sufficient to include all the substances in the scope of this restriction where a harmonised classification as skin sensitisers Category 1, 1A or 1B has been included in Annex VI of CLP.

Substances causing skin sensitisation are classified according to effects in animals or humans (ECHA (CLP Annex I)). When data is sufficient, a refined evaluation allows the allocation of skin sensitisers into sub-category 1A, strong sensitisers, or sub-category 1B for other skin sensitisers. The most relevant sub-categorisation for skin sensitisers in tattoo ink is based on potency i.e. the induction thresholds from animal tests or findings in humans:

- 1A: Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.
- 1B: Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.

Note that since by tattooing allergens are directly deposited in the dermis, and hence the absorption step is missing, potency information obtained by topical sensitisation cannot be used for judging the sensitisation potency of a substance after tattooing.

Mode of action of sensitising substances

Skin sensitisation may manifest as local effects in the skin. However, the nature of sensitisation is systemic.

The skin consists of two major compartments: the epidermis and the dermis. The epidermis is the outer layer and the dermis the inner layer of the skin. Certain cell types are required for induction of contact allergy. These cell types are Langerhans cells and dermal dendritic cells, which are present in epidermis and dermis, respectively.

Allergic contact dermatitis is a T cell mediated reaction. Contact allergens can be of various sizes, but are generally smaller molecules, also called haptens, that cannot induce T cell activation by themselves. Instead, contact allergens have to bind to the body's own proteins and thereby, modify them so that they appear foreign to the immune system and thereby induce T cell activation.

Once the immune system is activated, an allergic reaction in the skin may occur. Due to the systemic nature of sensitisation and development of immunological memory, allergic reactions

may also occur when a person is exposed to the contact allergen on other skin areas, or when exposed at a later point in life. See also the review by DEPA "Allergy and tattoos" (DEPA, 2017a).

Substances classified as skin sensitisers have been shown to induce and elicit contact allergy after contact with epidermis, the outer layer of the skin. However, contact allergy can also both be induced and elicited by injection of skin sensitisers into the dermis, where the tattoo inks are deposited.

Allergic reactions in tattoos have not been systematically investigated and reported in the literature, but seven population-based studies (Laumann & Derick, 2006) (Brady, et al., 2015) (Klügl, et al., 2010) (Kluger, 2016b) (Høgsberg, et al., 2013) (Hutton Carlsten & Serup, 2014) (Dybboe, et al., 2016), with different methodologies, concerning adverse skin reactions to tattoos have been identified and addressed in the review by DEPA (DEPA, 2017a). In some of these studies, questions were asked concerning allergic reactions observed in relation to tattoos, which were reported by 2.9%-8% of those with tattoos. No definitions were given of what was meant by an allergic reaction and no allergy tests were performed, which is a prerequisite of diagnosing allergy. Chronic skin reactions in permanent tattoos, defined as lasting more than 3 to 4 months, were reported by at least 5.9%-6.0% of random samples of tattooed persons and more transient acute reactions in 4.3%-12.5%. Even higher numbers of allergic reactions were obtained if subgroups of tattooed persons were studied such as sunbathers or tattooists. The tattooist generally has a larger area of the skin covered with tattoos, and this resulted in a higher risk for an allergic or allergy-like reaction. The same relation was also found in a study by the DEPA in 2013 (DEPA, 2013). Concerning sunbathers no explanation on the high number of allergy or allergy-like reaction was provided (Høgsberg, et al., 2013). Sun-induced complaints were reported with a frequency of 15%-23% of investigated subgroups. The severity of reactions was in most cases unknown. Contact allergic reactions may be among both acute and more chronic adverse reactions, but cannot be more precisely estimated, as it requires medical investigation to make the diagnosis (DEPA, 2017a).

In addition, two case studies were identified (DEPA, 2017a), which had a study population of more than two patients, where adverse skin reactions to tattoos were identified and where there was a systematic approach to obtain exposure information and perform patch testing. In one study (Serup & Hutton Carlsen, 2014), 79 patients with tattoo reactions were tested with expected problematic inks; 7 (8.8%) had a positive reaction. In addition, 74 of them were tested with selected textile azo dyes; 4 (5.4%) had a positive reaction. The compositions of the inks were unknown and therefore a causal relationship could not be firmly established. In the second study (Gaudron, et al., 2015), 6 patients with severe tattoo reactions were tested and one had a positive reaction to an ingredient of the ink. These studies demonstrate that contact allergic reactions exist in relation to tattoos, but also demonstrates the gap in knowledge concerning ingredients in the tattoo inks, which has caused reactions or are under suspicion. Numerous case studies which demonstrate a connection between tattoo ink and allergy have also been described by Serup and Bäumler in "Diagnosis and Treatment of Tattoo Complications" by Serup and Bäumler, Current Problems in Dermatology 2017 (Serup & Bäumler, 2017b).

The low number of reports, that identify sensitisation in relation to tattoos, may be due to limitations in the patch test methodology. A false negative result can occur if the test is not performed with the right substance, either due to the lack of ingredient information on tattoo inks or due to formation of the allergen in the skin. Another possibility is if the substance does

not penetrate the skin in sufficient amounts while testing, or if the reaction is due to photosensitivity, which is rarely tested.

From the description of the individual cases, the identified skin reactions in tattoos are often not typical for contact allergy and in many cases patch testing is negative. It may be that these reactions are due to other kinds of immune activation than contact allergy. The basic understanding of the non-allergic immune reactions to tattoos is very limited and no diagnostic test is available (DEPA, 2017a).

B.5.6. Repeated dosed toxicity and Specific target organ toxicity – repeated exposure (STOT RE)

The Dossier Submitter considers that it will be difficult to group all the substances with harmonised STOT RE classifications as they give rise to very diverse health risks. Therefore, it was decided to include in the scope only those STOT substances (not covered by other group or individual assessments) present in tattoo inks or PMU. There were only two such substances: 2,6-diamino-3-((pyridine-3-yl)azo)pyridine and uranium. However, both 2,6-diamino-3-((pyridine-3-yl)azo)pyridine and uranium were considered to be out of scope due to other reasons (2,6-diamino-3-((pyridine-3-yl)azo)pyridine is listed in CPR Annex III which is not in the scope of this restriction and uranium is considered to be a radioactive substance within the scope of Council Directive 96/29/Euratom and therefore exempted from REACH).

B.5.7. /8. Carcinogenicity and mutagenicity

The Dossier Submitter proposes that all substances classified as carcinogenic and mutagenic Category 1A, 1B and 2 (harmonised classification in Regulation 1272/2008 (CLP)) should be restricted in tattoo inks and PMU, except for carcinogens or mutagens in Cat 1A, 1B and 2 only with the hazard statements H350i (May cause cancer by inhalation), H351i (Suspected of causing cancer by inhalation), H340i (May cause genetic defects via inhalation) and H341i (Suspected of causing genetic defects by inhalation).

The carcinogenic and mutagenic substances in the scope of this restriction also includes primary aromatic amines (PAA) and polycyclic aromatic hydrocarbons (PAH) that have such classifications.

For all substances with inherent properties that may cause an effect with no threshold, it is not possible to do a quantitative hazard assessment, i.e. to identify a threshold for the given effect. Instead a qualitative assessment should be carried out. This is the case for the majority of substances with C and M classifications. These substances are therefore included in the proposal based on their non-threshold hazards.

It is assumed that multistage carcinogenesis develops in steps of tumour initiation, promotion and progression. Some substances act as initiators (DNA-reactive mutagens), while others promote proliferation of mutated cells without reacting with DNA (i.e. they are non-genotoxic carcinogens), or contribute to progression from benign to malignant cells/tumours. Many mutagens are also carcinogens, and act both as an initiator and a promotor. Where a harmonised classification has been included in Annex VI of CLP, this is seen by the Dossier Submitter as sufficient to apply a qualitative approach. It is proposed to include category 1A and 1B CM substances, as well as CM category 2 substances as the majority of the CM substances are assumed to be non-threshold substances, even if it is suspected that some of them work via a threshold, as may be the case for M aneugens (Elhajouji, et al., 2011) or C promotors (Neumann, 2009). For CM category 2 substances, there is a concern that they are suspected human carcinogens and may induce heritable mutations in the germ cells of

humans, based on limited evidence of carcinogenicity and mutagenicity. Substances may thus be assigned to C category 2 if evidence of carcinogenicity is restricted to a single experiment, or is only seen as benign neoplasms, or only as promoting activity in a narrow range of tissues or organs. Substances are not assigned to a particular hazard category based on whether or not they work via a threshold. However, even if a threshold did exist for some of the C substances in tattoo and PMU ink, the recommended RMM/OCs would still be to avoid contact with them (CSA Guidance R.8) (ECHA, 2012) and this would only be possible in tattoo inks by preventing the substances being in the inks. It is therefore proposed to treat all the classified substances as non-threshold; if any justification is provided in the Public Consultation that a threshold exists for specific CM substances in tattoo ink, these could be re-assessed on a case-by-case basis for their inclusion.

Certain substances that are azo dyes, and not classified as CM cat 1 or 2 but may undergo decomposition to aromatic amines or contain residual substances that are so classified, should also be restricted in tattoo inks and PMU based on the same qualitative argumentation (see below).

In relation to the substances classified as carcinogenic or mutagenic, these could potentially exert their effects both locally (e.g. skin cancer) and through systemic exposure when distributed in the body. A few case reports have been found in the literature investigating the connection between cancer and tattoos:

Malignancy was not observed by Sepehri et al. (Sepehri, et al., 2017b) when mice (immunocompetent C3.Cg/TifBomTac, hairless) were tattooed once with tattoo ink containing benzo(a)pyrene and 2-anisidine, and housed for one year. The authors concluded that the study did not support the hypothesis that tattooing causes cancer. The Dossier Submitter considers that the study adds little information to the risk assessment of substances in tattoo inks, as the number of mice and the follow-up time were limited. According to OECD TG 451 the number of animals should be 50 of each sex in each dose group. In the Sepehri study only 48 animals were used in total (11 mice tattooed black, 10 mice tattooed red, 5 controls, 22 mice tattooed and exposed to UV-radiation. The duration of the study was only 1 year, in contrast to the recommendation in OECD TG 451 of 18 month duration for C3H/J mice, representing the majority of the normal life span. The Dossier Submitter agrees with the author that a large epidemiology study would be required to exclude the risk of malignancy in the tattooed population.

Kluger and Koljonen (Kluger & Koljonen, 2012) extensively reviewed the literature and found 50 cases of skin cancer on tattoos: 23 cases of squamous-cell carcinoma and keratoacanthoma, 16 cases of melanoma, and 11 cases of basal-cell carcinoma. The review concluded that the number of skin cancers arising in tattoos is low, and this association has to be considered thus far as coincidental. The Dossier Submitter considers that due to the low power of the study, the probable underreporting and the lack of statistical analysis that the authors' conclusion may be questioned. There is no good epidemiological study that supports the conclusion of the authors.

Schmitz et al. reported the rare case of a 24-year-old woman who, seven months after getting a tattoo on the back of her foot, developed a squamous cell carcinoma in close proximity to the red dye used (Schmitz, et al., 2016). The dye implicated was azo pigment 5 CAS No. 6410-41-9.

Primary aromatic amines (PAAs) and azo colourants.

Since azo colourants are widely applied in tattoo inks (JRC 2015b), they have been specifically addressed in this dossier. The Dossier Submitter therefore proposes that azo colourants (due to inherent properties) that have a harmonised classification as carcinogenic are restricted. Further, it is know that some azo colourants can decompose into aromatic amines with carcinogenic properties. The Dossier Submitter therefore proposes that the azo colourants that decompose/degrade to these PAAs are included in the scope of this restriction based on their inherent properties (some of the PAAs are also classified as CMs), in the same way as other substances with C and M properties.

Thus, initially azo colourants with inherent negative health effects are proposed to be restricted. Further, azo colourants that decompose into carcinogenic primary aromatic amines (PAAs) are addressed and that specific limit values are established for these substances.

In addition to the azo colourants discussed above, the Dossier Submitter proposes to include 14 additional azo colourants in the restriction that are included in Table 2 of ResAP(2008) (see Table 6 below). The justification for this inclusion is that 7 Member States currently include these substances in their national legislation and not to cover them would potentially reduce the level of protection in those countries. There is limited information on 14 of the substances on Table 2 of CoE ResAP. They are currently not classified as CMR, skin sensitiser or skin/eye irritants or corrosives and none of them are registered under REACH. One of them, Basic Red 1, has been found in tattoo inks. (JRC, 2015b)

Table 6 Fourteen additional azo colourants on CoE ResAP Table 2 included in the scope of the proposed restriction

Substance Name	**Other regulatory name**	**EC#**	**CAS#**	**Entry#**	**Hazard classification with percent notifications**	**Notification#**
sodium 4-{[4-(diethylamino) phenyl][4-(diethyliminio) cyclohexa-2,5-dien-1-ylidene] methyl}naphthalene-2,7-disulfonate	Acid Green 16	603-214-8	12768-78-4	1	Not Classified (99.8%), Eye Irrit. 2 (0.2%), Skin Sens. 1 (0.2%)	1008
Disodium 1-(2,4-dimethylphenylazo)-2-hydroxynaphthalene-3,6-disulphonate	Acid Red 26	223-178-3	3761-53-3	2	Carc. 2 (100.0%), Eye Irrit. 2 (1.4%), Muta. 2 (1.4%), STOT SE 3 (1.4%), Skin Irrit. 2 (1.4%)	73
Hydrogen [4-[[4-(diethylamino)phenyl][4-[ethyl(3-sulphonatobenzyl)amino]phenyl] methylene]cyclohexa-2,5-dien-1-ylidene](ethyl)(3-sulphonatobenzyl)ammonium, sodium salt	Acid Violet 17	223-942-6	4129-84-4	3	Not Classified (65.7%), Aquatic Chronic 2 (30.5%), Eye Irrit. 2 (3.8%)	105
9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride*	Basic Red 1 , Basic red 1	213-584-9	989-38-8	8	Eye Dam. 1 (76.7%), Aquatic Chronic 1 (61.0%), Aquatic Acute 1 (56.9%), Acute Tox. 3 (51.1%), Acute Tox. 4 (35.7%), Aquatic Chronic 2 (14.0%), Muta. 1B (7.0%), Repr. 1B (7.0%), Muta. 2 (5.2%), Repr. 2 (5.2%), Not Classified (2.8%), Skin Irrit. 2	831

					(0.2%), Eye Irrit. 2 (0.1%), STOT SE 3 (0.1%)	
Ethanol, 2-[ethyl[3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]phenyl]amino]-	Disperse Blue 106	602-285-2	12223-01-7	14		
Disperse Blue 124	Disperse Blue 124	612-788-9	61951-51-7	15	Acute Tox. 3 (100.0%), Skin Sens. 1 (100.0%)	23
C.I. dDisperse Blue 35	Disperse Blue 35	602-260-6	12222-75-2	17	Skin Sens. 1 (100.0%)	23
Propanenitrile, 3-[[4-[2-(2,6-dichloro-4-nitrophenyl) diazenyl]phenyl]ethylamino]-	Disperse Orange 37	602-312-8	12223-33-5	19		
2-[ethyl[4-[(4-nitrophenyl)azo] phenyl]amino]ethanol	Disperse Red 1	220-704-3	2872-52-8	20	Skin Sens. 1 (93.3%), Not Class(6.7%), Skin Irrit.2(3.3%)	30
2,2'-[[3-methyl-4-[(4-nitrophenyl)azo]phenyl]imino]bis ethanol	Disperse Red 17	221-665-5	3179-89-3	21	Aquatic Chronic 2 (50.8%), STOT RE 2 (49.2%), Acute Tox. 4 (39.0%), Not Classified (5.1%), Skin Sens. 1 (5.1%)	59
N-(2,4-dinitrophenyl)benzene-1,4-diamine	Disperse Yellow 9	228-919-4	6373-73-5	23	Skin Sens. 1 (100.0%)	2
4-[(4-Aminophenyl)-(4-methyliminocyclohexa-2,5-dien-1-ylidene)methyl]aniline	Pigment Violet 3	603-635-7	1325-82-2	26	Aquatic Acute 1 (88.2%), Aquatic Chronic 1 (88.2%), Eye Dam. 1 (56.5%), Eye Irrit. 2 (31.8%), Not Classified (5.9%)	85
Methanaminium, N-[4-[bis[4-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, molybdatephosphate	Pigment Violet 39	264-654-0	64070-98-0	27		
4-dimethylaminoazobenzene	Solvent Yellow 2	200-455-7	60-11-7	34	Acute Tox. 3 (94.1%), Carc. 2 (85.3%), Skin Sens. 1 (8.8%), Muta. 2 (5.9%), Not Classified (5.9%), Eye Irrit. 2 (2.9%), STOT SE 3 (2.9%), Skin Irrit. 2 (2.9%)	34

Note: * reported in tattoo inks by JRC report (JRC, 2015b)

The same concentration limit for these substances as the other azodyes is proposed.

Chemically, a primary aromatic amine (PAA) consists of a nitrogen group (-NH2) attached to an aromatic backbone (DEPA, 2017c). PAAs are used in the production of azo colourants. Azo colourants are widely used since in general they possess a high degree of chemical and photolytic stability. Approximately 54% (67 in number) of the colourants used in tattoo inks and PMU are azo colourants (JRC, 2015b). Since the PAAs are used in the production of azo colourants, the PAAs might be present in the final colourant as impurities.

Degradation of azo colourants can generate PAAs. Azo colourants can be degraded by irradiation: sunlight or laser (JRC, 2015b). Enzymatic degradation or bacterial degradation has also been shown in the skin (Chacko & Subramaniam K, 2011), (Sudha, et al., 2014).

PAAs

In total 29 PAAs are within the scope of the current restriction proposal as they have a harmonised classification as carcinogenic/skin sens. (See Appendix B.2. on PAAs and azo colourants for detailed explanation). The PAAs can be expected to be present in tattoo inks as they may be formed due to cleavage of azo bond of one of the azo colourants listed in the CoE ResAP(2008)1. In addition, they may be present due to either cleavage of azo bond or amide hydrolysis of other azo colourants used in tattoo and PMU inks or originate from the production of the azo colourants used in tattoo and PMU inks. Some have also been detected in tattoo and PMU inks on the European market.

Table 7 The 29 PAAs in the scope of the restriction.

	CAS no.	**Primary Aromatic Amine**	**Carc.**	**Muta.**	**Skin sens.**
1	90-04-0	o-Anisidine	1B	2	
2	95-53-4	o-toluidine	1B		
3	91-94-1	3,3'-dichlorobenzidine	1B		1
4	95-80-7	4-methyl-m-phenylendiamine	1B	2	1
5	106-47-8	4-chloroaniline	1B		
6	99-55-8	5-nitro-o-toluidine	2		
7	119-90-4	3,3'-dimethoxybenzidine	1B		
9	119-93-7	4,4’-bi-o-toluidine	1B		
8	139-65-1	4,4'-Thiodianiline	1B		
10	95-69-2	4-chloro-o-toluidine	1B	2	
11	91-59-8	2-naphthylamine	1A		
12	62-53-3	Aniline	2	2	1
13	92-87-5	Benzidine	1A		
14	106-49-0	p-toluidine	2		1
15	95-70-5	2-methyl-p-phenylenediamine			1
16	92-67-1	Biphenyl-4-ylamine	1A		
17	97-56-3	4-o-tolylazo-o-toluidine	1B		1
18	615-05-4	4-methoxy-m-phenylenediamne	1B	2	
19	101-77-9	4,4'-methylenedianiline	1B	2	1
20	838-88-0	4,4'-methylenedi-o-toluidine	1B		1
21	120-71-8	6-methoxy-m-toluidine	1B		
22	101-14-4	4,4'-methylenebis[2-chloroaniline]	1B		
23	101-80-4	4,4'-oxydianiline	1B	1B	
24	137-17-7	2,4,5-trimethylaniline	1B		
25	60-09-3	4-Aminoazobenzene	1B		
26	106-50-3	p-Phenylenediamine			1
27	121-57-3	Sulphanilic acid			1
28	399-95-1	4-amino-3-fluorophenol	1B		1
29	87-62-7	2,6-xylidine	2		

Azo colourants

Some azo colourants have been classified as CM, cat. 1A, 1B or 2 based on their inherent properties, and should therefore be restricted. In addition, since some azo colourants may decompose to PAAs it is relevant to identify those specific azo colourants that may decompose into carcinogenic PAAs as these would be relevant to include in the scope of the restriction proposal. Two main decomposition routes are assumed here, either biologically (amide hydrolysis) or by photo-decomposition. Further, scientific evaluations and harmonised classification have been taken into account (see Appendix B.2 for more detail).

Based on the assessment detailed in appendix B.2 the following azo colourants listed in Table 8 should be restricted in tattoo inks and PMU.

Table 8. Azo colourants suggested to be included in the scope of the restriction proposal.

	CAS No	**CI no.**	**CI name**
1	6471-51-8	12420	Pigment Red 7 (PR7)
2	6410-38-4	12460	Pigment Red 9 (PR9)
3	6410-39-5	12465	Pigment Red 15 (PR15)
4	61932-63-6	12477	Pigment Red 210 (PR210)
5	85776-14-3	No CI no.	Pigment Orange 74 (PO74)
6	6528-34-3	11740	Pigment Yellow 65 (PY65)
7	6358-31-2	11741	Pigment Yellow 74 (PY74)
8	6410-32-8	12385	Pigment Red 12 (PR12)
9	6471-50-7	12380	Pigment Red 14 (PR14)
10	6655-84-1	12390	Pigment Red 17 (PR17)
11	6535-46-2	12370	Pigment Red 112 (PR112)
12	5468-75-7	21095	Pigment Yellow 14 (PY14)
13	6358-37-8	21096	Pigment Yellow 55 (PY55)
14	6041-94-7	12310	Pigment Red 2 (PR2)
15	6448-95-9	12315	Pigment Red 22 (PR22)
16	5280-68-2	12485	Pigment Red 146 (PR146)
17	67990-05-0	12466	Pigment Red 269 (PR269)
18	6505-28-8	21160	Pigment Orange16 (PO16)
19	2512-29-0	11680	Pigment Yellow 1 (PY1)
20	6358-85-6	21090	Pigment Yellow 12 (PY12)
21	15110-84-6	21107:1	Pigment Yellow 87 (PY87)
22	12225-18-2	11767	Pigment Yellow 97 (PY97)
23	3520-72-7	21110	Pigment Orange 13 (PO13)
24	15793-73-4	21115	Pigment Orange 34 (PO34)
25	5567-15-7	21108	Pigment Yellow 83 (PY83)
26	1229-55-6	12150	Solvent Red 1 (SR1)
27	1320-07-6	20170	Acid Orange 24 (AO24)
28	85-86-9	26100	Solvent Red 23 (SR23)

29	5413-75-2	27290	Acid Red 73 (AR73)
30	2832-40-8	11855	Disperse Yellow 3

Note: For additional 14 azo colourants on CoE Table 2, see Table 6.

B.5.9. Toxicity for reproduction

Reprotoxic substances classified in Category 1A/B

Substances classified for reproductive toxicity in hazard category Repr. 1A/B due to their effects on sexual function and fertility and development may exert their adverse effects when tattoo inks containing them are injected into dermis or other parts of the body (e.g. submucosal, intraocular, or under the tongue) of consumers. The Dossier Submitter proposes to restrict reprotoxic substances with concentration limits in tattoo inks and PMUs based on a quantitative hazard assessment approach that considers the group of all currently known Repro 1A/B-classified substances.

In contrast to carcinogenic and mutagenic substances, reprotoxic substances have been assumed to have an individual threshold level below which no adversity is expected. Thus, a quantitative approach to the justification for restriction is taken[7]. Within the scope of this restriction, the restriction proposal intends to cover all reprotoxic substances classified as Repr. 1 A/B. The approach covers only those reprotoxic substances (Category 1 A/B) here which are not also classified as carcinogen or mutagen or sensitiser (here named as reprotoxic "only" substances), as the justification for risk from those substances due to their intradermal injection in to the skin for the purpose of tattoos and PMU was already discussed in sections Sensitisation and /8. Carcinogenicity and mutagenicity

Thirty-four substances were identified in Annex VI of CLP as being classified as Repro. 1A/B without being also classified as carcinogen or mutagen or sensitiser. These substances and their classifications related to reprotoxicity are shown in Table 8. Four of them have been found in tattoo inks: bis(2-ethylhexyl) phthalate, dibutyl phthalate, mercury and disodium tetraborate, anhydrous (JRC, 2015b). For other reprotoxic compounds no information is available on their content in tattoo inks as ingredient or impurity. As mercury will be restricted based on it being listed both on Annex II (CPR) and in CoE Table 3 this substance was not addressed here.

[7] There are discussions whether endocrine disrupting substances act via a threshold mechanism or not. This has not been considered in this restriction proposal as it has not been decided how to risk assess this under REACH.

Table 9. Summary of reprotoxic substances which were selected for the development of the quantitative approach

Substance	**CAS Number / EC Number**	**Classification and labelling according to Regulation 1272/2008**	
		Hazard class and category codes	**Hazard statement codes**
bis(2-ethylhexyl) phthalate	117-81-7 / 204-211-0	Repr. 1B	H360FD
dibutyl phthalate	84-74-2 / 201-557-4	Repr. 1B	H360Df
diisobutyl phthalate	84-69-5 / 201-553-2	Repr. 1B	H360Df
disodium tetraborate, anhydrous	1330-43-4 / 215-540-4	Repr. 1B	H360FD
dihexyl phthalate	84-75-3 / 201-559-5	Repr. 1B	H360FD
n-pentyl-isopentylphthalate	--- / ---	Repr. 1B	H360FD
1,2-benzenedicarboxylic acid, di-C6-C8-branched alkyl esters, C7-rich	71888-89-6 / 276-158-1	Repr. 1B	H360D
1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear	68515-50-4 / 271-093-5	curr no	A current RAC opinion exists supporting classification as Repr. 1B (H360 DF).
tetraboron disodium heptaoxide, hydrate	12267-73-1 / 235-541-3	Repr. 1B	H360FD
boric acid, (orthoboric acid, sodium salt)	10043-35-3, 11113-50-1/ 233-139-2, 234-343-4 13840-56-7 / 237-560-2	Repr. 1B	H360FD
diboron trioxide	1303-86-2 / 215-125-8	Repr. 1B	H360FD
sodium perborate	13517-20-9, 15120-21-5 / 239-172-9	Repr. 1B	H360Df
sodium peroxometaborate	7632-04-4, 10332-33-9, 10486-00-7 / 231-556-4	Repr. 1B	H360Df
perboric acid, sodium salt	11138-47-9, 12040-72-1, 37244-98-7 / 234-390-0	Repr. 1B	H360Df
(2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol	94361-06-5 / 619-020-1	Repr. 2	H361d A current RAC opinion exists supporting the classification as Repr. 1B (H360D).

(4-ethoxyphenyl)(3-(4-fluoro-3-phenoxphenyl)propyl)dimethylsilane	105024-66-6 / 405-020-7	Repr. 1B	H360F
(R)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone, (S)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone	5543-58-8 / 226-908-9 5543-57-7 / 226-907-3	Repr. 1A	H360D
N,N-(dimethylamino)thioacetamide hydrochloride	27366-72-9 / 435-470-1	Repr. 1B	H360D
1,2-diethoxyethane	629-14-1 / 211-076-1	Repr. 1B (1)	H360Df
1-ethylpyrrolidin-2-one	2687-91-4 / 220-250-6	Repr. 1B	H360D
1-methyl-2-pyrrolidone	872-50-4 / 212-828-1	Repr. 1B	H360D
2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate	15571-58-1 / 239-622-4	Repr. 1B	H360D
4-tert-butylbenzoic acid	98-73-7 / 202-696-3	Repr. 1B	H360F
7-methoxy-6-(3-morpholin-4-yl-propoxy)-3H-quinazolin-4-one	199327-61-2 / 429-400-7	Repr. 1B	H360D
ammonium 2-amino-4-(hydroxymethylphosphinyl)butyrate	77182-82-2 / 278-636-5	Repr. 1B	H360Fd
chloro-N,N-dimethylformiminium chloride	3724-43-4 / 425-970-6	Repr. 1B	H360D
cyclic 3-(1,2-ethanediylacetale)-estra-5(10),9(11)-diene-3,17-dione	5571-36-8 / 427-230-8	Repr. 1B	H360F
Imidazole	288-32-4 / 206-019-2	curr no	A current RAC opinion exists supporting classification as Repr. 1B (H360D).
Ketoconazole	65277-42-1 / 265-667-4	Repr. 1B	H360 F
salts and esters of dinoseb, with the exception of those specified elsewhere in this Annex	--- / ---	Repr. 1B	H360Df
salts and esters of dinoterb	--- / ---	Repr. 1B	H360D
tetrahydrofurfuryl alcohol	97-99-4 / 202-625-6	Repr. 1B	H360Df
tributyltin compounds	--- / ---	Repr. 1B	H360FD
trixylyl phosphate	25155-23-1 / 246-677-8	Repr. 1B	H360F

(1) There is a mistake in the entry listed in Regulation (EC) No 1272/2008, Annex VI, part 3, Table 3.1, indicating the classification as 'Repr. 1**A**, H360Df". The correct classification is Repr. 1**B**, H360Df, which corresponds to Repr. Cat. 2; R61 and Repr. Cat. 3; R62 that are correctly stated in Annex VI, Table 3.2 (list of harmonized classification and labelling of hazardous substances from Annex I of Council Directive 67/548/EEC) of Regulation (EC) No 1272/2008. The classification was agreed by the Technical Committee C&L on the Classification and Labelling of Dangerous Substances at its meeting in September 2004.

A hazard assessment was performed for the 34 reprotoxic "only" substances related to their adverse effects to reprotoxicity. Adverse effects to reproduction included effects on sexual function and fertility of adults and development of offspring. Where available, key studies and the dose descriptors were taken from RAC opinions in CLH reports, restrictions or authorisations, unless new data indicate a different value. In cases no RAC opinions or CLH reports were available and in order to get up-to-date data, a literature search was performed for each substance using REACH registration data (IUCLID) and search engines such as PubMed, Scopus, TOXLINE, EMBASE and ChemIDplus Advanced. Additionally, data resources hosted by ECHA, NTP, EFSA and EPA have been mined.

The identified key studies and uncertainties are summarised and discussed in Appendix B.3. In Table 9 the NOAEL/LOAEL values which were identified as point of departure for the endpoint reproductive toxicity for each of the assessed substances are shown and key studies are shortly summarised.

A discussion for route-to-route extrapolation to account for the intradermal substance injection during the tattooing process has been included in the DNEL section (section B 5.14).

Reprotoxic substances classified in Category 2

In addition, to restricting Category Repr. 1A/B reprotoxicants in tattoo inks and PMU, the Dossier Submitter also proposes to restrict substances in category Repr. 2. It was not possible to quantitatively assess the individual Category 2 reprotoxicants due to the difficulty to estimate the dose descriptors for substances of concern for this endpoint (on which the accordant data were not sufficient to classify as 1A/B). Nevertheless, the Dossier Submitter proposes to use the same general approach as for category 1A and 1B reprotoxicants (see in section B.10).

Table 10. Summary of all derived NOAEL/LOAEL values selected as PODs

Substance	**CAS No.**	**Type of effects**	**NOAEL (LOAEL) [mg/kg bw/d]**	**Information on key study**
bis(2-ethylhexyl) phthalate	117-81-7	D	4.8	The NOAEL for developmental effects results from a three-generation toxicity study (similar to OECD TG 416 and according to GLP) in rats (Wolfe and Layton, 2004) and is based on small testes, small and/or aplastic epididymis and seminiferous tubular atrophy observed in offspring.
dibutyl phthalate (DBP)	84-74-2	D	2	The LOAEL for developmental effects results from a prenatal and postnatal developmental study in rats (Lee et al. (2004); supported as key study by RAC) and is based on reduced testicular spermatocyte development and mammary gland changes in offspring.
diisobutyl phthalate	84-69-5	D	2.5	Read-across from DBP
dihexyl phthalate	84-75-3	D	20	The LOAEL for developmental effects results from an oral postnatal developmental toxicity study in rat (Aydogan Ahbab and Barlas, 2013) and is based on significantly increased malformations of the reproductive tract e.g. tubular atrophy and atrophic and damaged tubules in testes.
n-pentyl-isopentylphthalate	--- / ---			No key study could be found
1,2-benzenedicarboxylic acid, di-C6-C8-branched alkyl esters, C7-rich	71888-89-6	D	100	The LOAEL for developmental effects results from a two generation reproductive toxicity study in rats (Exxon, 2003) and is based on reduction in sperm production rate and mean testicular sperm concentration in offspring.
1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear	68515-50-4	D	2	The LOAEL was obtained using read-across approach from dibutyl phthalate (DBP). The LOAEL for developmental effects results from a prenatal and postnatal developmental study in rats with DBP (Lee et al. (2004); supported as key study by RAC) and is based on reduced testicular spermatocyte development and mammary gland changes in offspring.
disodium tetraborate, anhydrous	1330-43-4	F	81.4	The NOAEL for fertility effects results from a read across from boric acid and a three generation study in rats (Weir (1966); supported as key study by RAC) and is based on testicular atrophy, reduced fertility.
tetraboron disodium heptaoxide, hydrate	12267-73-1	F	117.9 – 154.3	The NOAEL for fertility effects results from a read across from boric acid and a three generation study in rats (Weir (1966); supported as key study by RAC) and is based on testicular atrophy, reduced fertility.
boric acid	10043-35-3, 11113-50-1	F	100	The NOAEL for fertility effects results from a three generation study in rats (Weir (1966); supported as key study by RAC) and is based on testicular atrophy and reduced fertility.

diboron trioxide	1303-86-2	F	56.3	The NOAEL for fertility effects results from a read across from boric acid and a three generation study in rats (Weir (1966); supported as key study by RAC) and is based on testicular atrophy, reduced fertility.
sodium perborate	13517-20-9, 15120-21-5	D	100	The NOAEL for developmental effects results from read across approach from sodium perborate tetrahydrate and a prenatal developmental toxicity study in rats (OECD TG 414, GLP;Bussi (1995); supported as key study by European Chemicals Bureau (2007)) and is based on an increase in resorptions and reduction in foetal body weights.
sodium peroxometaborate	7632-04-4, 10332-33-9, 10486-00-7	D	100	The NOAEL for developmental effects results from read across approach from sodium perborate tetrahydrate and a prenatal developmental toxicity study in rats (OECD TG 414, GLP; Bussi (1995); supported as key study by European Chemicals Bureau (2007)) and is based on an increase in resorptions and reduction in foetal body weights.
orthoboric acid, sodium salt	13840-56-7	F	100	The NOAEL for fertility effects results from a read across from boric acid and a three generation study in rats (Weir (1966); supported as key study by RAC) and is based on testicular atrophy, reduced fertility.
perboric acid, sodium salt	11138-47-9, 12040-72-1, 37244-98-7	D	100	The NOAEL for developmental effects results from read across approach from sodium perborate tetrahydrate and a prenatal developmental toxicity study in rats (OECD TG 414, GLP; Bussi (1995); supported as key study by European Chemicals Bureau (2007)) and is based on an increase in resorptions and reduction in foetal body weights.
(2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol	94361-06-5	D	1.39	OECD TG 416, GLP, effects: dose-related increase in pre/perinatal mortality in the high-dose groups in the F0 and F1 generation (16.3% and 12.6%, respectively) (Eschbach et al., 1987)
(4-ethoxyphenyl)(3-(4-fluoro-3-phenoxphenyl)propyl)dimethylsilane	105024-66-6		n.a.	No key study could be found
(R)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone	5543-58-8	D	0.04	Clinical observation, nasal hypoplasia and vertebral stippling in offspring after warfarin application during pregnancy (Shaul et al., 1975)

(S)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone	5543-57-7	D	0.04	Clinical observation, nasal hypoplasia and vertebral stippling in offspring after warfarin application during pregnancy (Shaul et al., 1975)
N,N-(dimethylamino)thioacetamide hydrochloride	27366-72-9		n.a.	No key study could be found
1,2-diethoxyethane	629-14-1	D	50	The NOAEL for adverse effects on development was derived from a prenatal developmental study in mice which was performed in compliance with OECD TG 414 (George et al., 1988; J., 1992). Dose-related adverse effects on number of litters with malformed foetuses, foetal body weight and malformation incidence (Exencephaly, fused ribs) were observed.
1-ethylpyrrolidin-2-one	2687-91-4	D	50	The NOAEL for adverse effects on development was derived from a prenatal developmental study in rats which was performed in compliance with OECD TG 414 (Saillenfait et al., 2007). Dose-related adverse effects on number of litters with malformed foetuses, foetal body weight and incidences of rare cardiovascular malformation were observed.
1-methyl-2-pyrrolidone	872-50-4	D	150	one-generation reproduction toxicity study with Wistar rats (modified after OECD TG 415): reduced survival of pups, reduced body weight (Sitarek et al., 2012)
2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate	15571-58-1	D	15	The LOAEL for developmental effects results from a prenatal developmental study in mice (Anonymous, 2014b) and is based on a statistically significant positive trend on percentage of post implantation loss.
4-tert-butylbenzoic acid	98-73-7	F	1.6	The NOAEL for reproductive toxicity results from a 70 days male fertility study in rats (Hoechst AG, 1987) and is based on a dose-dependent decrease of male fertility/ability to impregnate females.
7-methoxy-6-(3-morpholin-4-yl-propoxy)-3H-quinazolin-4-one	199327-61-2 / 429-400-7		n.a.	No key study could be found
ammonium 2-amino-4-(hydroxymethylphosphin	77182-82-2 / 278-636-5	F	9.6	Embryotoxicity study, oral (by gavage), rabbit with GA (Baeder et al. (1983), as cited in EFSA (2005a))based on premature deliveries, abortions and dead foetuses

yl)butyrate				
chloro-N,N-dimethylformiminium chloride	3724-43-4 / 425-970-6		n.a.	No key study could be found
cyclic 3-(1,2-ethanediylacetale)-estra-5(10),9(11)-diene-3,17-dione	5571-36-8 / 427-230-8		n.a.	No key study could be found
imidazole	288-32-4	D	60	OECD TG 414 (prenatal developmental toxicity study), reduced mean foetal weight and increased number of resorptions and increased rate of variations and malformations at 180 mg/kg bw/d (BASF, 2002)
ketoconazole	65277-42-1	F	200	The LOAEL for fertility results from a subacute male fertility study (Waller et al., 1990) in rats (no guideline followed) and is based on loss of male fertility.
salts and esters of dinoseb, with the exception of those specified elsewhere in this Annex	--- / ---	D	1	This LOAEL for developmental toxicity results from a 3-generation rat reproductive study comparable to OECD guideline 416 (Dow Chemical Company, 1981), and is based on reduced pub weight in F0 to F1b littering groups.
salts and esters of dinoterb	--- / ---		n.a.	No key study could be found
tetrahydrofurfuryl alcohol	97-99-4	D	50	No key study could be found
tributyltin compounds, with the exception of those specified elsewhere in this Annex (here related to tributyltin chloride)	--- / ---	F	0.00017 - 0.001	repeated dose toxicity study with tributyltin chloride with focus on male fertility with KM mice, effects: dose dependent decrease of sperm count and viability (Chen et al., 2008; Si et al., 2012; Si et al., 2015; Yan et al., 2009)
trixylyl phosphate	25155-23-1 / 246-677-8	F	25	combined oral repeated dose and reproductive/developmental toxicity study according to OECD 422, effects: histological changes in reproductive organs (Experimur, 2004)

Lead compounds

Six lead compounds are classified only as reprotoxic category 1A/B; these are not covered in the quantitative approach above.

The Dossier Submitter proposes that all lead compounds should be restricted in tattoo inks and PMU based on their non-threshold effects (EFSA CONTAM Panel, 2013), acknowledged by RAC in the lead in jewellery and consumer article restrictions (ECHA, 2011a) and (ECHA, 2013a). EFSA (2013) concluded that there is no evidence for a threshold for a number of critical endpoints including developmental neurotoxicity (including from in utero exposure), increases in systolic blood pressure and renal effects (e.g., changes in proteinuria, glomerular filtration rate (GFR) or creatinine levels and clearance) in adults).

EFSA concluded that protection of children and women of child-bearing age against the potential risk of neurodevelopmental effects should be protective for all other adverse effects of lead, in all populations. EFSA also recommended work should continue to reduce exposure to lead, from both dietary and non-dietary sources. Therefore as it cannot be excluded that women of childbearing age would have tattoos and taking into account the non-threshold effects of lead, the Dossier Submitter proposes these lead compounds to be restricted in tattoo inks and PMUs.

A specific concentration limit has been calculated, see section Consumers.

B.5.10. Human data on health effects from tattoo inks and PMU

Health effects from the chemical substances in tattoo inks and PMU have been observed in clinics and been described by medical doctors. Reviews of these health effects mostly describe skin reactions but a clear classification of these reactions is difficult as they are often non-specific and there is much variability.

Earlier reviews of these effects group them according to histological patterns in granulomatous, lichenoid, or hypersensitivity allergic reactions (Wenzel, et al., 2013), also referred to as inflammatory/immune reactions (Brady, et al., 2015). This is also presented in detail in the JRC reports (JRC, 2016a) and (JRC, 2016b).

More recent reviews of adverse effects (CHDP, 2015), (Serup, et al., 2015b), (Serup, et al., 2016) have grouped them on the basis of clinical descriptive assessment and have submitted the classification to the World Health Organisation (WHO) as a proposal to the 11th revision of the International Classification of Diseases.

An overview of both ways of grouping these health effects and a description of the effects is given in Annex D.6.1 (human health impacts).

B.5.11. CPR Annex II, list of substances prohibited in cosmetic products

B.5.11.1. Background

Annex II of Directive 76/768/EEC (the Cosmetic Products Directive, CPD), later included as Annex II of the CPR (Cosmetic Products Regulation (EC) NO 1223/2009), is part of the Council of Europe Resolution ResAP(2008)1 and its predecessor ResAP(2003)2. Annex II of the CPR contains a list of substances prohibited in *cosmetic products* (see article 14 of CPR).

The ResAP recommends that tattoo and PMU must only be used if they do not contain substances listed in Annex II (in addition to other recommendations).

The ResAP (2008)1 and (2003)2 are the benchmark for those Member States having national legislation and for those taking restrictive measures against hazardous tattoo inks on the

market based on general safety requirements.

Annex II of the CPR includes substances with various hazardous properties, including amongst others CMR and skin sensitising substances, but also various other substances which may or may not have a harmonised classification. Although CMR and skin sensitising substances are covered in separate group justifications of the restriction proposal, the following justification provides a basis for inclusion of the entire list of substances in Annex II within the scope of the proposed restriction. Given the similarities in exposure potential (prohibited in cosmetic products which by definition (article 2 of CPR) are applied, among other, on the external parts of the human body, which include the epidermis), there is merit in considering all of these substances for a comparable restriction for use in tattoo inks and PMU.

B.5.11.2. Rationale

Annex I of REACH, para 0.5 states that "Where available and appropriate, an assessment carried out under Community legislation (e.g., risk assessments completed under Regulation (EEC) No 793/93) shall be taken into account in the development of, and reflected in, the chemical safety report. Deviations from such assessments shall be justified." Therefore the Dossier Submitter recommends that substances included in Annex II of the CPR based on an assessment of the SCCS and supported by the Member States when agreeing to an amendment of the CPR, should be restricted in tattoo inks and PMU taking into account section B.5.11.3. However, note that not all inclusions in annex II is based on SCCS opinions. E.g. if industry does not want to defend a substance or the substance is a drug or classified CMR, it can be included as well. The Dossier Submitter recommends that substances on Annex II of the CPR without an SCCS opinion should also be restricted in tattoo inks and PMU.

B.5.11.3. Justification for risk

The substances included in CPR Annex II are prohibited for use in cosmetics, regardless of the concentration expected to be applied/received. The information presented in Appendix B.4 indicates that the intradermal injection of a substance into the body through tattooing is expected to be at least as high, and in most cases higher, than an equivalent amount of the same substance administered to the skin in a cosmetic product. The CoE resolutions reflect this by requiring provisions for tattoo inks and PMU that are at least as strict as those for cosmetic products under the CPR. This is therefore also reflected by Member States that base their national legislation on CoE resolutions.

Therefore, taking into account the decisions of the Member States and recommendations of the expert committees for inclusion of substances in CPR Annex II, it may be concluded that:

- As the natural protection barrier of the epidermis is broken, the risks of a dose applied beneath the skin (in tattoo inks) is likely to pose at least as high (if not higher) risk to human health than an equivalent dose applied on the skin.
- The CPR Annex II prohibits the use of a number of substances for use in cosmetic products. It does not establish a safe dose in cosmetic products for the application of these Annex II substances on the skin.
- There is therefore a basis for recommending that these substances should be restricted in tattoo inks and PMU relying on the decisions made for inclusion of the substances under CPR Annex II without a detailed risk assessment of each substance.

B.5.12. CPR Annex IV, colourants in cosmetic products

B.5.12.1. Background

Annex IV of Directive 76/768/EEC (the Cosmetic Products Directive, CPD), which later became Annex IV of the CPR (Cosmetic Products Regulation (EC) NO 1223/2009), is part of the Council of Europe Resolution ResAP(2008) and its predecessor ResAP(2003)2.[8] The ResAP recommends that tattoo and permanent make up (PMU) products only be used if they do not contain substances listed in column 2 to 4 of Annex IV of the CPD), now reflected in Annex IV of the CPR, column 'g'. The ResAP (2008)1 and (2003)2 are the benchmark for those Member States having national legislation and for those taking restrictive measures against hazardous tattoo inks on the market based on general safety requirements.

Article 14 of the CPR establishes that cosmetic products shall not contain any colourants other than those listed in Annex IV (List of colourants allowed in cosmetic products). For a number of these substances, Annex IV also establishes specific conditions outside of which their use in cosmetics is prohibited. Such conditions are specified, in terms of product type (rinse-off or leave-on) and of body parts for which the use of substances is allowed or prohibited (e.g., lips, eyes, etc.), the maximum concentration allowed in ready for use preparation, as well as other conditions (e.g., purity requirements).

The conditions are specified in columns "g" to "i" in Annex IV of the CPR:

- Column "g" in the CPR: "Product type/Body part" contains information formerly summarised in columns 1 to 4 of the CPD: "Field of application" as follows:
    - Column 1 of the CPD – Colouring agents allowed in all cosmetic products.
    - Column 2 of the CPD – Colouring agents allowed in all cosmetic products except those intended to be applied in the vicinity of the eyes, in particular eye make-up and eye make-up remover. CPR labels these colourants in column g as colourants "not to be used in eye products"
    - Column 3 – Colouring agents allowed exclusively in cosmetic products intended not to come into contact with the mucous membranes. CPR labels these colourants in column g as colourants "not to be used in products applied on mucous membranes".
    - Column 4 – Colouring agents allowed exclusively in cosmetic products intended to come into contact only briefly with the skin. CPR labels these colourants in column g as colourants allowed in "rinse-off products".
- Columns "h" and "i" in CPR,[9] respectively "Maximum concentration in ready for use preparation" and "other" correspond to the former column "Other limitations and requirements".

According to these specific conditions for use, the following groups of colourants are proposed to be included in the scope of the restriction, as follows:

- The use of the following colourants in tattoo inks to be restricted (i.e., not to be

[8] Council of Europe Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up (superseding Resolution ResAP(2003)2 on tattoos and permanent make-up), 20 February 2008.

[9] One additional column has been added in the CPR: "j": "Wording of conditions of use and warnings". To date, no conditions have been specified in this column for any of the colourants on Annex IV.

allowed):

  - Colourants allowed in rinse-off products only;
  - Colourants not to be used in products applied on mucous membranes;
  - Colourants not to be used in eye products;
- The use of the following colourants to be allowed in tattoo inks under the conditions specified for use in cosmetic products:
  - Colourants allowed in all cosmetic products in concentrations not exceeding the limits specified in Annex IV or subject to other conditions specified in columns "h" to "i" of the Annex (e.g., purity requirements).

Substances allowed only in rinse-off products are considered to pose risks to human health when their use leads to a prolonged exposure. Substances that must not be used in products applied on mucous membranes or in the vicinity of the eyes are considered to pose risks to human health when used via bypassing of the epidermal barrier (or rather providing conditions for an easier penetration of the epidermal layer, in comparison to skin). As use of inks in tattoo applications leads both to prolonged exposure and to circumvention of the skin barrier, the use of these substances in tattoo applications is considered to pose at least equal risks as the above uses (for an equivalent dose). Given this, there is merit in adopting comparable measures in Annex XVII to the conditions in Annex IV of the CPR on colourants in use in tattoo inks and PMU.

In addition, some colourants used in cosmetic products have been shown to pose a risk to human health when applied to the skin in concentrations exceeding the limits specified in Annex IV or other conditions specified in columns "g" to "i" of the Annex (e.g., purity requirements). Therefore, given the similarities in exposure potential (i.e., prohibited or allowed to be used under specific conditions in cosmetic products which by definition (Article 2 of CPR) are applied, among other, on the external parts of the human body, which include the epidermis), there is merit in adopting comparable measures for the use of these colourants in tattoo inks and PMU. This is also the basis of a similar argumentation for including substances on Annex II of the CPR in the scope of the proposed restriction.

The following justification provides a more detailed explanation for inclusion of the list of substances on Annex IV column 'g' to 'i' and which are included in the categories described above within the scope of the proposed restriction.

B.5.12.2. Rationale

Annex I of REACH, paragraph 0.5 states that "Where available and appropriate, an assessment carried out under Community legislation (e.g., risk assessments completed under Regulation (EEC) No 793/93) shall be taken into account in the development of, and reflected in, the chemical safety report. Deviations from such assessments shall be justified." Therefore, substances included in Annex IV of the CPR with specific conditions on field of application, concentration limit, purity requirements, etc. based on an assessment of the SCCS and supported by the Member States when agreeing to an amendment of the CPR. Therefore, comparable measures are proposed to the use of these substances in tattoo inks in Annex XVII of REACH. However, it should be noted that not all inclusions in Annex IV of CPR are based on SCCS opinions.

B.5.12.3. Justification for risk

The evidence presented in Appendix B.5 indicates that the substances in Annex IV proposed to

be included in the scope of this restriction may present equal or greater risk when used in tattoo applications. CoE ResAP(2008)1 seems to take this into account by requiring provisions for tattoo inks and PMU that are at least as strict as those for cosmetic products under the CPR/CPD. This is therefore also reflected by member states that base their national legislation on CoE ResAP(2008)1 or its predecessor.

Taking into account the decisions of the relevant authorities and recommendations of the expert committees for inclusion of substances in CPR Annex IV, it may be concluded that:

- For one group of substances, relevant authorities has concluded that the use of these should only be allowed in rinse-off cosmetic products. These should only be in contact with the skin for short periods of time, and substances present are therefore less bioavailable than in leave-on cosmetics products. It is therefore considered appropriate that such substances should not be allowed in tattoo inks which remain in prolonged (almost indefinite) contact with the dermis.
- For another group of substances, the relevant authorities have concluded that there is a higher risk when these are applied in the vicinity of the eyes or on mucous membranes as compared to applications on the skin. It can therefore be argued that these substances should also not be used in tattoo inks which equally bypass the protective skin layer.
- For another group of substances, the relevant authorities have concluded that there is higher risk when these are applied on the skin in concentrations exceeding those specified in column "h" or not meeting content or purity requirements specified in column "i". It can therefore be considered appropriate that such substances should not be allowed also in tattoo inks or PMU if they do not meet these conditions specified in columns "h" and "i" of Annex IV of the CPR.
- There is therefore a basis for recommending a restriction (i.e., a prohibition of use or use under specific conditions) of the above substances in tattoo inks (see Appendix B5 for further details) relying on the conditions for the substances under CPR Annex IV.

B.5.13. Council of Europe Resolution ResAP(2008)1, Table 3, impurities in tattoo inks and PMU

In the CoE Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up, a list of maximum allowed concentrations of impurities in products for tattoos and PMU can be found (Table 3 in the ResAP(2008)1). This list comprises the following substances:

Table 11 Substances from Table 3 in CoE ResAP(2008)1

Substance on the list
Arsenic (As)
Barium (Ba)
Cadmium (Cd)
Cobalt (Co)
Chromium (Cr) (VI)
Copper (Cu) soluble
Mercury (Hg)
Nickel (Ni)
Lead (Pb)
Selenium (Se)
Antimony (Sb)
Tin (Sn)
Zinc (Zn)
Policyclic aromatic hydrocarbons (PAH)
Benzene-a-pyrene (BaP)

The Dossier Submitter has assessed certain of these substances (see Table 12 to determine the need for risk-based concentration limits for these substances in tattoo ink and PMU.

Table 12. Substances assessed and Points of Departure (POD) chosen to derive DN(M)ELs.

Substance	**Point of departure, POD**	**Information on key study**	**Detailed assessment**
Arsenic (As)	Excess lifetime risk of lung tumours = 1.7 x 10^{-3} per µg As/kg bw/day (as a systemic exposure)	Based on the WHO/FAO risk estimates from the Taiwanese drinking water cohort, using data from the most recent publications of Chen et al (2010a, 2010b), and 10^{-6} as an indicative tolerable risk level.	Appendix B.6. Risk assessment of arsenic (As)
Barium (Ba)*	NOAEL 60 mg/kg bw/d	Nephrotoxicity in male rats at 60 mg/kg bw/d in NTP 13 week study, also supported by findings in female rats and in male/female mice (NTP 13 week study), as well as interim findings in female rats in the NTP 2 year study	Appendix B.7. Risk assessment of barium (Ba)
Copper (Cu)*	2 mg/L drinking water, equalling 2.2 mg Cu/day	Two mg/l equals a mean total copper intake of 2.2 mg/day (95th percentile would be 5.6 mg), if assuming a bw of 60 kg and a water intake of 1.1 l/d (or with the 95th percentile 2.8 l/d) to avoid GI irritation (WHO guidelines for drinking-water quality, 2004)	Appendix B.8. Risk assessment of copper (Cu)
Lead (Pb)	$BMDL_{01}$ 0.50 ug Pb/kg day	Effects on the developing nervous system including in utero (EFSA 2010/2013), applied by RAC (ECHA 2011; 2013).	Appendix B.10. Risk assessment of lead (Pb)
Zinc (Zn)*	NOAEL 0.83 mg/kg bw/d	An EFSA report from 2006 (EFSA 2006) and supported by the SCCS opinion from 2017 (SCCS/1586/17) adopted a NOAEL of 50 mg/day or 0.83 mg Zn^{2+}/kg bw/day which is based on the absence of any adverse effects on a wide range of relevant indicators of copper-status as critical endpoint.	Appendix B.11. Risk assessment of zinc (Zn)

* Soluble

Certain other substances on the list were included in the scope of the restriction due to the CM/SS qualitative approach (cadmium, cobalt, chromium (VI), mercury, nickel, polycyclic aromatic hydrocarbons (PAHs) and benzene-a-pyrene (BaP)). For practicality purposes the Dossier Submitter proposes to restrict these substances as they are regulated in some Member States national restrictions based on CoE ResAP Table 3. No assessment was carried out for the remaining substances on Table 3 (selenium, antimony, and tin). However, the Dossier Submitter proposes that these substances are included in the scope of the proposed restriction as well as they are also included in Member State's national restrictions, see the section on Risk Characterisation below (see Human health).

B.5.14. Derivation of DNEL(s)/DMEL(s)

Derived No-Effect Levels (DNELs) derived under REACH usually refer to the level of the daily external dose where no adverse effects are anticipated. However, for tattoos the dose is injected into the dermis, so only internal DNELs are relevant. Thus, available external DNELs are converted to internal DNELs, by applying absorption rates etc.

It was possible to conduct quantitative risk assessments for some of the substances. The leading health effect was identified and the corresponding DNEL was derived for substances

with threshold effects when a dose-descriptor such as a NOAEL or LOAEL was available, e.g. for substances toxic to reproduction. This approach is in accordance with ECHA Guidance R.8 (ECHA, 2012).

For other substances with non-threshold effects such as for most mutagenic and carcinogenic substances, the risk was only assessed in a qualitative way. Some of the PAAs, arsenic and lead as impurities were however assessed in a semi-quantitative manner with derivation of Derived Minimal Effect Levels (DMELs).

The Dossier Submitter recognises that it is not possible to introduce a complete ban of all hazardous substances in tattoo inks and PMU. Therefore, in some cases there was a need for setting a concentration limit for hazardous substances and impurities in tattoo inks to protect the consumers from adverse health effects. The concentration limits were based on the DNELs and DMELs for these substances. To derive the DMEL values for non-threshold carcinogenic substances, the maximum level of indicative tolerable lifetime excess cancer risk for consumers was assumed to be 10^{-6}, in accordance with ECHA guidance R.8.1.1 (ECHA, 2012).

All DNELs and DMELs were derived for systemic effects via intradermal injection.

Assessment factors were applied in accordance with ECHA Guidance R.8 (ECHA, 2012). In most of the assessments an assessment factor of 10 was applied for intra-species variation and another assessment factor of 10 was applied for inter-species variation. Modification of the dose descriptors and the application of additional assessment factors are given in the respective chapters for the relevant endpoints/substances (such as assessment factors for differences in exposure duration, issues related to dose-response, quality of whole database.).

Derivation of DNEL for methanol

The Dossier Submitter proposes to derive the DNEL on the basis of on the IOEL value in line with Appendix R.8-13 (Deriving DNELs when community/national Occupational Exposure Limit (OEL) is available) to Chapter R.8 (Characterization of dose [concentration]-response for human health of Guidance on information requirements and chemical safety assessment (ECHA). A NOAEL/LOAEL as basis for the OEL is not available. Exposure to 260 mg/m^3 during a working shift is equivalent to a dose of 2.6 g/person/day (40 mg/kg b. w. and day) which may be considered as a systemic DNEL (40 mg/kg bw/day). An additional assessment factor of 5 is used to take into account possible higher sensitivities and possible longer exposure duration for the general population verses workers.

Table 13. DNEL for methanol

Substance	**CAS No.**	**Type of effects**	**Dose descriptor**	**DNEL** general population, [mg/kg bw/d]	**Remark**
Methanol	67-56-1	STOT SE1 (ocular and CNS)	OEL: 260 mg/m^3	8	-

Derivation of DMELs for PAAs

A hazard evaluation was performed for the ten PAAs found in a Danish survey of tattoo inks (DEPA, 2012) to determine a DMEL for the carcinogenic effects, see Table 13. For more information on the derivation of the DMELs or the other assessments, see Appendix B.2. PAAs and azo colourants

Table 14. DMEL values for PAAs found in tattoo inks.

Substance	CAS No.	Classification	Point of Departure (POD), Dose descriptor	DMEL general population, carcinogenic effects	Remark
Aniline	62-53-3	Carc 2 Muta 2 Acute tox 3 STOT RE1 Eye damage 1 Skin sens 1	HT25, 4.6 mg/kg bw/day	2×10^{-5} mg/kg bw/day	The DMEL was based on HT25 and application of a HtLF (High to low dose risk extrapolation factor) of 250.000 (the 'default' for the 10^{-6} lifetime risk when T25 is used as a PoD (ECHA Guidance chapter 8 appendix 8-6 and 8-7)),
o-Anisidine	90-04-0	Carc 1B Muta 2 Acute tox 3	HT25 9.9 mg/kg bw/day	4×10^{-5} mg/kg bw/day	
4-chloroaniline	106-47-8	Carc 1B Acute tox 3 Skin sens 1		-	DNEL/DMEL for the critical effects could not be established.
4-chloro-o-toluidine	95-69-2	Carc 1B Muta 2 Acute tox 3		-	
3-3'-dichloro-benzidine	91-94-1	Carc 1B Acute tox 4 Skin sens 1		-	
4-methyl-m-phenylene-diamine	95-80-7	Carc 1B Muta 2 Repr 2 STOT RE 2 Acute tox 3 Acute tox 4 Skin sens 1		-	
4-methoxy-m-phenylene-diamine	615-05-4	Carc 1B Muta 2 Acute tox 4			
2-naphthyl-amine	91-59-8	Carc 1A Acute tox 4			
5-nitro-o-toluidine	99-55-8	Carc 2 Acute tox 3			
o-toluidine	95-53-4	Carc 1B Acute tox 3			

		Eye irrit 2			

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

For the evaluated PAAs, it is considered that there is no threshold for the carcinogenic effects and, therefore, a DNEL cannot be established. Instead, DMELs may be derived.

For two of the PAAs (aniline and o-anisidine), a DMEL could be established. For the remaining eight PAAs, a DMEL could not be established based on the available data.

The DMEL for aniline is derived at approximately 2 x 10^{-5} mg/kg bw per day for the carcinogenic effects. The DMEL for o-anisidine is derived at approximately 4 x 10^{-5} mg/kg bw per day.

Since all the PAAs with a harmonised classification as carcinogenic are very similar, a grouping approach is applied and the lowest DMEL value of 2 x 10^{-5} mg/kg bw per day of aniline is applied for the group.

Taking the considerations on potency of the PAAs into account, note that the DMEL value for aniline is only half that of o-anisidine, which could be compared with the other cancer potency indicators as discussed in Appendix B.2. PAAs and azo colourants

Sensitisation was also considered as a critical effect in relation to tattooing for aniline, 4-chloroaniline, 3,3'-dichlorobenzidine and 4-methyl-m-phenylenediamine. In the EU, these substances are classified Skin Sens. 1 (H317: May cause an allergic skin reaction) according to Annex VI of the CLP Regulation (EC no. 1272/2008). The health effect assessment of chemical contact allergens can only be performed if the potency and the threshold value have been carefully examined for the specific chemical allergen (Nielsen, et al., 2005). For the selected substances, the available data is not sufficient for an evaluation of either the potency or the threshold value and, therefore, a DNEL for sensitisation cannot be established for these substances.

Derivation of DNELs for reprotoxic substances

In the present section critical DNELs for the substances toxic to reproduction assessed in section B5.9 are presented and discussed. An overview of all DNELs derived is given in Table 14. Moreover, a general DNEL representing the group of classified Repro 1 A/B "only" substances is proposed. The point of departures and the assessment factors applied for the individual substances are shown and discussed in the appendices, see Appendix B.3. Hazard assessment for reprotoxic substances

Table 15. Overview of critical DNELs for substances toxic to reproduction

Substance	**CAS No.**	**Type of effects**	**Dose descriptor (LOAEL/NOAEL) [mg/kg bw/d]**	**DNEL** general population, reproductive effects [mg/kg bw/d]	**Remark**
bis(2-ethylhexyl) phthalate	117-81-7	D	4.8	0.048	
dibutyl phthalate	84-74-2	D	2	0.0067	
diisobutyl phthalate	84-69-5	D	2.5	0.0083	
dihexyl phthalate	84-75-3	D	20	0.067	The DNEL for fertility effects is higher.
n-pentyl-isopentylphthalate	--- / ---			n.a.	No key study could be identified.
1,2-benzenedicarboxylic acid, di-C6-C8-branched alkyl esters, C7-rich	71888-89-6	D	100	0.33	
1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear	68515-50-4	D	2	0.0067	
disodium tetraborate, anhydrous	1330-43-4	F	81.4	0.407	The DNEL for developmental effects is higher
tetraboron disodium heptaoxide, hydrate	12267-73-1	F	117.9 – 154.3	0.59 – 0.77	The DNEL for developmental effects is higher. Range: hydrate dependant.
boric acid	10043-35-3, 11113-50-1 & 13840-56-7	F	100	0.5	The DNEL for developmental effects is higher.
diboron trioxide	1303-86-2	F	56.3	0.28	The DNEL for developmental effects is higher
sodium perborate	13517-20-9, 15120-21-5	D	100	1	
sodium peroxometaborate	7632-04-4, 10332-33-9, 10486-00-7	D	100	1	
perboric acid, sodium salt	11138-47-9, 12040-72-1, 37244-98-7	D	100	1	
(2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol	94361-06-5	D	1.39	0.014	

(4-ethoxyphenyl)(3-(4-fluoro-3-phenoxphenyl)propyl)dimethylsilane	105024-66-6		n.a.	n.a.	No key study could be identified.
(R)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone and (S)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone	5543-58-8 & 5543-57-7	D	0.04	0.001	
N,N-(dimethylamino)thioacetamide hydrochloride	27366-72-9		n.a.	n.a.	No key study could be identified.
1,2-diethoxyethane	629-14-1	D	50	0.29	
1-ethylpyrrolidin-2-one	2687-91-4	D	50	0.5	
1-methyl-2-pyrrolidone	872-50-4	D	150	0.5	
2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate	15571-58-1	D	15	0.03	
4-tert-butylbenzoic acid	98-73-7	F	1.6	0.0027	
7-methoxy-6-(3-morpholin-4-yl-propoxy)-3H-quinazolin-4-one	199327-61-2 / 429-400-7		n.a.	n.a.	No key study could be identified.
ammonium 2-amino-4-(hydroxymethylphosphinyl) butyrate	77182-82-2 / 278-636-5		6.3	0.0175	
chloro-N,N-dimethylformiminium chloride	3724-43-4 / 425-970-6		n.a.	n.a.	No key study could be identified.
cyclic 3-(1,2-ethanediylacetale)-estra-5(10),9(11)-diene-3,17-dione	5571-36-8 / 427-230-8		n.a.	n.a.	No key study could be identified.
imidazole	288-32-4	D	60	0.6	
ketoconazole	65277-42-1	F	200	0.11	
salts and esters of dinoseb, with the exception of those specified elsewhere in this Annex	--- / ---	D	1	0.0033	
salts and esters of dinoterb	--- / ---		n.a.	n.a.	No key study could be identified.
tetrahydrofurfuryl alcohol	97-99-4	D	50	0.5	
tributyltin	--- / ---	F	0.00017 - 0.001	5.4×10^{-8} – 3.7×10^{-7}	
trixylyl phosphate	25155-23-1 / 246-677-8	F	25	0.014	

Overall, for 27 of the 34 substances $DNEL_{s_{general\ population,\ reproductive\ effects}}$ could be derived. If a

substance was classified as Repr. 1B for more than one reprotoxic endpoint (e.g. developmental and fertility effects) the lowest DNEL value was considered as the critical DNEL for this substance.

For 7 substances no data were available related to the endpoint toxicity to reproduction. Thus a key study and DNEL value could not be identified. Only one DNEL value was derived for substances having two entries with different CAS numbers but which were identified as chemically and toxicologically identical (boric acid CAS No. 10043-35-3 and CAS No. 11113-50-1, warfarin R and S racemates CAS No. 5543-58-8 and CAS No. 5543-57-7).

For 96% of the substances DNEL values between 1 and 0.001 mg/kg bw/d were obtained. For only one substance, tributyltin chloride, a lower DNEL was found which is five levels of magnitude lower than 0.001 mg/kg bw/d (0.000000053 - 0.00000031 mg/kg bw/d). This DNEL was based on NOAEL values for fertility effects observed in subacute studies in one mice species (Kun Ming mice) at low dose levels. High AF had to be applied for the obtained threshold indicating a high uncertainty for the DNEL derived.

In the following generally applied AF to derive the $DNEL_{general\ population,\ reproductive\ effects}$ are discussed. To account for chronic exposure generally an AF was applied to extrapolate for chronic fertility effects. For developmental effects, as appearing in a restricted (defined) period of life ('sensitive window for developmental effects') and as prenatal developmental toxicity studies mostly do cover this sensitive window for developmental effects, no general AF was applied to extrapolate to chronic effects but AF were applied individually if necessary depending on available data. Other AF applied were default to cover intraspecies differences, interspecies differences (allometric scaling and remaining differences) and dose-response relationships (LOAEL/NOAEL). The AF applied for the individual substances are shown in Appendix B.3.

A substance-related restriction and entry in REACH Annex XVII based on individual risk assessments and specific concentration limits for each of the 34 substances was considered not to be appropriate for the restriction of reprotoxic substances in tattoo inks and PMU. This would trigger a continuous updating of the restriction to account for reprotoxic substances which are classified in the future which is considered to be not feasible. This is further discussed in the risk characterisation section B.10. Thus, the quantitative risk assessment approach as described here (RO 1, see Human health) intends to establish a general concentration limit for reprotoxic "only" substances in tattoo inks and PMU based on the most sensitive DNEL identified among the known 34 members of reprotoxic "only" compounds that are considered to be representative for reprotoxic substances classified as Repro. 1 A/B.

As the DNEL for tributyltin compounds is considered as an outlier due to high uncertainties which may lead to overestimation of the risk for most substances, the **overall $DNEL_{general\ population,\ reproductive\ effects}$ of 0.001 mg/kg bw/d** is proposed as the most sensitive DNEL for risk assessment of reprotoxic substances in tattoo inks and PMU. The DNEL was derived from the substance (R)- and (S)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone based on a LOAEL of 0.04 mg/kg bw/d and an overall AF of 30. (See Appendix B.3. (section A2.12) for details). This DNEL further is supported by a 'threshold of toxicological concern' (D of 0.001 mg/kg bw/d for developmental toxicity which was published by Bernauer and colleagues in 2008 (Bernauer et al., 2008) using a TTC concept for reproduction toxicity based on data from 91 chemicals.

The substances classified in CLP in category Repr.2 have not been assessed individually due to the difficulty to estimate the dose descriptors for substances of concern for this endpoint. However, the Dossier Submitter proposes that as a starting point the resulting group DNEL for the Repr.1A/B substances is also applied to Repr.2 substances.

Almost all NOAELs/LOAELs were derived from studies with oral substance administration. If

assumed bioavailability of substances after intradermal injections and oral uptake is 100%, no AF to correct for route is needed.

Thus, it was assessed based on available physico-chemical and toxicokinetic data, whether for substances which represent the range of the lowest DNELs (DNEL values ≤ 0.1) namely (R)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone, 4-tert-butylbenzoic acid, salts and esters of dinoseb, dibutyl phthalate, 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear, (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol a 100% oral bioavailability could be assumed. For those substances toxicokinetic data (not presented here) did not suggest a deviation from the default assumption of a 100% oral bioavailability. Thus, no route specific correction of the NOAEL/LOAELs was performed to account for intradermal injection reflecting the tattooing process.

Derivation of DN(M)ELs for substances on the CoE ResAP(2008)1, Table 3, impurities in tattoo inks and PMU

A hazard evaluation was performed for the following substances on CoE ResAP(2008)1 Table 3 to determine a DN(M)EL for the relevant critical effects, see Table 15. For more information on the derivation of the DN(M)ELs, see appendix B.6-B.11.

Table 16 Point of Departure (POD) and DN(M)ELs derived for selected substances on the CoE ResAP(2008)1, Table 3

Substance	**Point of departure, POD**	**Information on key study**	**DMEL, general population, carcinogenic effects or DNEL STOT-RE**
Arsenic (As)	Excess lifetime risk of lung tumours = 1.7 x 10^{-3} per µg As/kg bw/day (as a systemic exposure)	Based on the WHO/FAO risk estimates from the Taiwanese drinking water cohort, using data from the most recent publications of Chen et al (2010a, 2010b), and 10^{-6} as an indicative tolerable risk level.	DMEL 0.0005882 µg As/kg bw/d
Barium (Ba)*	NOAEL 60 mg/kg bw/d	Nephrotoxicity in male rats at 60 mg/kg bw/d in NTP 13 week study, also supported by findings in female rats and in male/female mice (NTP 13 week study), as well as interim findings in female rats in the NTP 2 year study	DNEL 0.60 mg/kg bw/d
Copper (Cu)*	2 mg/L drinking water, equalling 2.2 mg Cu/day	Two mg/l equals a mean total copper intake of 2.2 mg/day (95th percentile would be 5.6 mg), if assuming a bw of 60 kg and a water intake of 1.1 l/d (or with the 95th percentile 2.8 l/d) to avoid GI irritation (WHO guidelines for drinking-water quality, 2004)	DNEL 0.037 mg/kg bw/d
Lead (Pb)	$BMDL_{01}$ 0.50 ug Pb/kg day	Effects on the developing nervous system including in utero (EFSA 2010/2013), applied by RAC (ECHA 2011; 2013).	DMEL 0.05 µg
Zinc (Zn)*	NOAEL 0.83 mg/kg bw/d	An EFSA report from 2006 (EFSA 2006) and supported by the SCCS opinion from 2017 (SCCS/1586/17) adopted a NOAEL of 50 mg/day or 0.83 mg Zn^{2+}/kg bw/day which is based on the absence of any adverse effects on a wide range of relevant indicators of copper-status as critical endpoint.	DNEL 0.166 mg/kg bw/d

* Soluble

B.6. Human health hazard assessment of physicochemical properties

B.6.1. Explosivity

Not relevant for this Dossier.

B.6.2. Flammability

Not relevant for this Dossier.

B.6.3. Oxidising potential

Not relevant for this Dossier.

B.7. Environmental hazard assessment

B.7.1. Aquatic compartment (including sediments)

Not relevant for this Dossier.

B.7.2. Terrestrial compartment

Not relevant for this Dossier.

B.7.3. Atmospheric compartment

Not relevant for this Dossier.

B.7.4. Microbiological activity in sewage treatment systems

Not relevant for this Dossier.

B.7.5. Non compartment specific effects relevant for the food chain (secondary poisoning)

Not relevant for this Dossier.

B.8. PBT and vPvB assessment

B.8.1. Assessment of PBT/vPvB Properties – Comparison with the Criteria of Annex XIII

Not relevant for this Dossier.

B.8.2. Emission Characterisation

Not relevant for this Dossier.

B.9. Exposure assessment

B.9.1. General discussion on releases and exposure

B.9.1.1. Summary of the existing legal requirements

See Section 1.1 of the report and D.1.3 for a summary of these requirements.

B.9.1.2. Summary of the effectiveness of the implemented operational conditions and risk management measures

Not relevant.

B.9.2. Manufacturing

Not relevant for this Dossier.

B.9.3. Use 1: Intra-dermal injection of tattoo inks

B.9.3.1. General information

Tattoo ink is injected into the dermis by puncturing the epidermis at a rate of 50 to 5 000 times per minute. Capillary action acts to draw ink further into the dermis (see Annex A for more details). This exposure route is somewhat unique in the scope of REACH risk assessments.

B.9.3.2. Exposure estimation

The exposure assessment has been performed in order to address hazardous constituents used in tattoo inks, as well as unavoidable hazardous impurities. The aim is to use the assessment to determine if there is a risk from those constituents and impurities and in order to derive proposals for limit values of the hazardous constituents to control risk.

In the exposure assessment only one exposure scenario has been developed. This exposure scenario consists of isolated single tattoo sessions on 300 cm^2 skin repeated until most of the body is covered. This exposure scenario will be protective for both people getting full body tattoos and for others getting single or a few tattoos.

B.9.3.2.1. Consumer exposure

Amount of Ink Injected

Very limited data on the amount of tattoo ink deposited in the skin during the tattooing process is available. Still an estimate of 14.36 mg tattoo ink/cm^2 tattooed skin has been determined.

Based on the information available, there are indications of a difference concerning the amount of ink placed in the skin during tattooing by experienced tattoo artists and by unexperienced (amateur) tattoo artists. Naturally, a professional tattoo artist is anticipated to be more experienced than an amateur only tattooing occasionally and we assume that an experienced tattoo artist uses less ink per cm^2 than the unexperienced tattoo artists. However, robust data to distinguish between the tattoo artists regarding the amount of tattoo ink applied is not available, so no distinction between these two groups has been done in this exposure assessment. For the purpose of the exposure assessment, a tattoo ink containing 25% pigment is considered to represent a realistic composition. This concentration is within the typical range of pigment in tattoo inks, which is between 20% and 45% (JRC, 2015b). For phthalocyanines the content in four samples analysed in a survey by DEPA (DEPA, 2012)varied from 4.65% to 18.9%. The Dossier Submitter still considers 25% to be appropriate in the assessment.

In a study performed on pig and human skin, Engel et al. (Engel, et al., 2008) have determined the amount of pigment red 22 (PR 22; CAS 6448-95-9) placed in the skin after tattooing performed by researchers and professional tattoo artists. In a number of experiments, the group used different grades of PR 22 (synthesised: purity >98% and commercial: purity 80%), different methods of tattooing and different equipment. Using a self-developed extraction method with a recovery rate ~98%, the amount of pigment deposited was determined to be in the range of 0.60-9.42 mg/cm^2. For the experiments a suspension of PR 22 in 10% (w/v) glycerol in water/isopropanol was used with concentrations of 10 or 25% (w/v) of the pigment. In the study the concentration of PR 22 was only given as mass/volume, this is however considered an appropriate approximation for the mass fraction (w/w) in this case, see textbox below.

Textbox 2. Consideration on the pigment red concentration given as mass/volume (Engel et al., 2008 and https://echa.europa.eu/registration-dossier/-/registered-dossier/10472/4/5)

Assuming complete insolubility of pigment red 22 in the vehicle and approximating the density of the vehicle as d ≈ 1 g/cm^3 the mass fraction of pigment red (PR 22) was ~ 24% (w/w). The density of pigment red 22 (PR 22) is 1.38 g/cm^3, thus 25 g ≈ 19 ml (pigment red (PR 22)). Thus the volume of the vehicle in 100 ml must be 81 ml (100 ml-19 ml). Then the mass of 100 ml of solution becomes 106 g (25 g pigment red (PR 25) + 81 g vehicle) = 106 g. The percentage of pigment red then becomes 24% (100 * (25 g/106 g))

Engel et al. investigated the resulting amount of pigments in pig and human skin after in vitro tattooing by using two different concentrations of pigments (10 and 25% pigment (w/v)) (Engel, et al., 2008). Since we assume 25% pigments in tattoo ink, the Dossier Submitter selected only the results from the Engel study on the concentrations of 25% pigment to

estimate the resulting amounts in the skin. The mean value for pigment in the skin in all experiments by (Engel, et al., 2008) combined with a 25% concentration of pigment red (PR 22) resulted in 3.2 mg pigment/cm^2. The median was 2.6 mg pigment/cm^2, the 95th percentile was found to be 7.73 mg pigment/cm^2 and the 75th percentile is found to be 3.59 mg pigment/cm^2 (values calculated by the Dossier Submitter).

Table 17. Original table from (Engel, et al., 2008):

Contact Dermatitis 2008: 58: 228–233

Table 1. Concentrations of pigments in skin[a]

Method	Needle size	Applied concentration (w/v) (%)	Amount per tattooed area (mg/cm^2)	RSD (%)
A	8R	10	0.63	13.5
	8R	25	1.42	7.8
	4R	10	1.75	5.9
	4R	25	5.19	15.8
	8F	10	1.02	30.0
	8F	25	2.60	21.6
	4F	10	2.49	4.9
	4F	25	3.44	13.4
B	8R	10	1.90	32.9
	8R	25	3.59	14.1
	4R	10	2.90	45.3
	4R	25	9.42	11.8
C	9R	25	0.60	14.7
D	8R	25	0.95	23.9
E	8R	25	1.69	7.4
Mean value			2.53	17.9

RSD, relative standard deviation.
[a]The amount of PR 22 deposited in 1 cm^2 pigskin and human skin each. Researchers obtained concentration values in experiments with (A) synthesized and (B) commercial PR 22 in pigskin. The values in (C) stand for experiments performed by tattoo artists using synthesized PR 22 in pigskin. The values for human skin are displayed using commercial (D) or synthesized (E) PR 22. RSD is given for each experimental setting. The last line of the table shows the respective mean of the values for each different setting.

Table 18. Extract of data from the Engel study (Engel, et al., 2008). Data rearranged by the Dossier Submitter only showing the results when applying 25% pigment red (PR 22):

Experiment number	**Amount of pigment recovered (mg/cm^2)**
1	0.6
2	0.95
3	1.42
4	1.69
5	2.6
6	3.44
7	3.59
8	5.19
9	9.42

Other information is also available on this topic. In a recent review article, the authors assumed that an amount of 1 mg of ink per cm^2 of skin is injected (Laux, et al., 2016). In the survey made by the Danish EPA expert judgement by tattoo artists came to the same conclusion (DEPA, 2012). Prior (Prior, 2015) experimentally determined an average value of 0.4 mg/cm^2 ink using an indirect quantification method. The highest amount in this study was determined to 1.2 mg/cm^2 (Prior, 2015). However, the Engel study gives the highest confidence as the value was experimentally derived and is likely, in the judgement of the Dossier Submitter, a realistic worse case situation.

Normally, the 95th percentile is applied in REACH consumer exposure assessment. However, since the REACH guidance document R15 on consumer (ECHA, 2016c) exposure doesn't cover this exposure situation well, the principles from the R14 guidance document on occupational exposure might be applied (ECHA, 2016b). According to R14, *"in general the 90th percentile value, representing the reasonable worst case exposure level of a distribution within a generally suitable dataset (i.e. a dataset corresponding to the conditions described in a contributing scenario), should be used as the exposure value for the risk characterisation. Under particular conditions other percentiles may be applicable as well. A justification should be provided in the CSR. For instance, the use of the 75th percentile may be justified when the data set reflects worst case situation only (e.g. data sets taken in companies suspected of being non-compliant)".*

As the R14 guidance suggests, deviation from the 90^{th} percentile can be justified if the data set reflect worst case only. As there is very limited data to assess the amount of pigment in the skin after tattooing, and since the data from the Engel study (Engel, et al., 2008) compared with expert judgement are rather high (see the text below), it is justified that the data-set is comparable to the situation where only the worst case situation is reflected. Thus, the Dossier Submitter proposes to apply the 75th percentile. The 75th percentile is found to be 3.59 mg pigment/cm^2.

As we assume that the content of pigment in the ink is 25%, the corresponding amount of tattoo ink containing the pigment is calculated to be 14.36 mg ink/cm^2 (4 x 3.59). This value will be used in the risk characterisation and in the derivation of concentration limit values for safe use of hazardous substances in tattoo ink.

In the Engel et al. study (Engel, et al., 2008), tattooing on pig skin was performed by researchers and by two professional tattoo artists and in this case the amount of pigment red (PR 22) found in the skin was lower, only 0.6 mg/cm^2, but still comparable to the values achieved by the researchers. This corresponds to 2.4 mg ink /cm^2, which is also higher than the amounts of 0.4 to 1.2 mg ink/cm^2, which have been estimated in other studies.

Table 19. Summary of studies on the amount of ink injected.

Source	Value	Remark
(Laux, et al., 2016)	Ink: 1 mg/cm^2	Expert judgement
(Prior, 2015)	Ink: 0.4 mg/cm^2	Ink with 67% carbon black
(Engel, et al., 2008)	Pigment: range - 0.60-9.42 mg/cm^2	Human and pig skin, experiments by tattoo artist and researchers and with different equipment
	Mean: 3.2 mg/cm^2	
	75th percentile: 3.59 mg/cm^2	
	95th percentile: 7.73 mg/cm^2	
This proposal	Ink: 14.36 mg/cm^2	Calculated from (Engel, et al., 2008) for 25% pigment and applying 75th percentile

The amount of ink per cm^2 is of major importance in the calculations in the risk assessment and as such it is included in the uncertainty and the sensitivity analysis.

Tattooed Skin Area in single and multiple tattoo sessions

Several estimates of the typical size of tattoos are available in the literature. The Danish EPA has presented results of a clinical investigation with 72 tattooed male and female persons in Denmark (DEPA, 2012). The average area of tattooed skin was estimated to be approximately 2.5% of the skin surface corresponding to 423 cm^2 for women and 485 cm^2 for men. In another Danish study the tattoos of 154 young individuals (mean age 27.5 years) were investigated (Høgsberg, et al., 2013). The total number of tattoos was 342. Most of these tattoos were defined individual tattoos and the covered skin area was in the range of 0.1-1% of the body surface. In an exceptional case a male study participant had tattoos covering over 72% of the skin surface. An internet survey in German speaking countries showed that most tattooed participants (61 %) have tattoos bigger than 300 cm² and 16% larger than 900 cm^2.

In general, with respect to exposure estimation, former studies and reports (JRC, 2015b) (JRC, 2016b) and references within) have focused on the size of the final tattoo. However, since data on the absorption kinetics is very limited, which implies an assumption of 100% uptake and since a relative fast excretion is assumed, it is more appropriate to base the exposure assessment on the total amount of tattoo ink injected during a single tattoo session.

A recent unpublished Danish survey reported in Appendix F.1 Questionnaire on the tattoo process showed that repeated tattooing is common. Thus, the customers visit the tattoo artist on a regular basis. This supports the use of the exposure that takes place in separate tattoo sessions, and the assumption of repeated tattooing.

Further, the survey showed that the concept of a "large tattoo" from JRC is not applicable. Rather, the tattoo artist covers a full body part as a lower leg, an arm or the back. These large body parts are tattooed during a series of tattoo sessions.

Based on the recent Danish survey and information gathered by (JRC, 2016b), tattoos can roughly be divided into two types of tattoos:

1. Small tattoos, which are very common and frequent. This type of tattoo is estimated to have an average size of 140 cm^2 (see Appendix F.1 Questionnaire on the tattoo process). It is within the range of a medium tattoos as estimated by (JRC, 2015b) (between 30 and 300 cm^2). This type of tattoo is probably at the most made once a year or once every second year (see Appendix F.1 Questionnaire on the tattoo process) and a rough estimate is that during a lifetime a person is could have a maximum of around 5 to 15 tattoos.
2. Large tattoos are covering full body parts like arm, leg and back or a full body tattoo. As explained above, these tattoos are performed during a series of tattoo sessions. This corresponds to a large tattoo as estimated by (JRC, 2016b) (> 300 cm^2)

In the survey on the practice of tattooing that was recently conducted in Denmark, the results from the survey were discussed with tattoo artists (See Appendix F.1 Questionnaire on the tattoo process). This discussion confirmed that repeated tattooing appears to be normal. Thus, the customers visit the tattoo artists on a regular basis – such as once a month through a year - in order to complete a larger tattoo (e.g. full arm or leg). This information combined with the lack of knowledge on the toxicokinetics of ink in the body, suggests that it would be appropriate to apply the exposure that takes place in a single tattoo session, which should then be used in the exposure assessment. The repeated exposure in each session to obtain the final size of the tattoo supports the use of DNEL/DMEL related to lifetime exposure.

The studies so far (as reported in (JRC, 2015b) (JRC, 2016b)) have not considered the percentage of pigmentation coverage. A simple line tattoo (in e.g. a poem) doesn't take as much ink as a full colour tattoo and the tattoo is completed much faster. This is an essential finding in the recent Danish survey (see Appendix F.1 Questionnaire on the tattoo process).

Depending on the picture and the colour intensity, the number of sessions needed to complete a large tattoo varies. Considering, e.g., a full arm and a complex/high colour density tattoo, up to 10 sessions may be needed. In contrast the same area for a low colour density tattoo can be completed in one session.

In the survey presented in Appendix F.1 Questionnaire on the tattoo process , the tattoo artists explained that in principle as long as there continuously is a new skin area to fill, the tattoo artist can just continue to fill the whole body. However, the tattoo artists explained that there are factors limiting the length of a tattoo session, such as the ink capacity of the skin and the pain for the consumer. Thus, there is a limit for how much a tattoo artist can tattoo in one day. The typical maximum area of a full colour tattoo that can be made in one session (in one day) is estimated to be 300 cm^2 (Appendix F.1 Questionnaire on the tattoo process). However, in a few cases the limit of 300 cm^2 per session or day may be exceeded.

Further, due to the healing process, the tattoo artist (see Appendix F.1 Questionnaire on the tattoo process) in general recommends at least 25 days between tattoo sessions. However, sometimes tattoo artists tattoo a person every day in a week (every day a new piece of skin). Afterwards, the customer is advised to take a long break with respect to new tattoos.

The numbers identified in the survey presented in Appendix F.1 Questionnaire on the tattoo process are in line with the observation reported in the literature (see e.g. (JRC, 2016b)).

Conclusion - The Realistic Worst Case Exposure Scenario

The exposure will be assessed as the exposure from a single tattoo session in this dossier. The Dossier Submitter assumes that the typical maximum area of a full colour tattoo that made in one session is 300 cm^2. The amount of ink injected in a single session is estimated to be 14.36

mg ink/cm^2. This corresponds to 3.59 mg pigment/cm^2 (25%) when the 75th percentile from the Engel study is applied. The absolute amount of tattoo ink in a single session would then be 300cm^2 x 14.36 mg ink/cm^2 = 4 308 mg ink, assuming that the size of the tattoo is 300 cm^2.

This scenario is based on a realistic worst case situation where the exposed person repeatedly gets the maximum size tattoo that is possible in one session (300 cm^2), until the person has a full coloured full body tattoo.

It normally takes several tattoo sessions over a period of time to get a full colour, full body tattoo. Only a small part of the full body tattoo is normally completed in each session. In this scenario, the person will (on average) go to the tattoo artist once a month, which according to the survey (Appendix F.1 Questionnaire on the tattoo process) can be considered a typical behaviour in relation to having full body parts tattooed.

Comparison of the exposure with the long-term DNEL

The full body tattoo will be completed in 61.5 months (18 440 cm^2 [10]/(300 cm^2/session)/month), which is equal to ca. 5.2 years. The repeated exposure over a period of ca. 5 years supports that, in the risk characterization, the exposure with 4 308 mg ink should be compared with a DNEL/DMEL related to lifetime exposure (ECHA, 2016c).

Further, according to ECHA CSA Guidance R15 "as a conservative approach, the risk for a consumer exposure scenario can be characterised by comparing the event exposure over a day to this DNEL" ((ECHA, 2016c), p. 17, last paragraph). Accordingly, in the risk characterisation the DNEL/DMEL related to lifetime exposure is still relevant even if the exposure event results from an "only one use" or "infrequent"-event. Thus, it is proposed not to adjust the exposure and apply the DNEL/DMEL related to lifetime exposure.

This further assures a higher protection for the consumers than to adjust the exposure estimate over a long period of time. The continuous release of impurities from some of the pigments also supports that the DNEL/DMEL is based on lifetime exposure.

Exposure Scenario – Summary

A realistic worst case scenario has been developed. In the table below the data for the scenarios has been summarised.

[10] For a woman aged 50-60 years with a skin size equal to the 95 percentile, the tattooed body surface can be calculated to be 18 440 cm^2 (23,800 cm^2 – 1 140 cm^2 – (2 x 890 cm^2) – (2 x 1 220 cm^2) = 18,440 cm^2).

Table 20. Parameters to be applied in the exposure calculation for tattoo inks.

Parameter	**Value**
Size of tattoo per session (cm^2)	300
Pigmentation covering (%)	100
Weight of tattooed person (kg)	60
Amount of ink used per cm^2 (mg)	14.36
Amount of ink used per session (mg)	4 308
Bioavailability of pigments - Percentage of pigment removed from tattoo area by body fluids	100%
Bioavailability of impurities - Percentage of ink-fluids and soluble substances including impurities removed from the tattoo area	100%
Excretion of pigments	100%
Excretion for soluble substances incl. impurities	100%

Body surface area and body weight

Data on the body surface area and body weight used in the exposure assessment is taken from the US EPA Exposure factors handbook (US EPA, 2011), as referred to in ECHA guidance, section R.15.3 (ECHA, 2016c).

Table 21. Mean Surface Area by Body Part (cm^2) from the US EPA Exposure Factor handbook (US EPA, 2011)

Mean Surface Area by Body Part cm^2						
	Head	**Trunk**	**Arm**	**Hands**	**Legs**	**Feet**
Age Group						
Adult Male 21 + years	1360	8270	3140	1070	6820	1370
Adult female 21 + years	1140	6540	2370	890	5980	1220

Table 22. Body size in cm^2 from the US EPA Exposure Factor handbook (US EPA, 2011).

	Body area (cm^2)	**Body weight (kg)**	**Body area/body weight (cm^2/kg)**
Male 40 – 50 years			
Full body -95 percentile	25600	70	366
Female 50 – 60 years			
Full body -95 percentile	23800	60	397

In a Nordic report, the default value of body weight for use in exposure assessment is recommended to be 70 kg for men and 60 kg for women (The Nordic Exposure Group, 2011). In the guidance document for consumer exposure (ECHA, 2016c), in example R.15-1, 60 kg is applied for women, however no overall recommendation is given. In this Annex XV report a body weight of 60 kg is applied for all. Further, the same body weight is applied for all ages. This strengthens the support for using 60 kg and not 70 kg as a default body weight, as many young people/teenagers get tattoos.

The largest skin area per kg body weight is found in women in the 95th percentile of the age interval 50 – 60 years. This equals a skin area of 23 800 cm^2 which is applied in the calculation, and is presented in the section on the exposure scenario.

Since the area per kilo is higher for women than for men and since both men and women should be equally protected, the value for women is applied.

However, as skin area is only used to estimate the number of tattoo sessions needed to get a full body tattoo and not used in the calculation of the risk when comparing a single exposure session to the DN(M)EL, and thus are not critical for other numerical results, no uncertainty or sensitivity analysis are performed for these default values.

Measured content of selected substances in tattoo inks reported by JRC

In addition to the exposure scenario above, the Dossier Submitter assessed the actual content of selected substances found in tattoo inks. The source for data on content of substances in tattoo inks results from national surveys and market surveillance activities compiled by JRC (JRC, 2015b):

Table 23. Content of selected substances in tattoo inks (facsimile from JRC 2015b)

Table 4.38: PAAs presence in tattoo and PMU inks.

Substance	CAS nr	Number of analysed samples	% non compliant samples	ResAP (2008)1 limit (mg/kg)	Range (min-max) (mg/kg)
PAA (total)		3283	14 (468)		0.1-68
4-Aminoazobenzene	60-09-3			0	>0
Aniline	62-53-3			0	5-61
o-Anisidine	90-04-0	3655	10 (347)	0	0.52-2197

Table 4.39: Metals present in tattoo and PMU inks.

Substance	CAS nr	Number of analysed samples	% non compliant samples	ResAP (2008)1 limit (mg/kg)	Range (min-max) (mg/kg)
Antimony (Sb)	7440-36-0	932	7 (70)	2	0.02 - 147
Arsenic (As)	7440-38-2	1164	5 (62)	2	0.2-60
Barium (Ba)	7440-39-3	886	20 (180)	50	50-17737
Cadmium (Cd)	7440-43-9	1863	5 (93)	0.2	0.01-7.84
Cr (VI)	7440-47-4			0.2	0.3-147
Cobalt (Co)	7440-48-4	350	4 (14)	25	0.003-31310
Copper (Cu) soluble	7440-50-8	283	32 (90)	25	2.5-45000
Lead (Pb)	7439-92-1	2175	8.5 (195)	2	0.015-401.5
Mercury (Hg)	7439-97-6	809	2.5 (20)	0.2	0.2-0.253
Nickel (Ni)	7440-02-0	886		ALTA	0.03-78
Selenium (Se)	7782-49-2	166	17 (28)	2	2.0-290
Tin (Sn)	7440-31-5	277	1.4 (4)	50	0.5-101
Zinc (Zn)	7440-66-6	459	21 (99)	50	0.3-1690

Table 4.40: Preservatives, nitrosamines and phthalates presence in tattoo and PMU inks.

Chemical class	Substance	CAS nr	Number of analysed samples	% non compliant samples	Range (min-max) (mg/kg)
Phthalates	Dibutyl phthalate (DBP)	84-74-3	25		0.12-691.2
Phthalates	Di-(2-ethylhexyl) phthalate (DEHP)	117-81-7	11		0.2-19.3

B.9.3.2.2. Workers exposure

Not relevant for this Dossier.

B.9.3.2.3. Indirect exposure of humans via the environment

Not relevant for this Dossier.

B.9.3.2.4. Environmental exposure

Not relevant for this Dossier.

B.9.4. Other sources (for example natural sources, unintentional releases)

Not relevant for this Dossier.

B.9.5. Overall environmental exposure assessment

Not relevant for this Dossier.

B.9.6. Combined human exposure assessment

Not relevant for this Dossier.

B.10. Risk characterisation and derivation of concentration limits for chemical substances in tattoo inks and PMU

B.10.1. Manufacturing

Not relevant for this Dossier.

B.10.2. Use 1: Intra-dermal injection of tattoo inks

B.10.2.1. Human health

Quantitative risk assessments and derivation of DNELs were made for a number of threshold substances, such as substances toxic to the reproduction and selected impurities with other threshold effects. Some impurities and non-threshold substances were risk assessed in a semi-quantitative way with derivation of DMELs, primarily for the derivation of concentration limits but also for risk characterisation.

The remaining substances in the scope were assessed by a **qualitative** approach and the exposure assessment described in Annex B.9 was not applied numerically in the risk assessment.

According to ECHA guidance Part E (ECHA, 2016d) and R.8 (ECHA, 2012), a qualitative approach has to be chosen when no reliable dose descriptor (without identified thresholds) can be set for a given endpoint. In this proposal this applies to the effects skin irritation/corrosion, eye damage/eye irritation, sensitisation, and mutagenicity/carcinogenicity, with a few exceptions for substances for which a (semi-) quantitative approach was applied. The purpose of the qualitative risk assessment is to assess 'the likelihood that effects are avoided when implementing the exposure scenario…' as expressed in REACH Annex 1, Section 6.5.

> "6.5. For those human effects and those environmental spheres for which it was not possible to determine a DNEL or a PNEC, a qualitative assessment of the likelihood that effects are avoided when implementing the exposure scenario shall be carried out."

The exposure assessment indicates that significant exposure can occur and since these are non-threshold substances it cannot be excluded that risks to consumers can occur.

There is no single, standardised methodology for performing a qualitative assessment. The purpose of this qualitative risk characterisation is to assess the likelihood that these effects are avoided when receiving a tattoo. However, traditional operational conditions (OC) and risk managements measures (RMM), such as level of containment and use of personal protective equipment, do not have relevance to the intradermal injection of tattoo inks and PMU. This makes the hazard bands presented in ECHA Practical Guide 15 (ECHA, 2017c) and ECHA guidance Part E (ECHA, 2016) depending on the EU hazard classification unsuitable to apply as such. The only way to manage the risk in the case of receiving tattoos is to limit the presence of unwanted substances in the tattoo inks.

This use of a qualitative approach is consistent with the approach taken in REACH Annex XVII entries 28, 29 and 30 (restriction of substances classified as CMRs cat 1A and 1B to the general public, CL/SCL apply).

The Dossier Submitter therefore proposes that the substances should be restricted in tattoo inks based on the risk from exposure to substances classified with regard to skin irritation/corrosion, eye damage/ irritation, sensitisation, mutagenicity and carcinogenicity and with consideration to the exposure as described in Annex B.9, even if a quantitative risk assessment could not be performed. A total ban is not realistic, as this would ban tattooing as such, so the risk should be managed by setting concentration limits for the chemical substances in tattoo ink, as proposed in the chapter on risk management options (see 2.2).

The output of the quantitative assessment is a proposal for setting concentration limits for hazardous substances detected in tattoo ink.

The use of the approach in this dossier to base the restriction on classifications will ensure that substances classified in the future also will be restricted in tattoo inks and PMU.

For the substances assessed in a (semi-)quantitative manner, DN(M)ELs were derived and compared to the exposure assessment in the exposure scenario (see B.9). The DN(M)ELs were compared to the exposure from receiving a tattoo and the maximum content of each substance corresponding to where exposure is controlled to a risk level of low concern.

When the content of the substances in tattoo and PMU ink is limited to the proposed concentration limits described below, the risk from exposure described in the exposure scenario for tattoos is considered to be adequately controlled for threshold substances with a quantitative approach. For non-threshold substances, such as carcinogens, a cancer risk level of 10^{-6} could be seen as indicative tolerable risk level when setting DMELs for the general population and has been used by the Dossier Submitter ((ECHA, 2012) R. 8-14 Evaluating carcinogenicity risk levels).

The non-threshold critical effect of developmental neurotoxicity for lead is described in an opinion adopted by the ECHA Committee for Risk Assessment (RAC), as 0.05 µg Pb/kg bw per day as a maximum exposure value based on benchmark dose (BMD) approach (ECHA, 2011b). This value was use by the Dossier Submitter in the risk characterisation.

In the risk characterisation, the risk arising from current content in tattoo inks when applying the exposure scenario described in section B.9 has been compared with the derived DNELs described in section B.5.14 for selected substances. For non-threshold carcinogens, the risk arising from current content in tattoo inks when applying the exposure scenario has been compared with the cancer risk level of 10^{-6} (see Table 29 and Table 30).

Related to the discussion on concentration limits, two different restriction options (RO1 and RO2) are included in this restriction proposal. The two options differ mainly in terms of the

concentration limits proposed, with RO1 having much stricter limits for some substances that RO2 (for more detailed information see 2.3 and Annex D). The restriction options and concentration limits are presented in Table 27).

It should be noted that the concentration limit values arise from various sources, such as limits in CPR, CLP, CoE ResAP and concentration limits derived specifically for this restriction proposal. For substances covered by more than one concentration limit, the lower limit applies.

B.10.2.1.1. Workers

Not relevant for this Dossier.

B.10.2.1.2. Consumers

Qualitative risk characterisation and derivation of concentration limits

The following groups of substances proposed to be restricted in tattoo inks and PMU were assessed by a qualitative approach due to their hazard profile as predominantly non-threshold substances.

- Substances classified as eye irritant/damaging and skin irritant/corrosive
- Substances classified as skin sensitisers 1/1A/1B
- Substances classified as CM category 1A, 1B or 2, including PAHs

The following groups of substances can best be assessed in a qualitative manner in the context of this restriction, due to their restriction in the cosmetics regulation and based on the assumption that substances not allowed to be used in cosmetic products on the surface of the skin should also not be allowed to be injected into the skin:

- Substances on Annex II of the Cosmetics regulation (list of substances prohibited in cosmetic products).
- Substances on Annex IV to the Cosmetics regulation that are not allowed to be used in contact with mucous membranes, eyes or in prolonged contact with the skin (column "g") or subject to other conditions specified in columns "h" to "i" of the Annex (e.g., purity requirements).

Based on the harmonised classification and the conclusion that intradermal exposure poses at least the same or higher risk as dermal exposure, these substance groups are proposed to have the concentration limits as described in the text below.

Eye irritant/damaging and skin irritant/corrosive substances

The Dossier Submitter proposes under RO1 a practical concentration limit of 0.1% w/w to discourage intentional use and an alternative limit under RO2: the concentration limit for classification in a mixture as specified under CLP Regulation.

In CLP, the GCL for substances classified as Cat. 1: Irreversible effects on the eye (Eye Dam. 1) or Skin corr 1A/B/C is ≥ 3% in a mixture classified as Irrev Eye Effects 1 and ≥ 1% but <3% in mixtures classified as Cat. 2: Irritating to eyes (Eye Irrit. 2). The GCL for substances classified as Eye Effects 2 is ≥ 10% in a mixture classified as Rev Eye Effects 2.

In CLP, the GCL for substances classified as Skin Corr 1A/B/C is ≥5% in a mixture classified as Skin Corr 1 and ≥ 1% but < 5% in mixtures classified as Skin Irr 2. The GCL for substances classified as Skin Irr 2 is ≥ 10% in a mixture classified as Skin Irr 2.

In addition to this rules of addition apply. See page 290 and 316 in the CLP guidance on the application of the CLP criteria.

Skin sensitising substances

Induction as well as elicitation of contact allergy is dose-dependent and the threshold dose differs between different sensitizers. The threshold dose of a number of sensitizers has been investigated in human and animal test systems as well as in clinical studies of sensitized individuals. In most studies, the allergens are applied on the skin (epicutaneously), however it is known that if allergens are deposited into the dermis (intradermally), stronger reactions will occur and with lower doses. The limits established based on epidermal exposures cannot be used to set risk based limit values for tattoo inks, as even very small levels of allergens injected into the skin may pose a problem. For further details please consult the review "Allergy and Tattoos" (DEPA, 2017a).

The Dossier Submitter proposes under RO1 a practical concentrating limit of 0.1% w/w to discourage intentional use and under RO2: the generic and specific concentrations limits for classification in a mixture as specified under CLP Regulation. In CLP the generic concentration limit for skin sens 1 is 1.0%, for skin sens 1A 0.1% and for skin sens 1B 1.0%. Specific concentration limits are substance specific and lower than the generic limits.

Carcinogenic and mutagenic substances

Since carcinogenic and mutagenic substances eventually will be added to CPR Annex II, similar concentration limits (depending on the RO taken) should apply to at least category 1A/B. Therefore, under RO1, the Dossier Submitter proposes that tattoo inks and PMU shall not contain substances in category 1A/B. The same is proposed for category 2 carcinogenic and mutagenic substances under RO1.

For RO2, the Dossier Submitter proposes that the generic concentration limits (GCL) as well as the specific concentration limits (SCL) under CLP will be followed for the carcinogenic and mutagenic substances. The CLP GCLs are: 0.1% w/w for category 1A/B and 1% w/w for category 2.

Polyaromatic hydrocarbons (PAHs)

For the PAHs, under both RO1 and RO2, the Dossier Submitter proposes the same concentration limit for all PAHs with harmonised classification as CM as for the eight PAH substances in REACH Annex XVII, entry #50 (6), for toys and childcare articles, namely: Shall not contain more than 0.00005% w/w.

This approach is taken to be consistent with previous regulatory decisions. It should be noted that entry 50 is currently being reviewed and any changes to this limit should be reflected in this restriction.

CPR Annex II substances prohibited in cosmetic products

As stated in Appendix B.4, substances on Annex II are prohibited in cosmetic products; therefore, they are currently enforced at a limit of detection (LoD) by Member States with national legislation. As the justification for risk is based on conclusions that intradermal exposure is at least as risky as dermal exposure, the appropriate measure would be to restrict

these substances in the same way as under the CPR, i.e. tattoo inks shall not contain substances on annex II to the CPR (RO1).

The one disadvantage to this approach is that it would be difficult to differentiate between intentional and non-intentional use, which the CPR does effectively by allowing traces of prohibited substances if not intentionally added but found in cosmetic products, due to e.g., impurities or as a result of the manufacturing process. Therefore, the Dossier Submitter proposes a second restriction option (RO2), which allows small amounts of these substances, i.e., less than 0.1% w/w, in tattoo inks and PMU. The 0.1% w/w concentration limit is proposed as a practical limit aiming to discourage intentional use.

CPR Annex IV substances allowed in cosmetic products with restrictions

Following the same rationale for substances on Annex II, under RO1 it is proposed that those substances on Annex IV with specific use restriction (i.e., allowed in cosmetic products with restrictions on their use on mucous membranes or eye products, and allowed in rinse-off products only) are not allowed in tattoo inks and PMU.

Again, in order to allow the unintentional presence of small traces of these substances, a second restriction option is proposed – RO2 – with a practical limit of 0.1% w/w. It is worth noting that Annex IV substances are colourants and therefore, more likely to be found in tattoo inks and PMU only if intentionally added, although some exceptions are possible.

For the remaining 119 substances with conditions on their use in columns h and i of annex IV, it is proposed, under both RO1 and RO2, that those substances are also allowed in tattoo inks and PMU if the specified requirements for their use in columns h to i are met (e.g., for purity, constituents, concentration limits, particle size, etc.).

<u>(Semi-)quantitative risk characterisation and derivation of risk-based concentration limits</u>

The following groups of substances proposed to be restricted in tattoo inks and PMU were assessed or grouped by a (semi-) quantitative approach.

- Methanol (STOT SE)
- PAAs and azo colourants
- Substances toxic to reproduction (Repr. 1A/B and 2)
- Substances on Table 3 of the CoE ResAP(2008)1

General approach for derivation of risk-based concentration limits:

DN(M)ELs for the general population expressed as daily dose of the substance per kg bw were derived based on available information. The DN(M)ELs were compared to the exposure from receiving a tattoo and the maximum content of each substance corresponding to where exposure is controlled to a risk level of low concern was calculated:

The DN(M)EL expressed as mg/kg/d

Bodyweight 60 kg

Maximum Dose received in a tattoo session (RCR≤1) = DN(M)EL x 60 kg

For a single 300 cm^2 tattoo, 4 308 mg (14.36 mg ink/cm^2 x 300 cm^2) ink is injected.

The concentration limit (CL) becomes (maximum dose mg /4 308 mg) = X

X multiplied by 100% w/w = concentration limit in % w/w or by 10.000 ppm w/w = concentration limit in ppm w/w.

This can also be expressed in the following manner:

Exposure Scenario	
Tattoo Size	$300\ cm^2$
Amount of ink per cm^2	$14.36\ \frac{mg_{ink}}{cm^2}$
Amount of ink per kg bw (60 kg/person)	$72.00\ \frac{mg_{ink}}{kg_{bw} \times d}$
Amount of substance per kg bw	$72.00\ \frac{mg_{ink}}{kg_{bw} \times d} \times c_{substance}$
Concentration limit	
$c_{substance}$ shall result in RCR < 1	$RCR = \frac{Exposure}{DNEL} = \frac{72.00\ \frac{mg_{ink}}{kg_{bw} \times d}}{DNEL\left[\frac{mg_{substance}}{kg_{bw} \times d}\right]} \times c_{substance} < \mathbf{1}$
concentration limit ($c_{substance}$)	$c_{substance} < \frac{DNEL\left[\frac{mg_{substance}}{kg_{bw} \times d}\right]}{72.00\ \frac{mg_{ink}}{kg_{bw} \times d}}$

Methanol (STOT SE)

In the JRC report (JRC, 2015b), ethanol is reported to be used in high percentages in the formulation of inks in Germany: "ethanol seems to be used in high percentages (Germany reported a concentration of 48% while a concentration range of 10-30% was described by Canada)".

Since methanol is used as denaturing agent of ethanol (see annex III of Cosmetic Regulation) up to a concentration of 5%, in the worst case we could assume that a maximum concentration of 2.4% of methanol could be reached in the formulation of ink.

Using the previous assumptions where 4 308 mg of tattoo ink is injected that means at maximum this could entail 4 308 x 0.024 = 103.4 mg, which considering a 60 kg person means a maximum dose of 1.7 mg/kg bw. This exposure can be compared to the DNEL derived in section 5.14 (8 mg/kg bw/day). As methanol is soluble exposure is likely to be very rapid so this is likely to be within 1 day (worst case). Hence the RCR for methanol would be 0.22 and there is no risk presuming the assumptions on exposure are correct.

The general approach for derivation of risk-based concentration limits described above was used to derive a concentration limit of 10.9% w/w. This figure (equals RCR=1) has been applied for both RO1 and RO2.

Primary aromatic amines (PAAs) and azo colourants

PAAs:

For primary aromatic amines (PAAs), the $DMEL_{general\ population,\ carcinogenic\ effects}$ of 2×10^{-5} mg/kg bw/day for aniline (see Table 13) was the lowest of the derived DMELs. This DMEL was carried forward to the risk characterisation as the most sensitive DMEL and used to establish a general concentration limit for all PAAs. The general approach for derivation of risk-based concentration limits described above was then used to derive a concentration limit. This results in a risk-based concentration limit for PAAs in the ink of 0.00003% w/w (dissolved fraction) for each individual PAA. However, due to socio-economic reasons another CL is proposed in RO1 and RO2, see Annex D.

Azo colourants:

For the azo colourants a practical approach is chosen. A minimum concentration of azo colourants of 5-10 percent in the tattoo ink is normally required in order to be able to colour the skin. Thus, a practical limit of 0.1% will prevent the use of the azo colourants that are in the scope of the restriction, see Table 8. This limit is proposed for both RO1 and RO2.

Substances toxic to reproduction (Repr. 1A/B)

The approach is based on risk estimate of a group of 34 reprotoxic substances of diverse structures which currently are included in Annex VI and which are not also classified as carcinogen, mutagen or sensitiser. The lowest DNEL identified from the group of reprotoxins classified as category 1A/B is assumed sufficiently conservative to represent potential risks from all substances which will be classified as Repr. 1 A/B in the future but currently do not have a harmonised classification as reprotoxins (Cat. 1 A/B). To enable an equal regulation for reprotoxic substances classified currently or in future as Repro. 1A/B the Dossier Submitter proposes, as risk management option 1, a quantitative risk assessment approach based on an overall $DNEL_{general\ population,\ reproductive\ effects}$ which represents the relevant most critical DNEL derived within the group of currently known reprotoxic "only" substances (see section B.5.9).

As the presence of reprotoxic substances in tattoo inks and PMU as ingredient or impurity has not been analysed for most of the assessed substances, the actual risk of those cannot be demonstrated. However, concentration limits can be derived for reprotoxic substances as risk regarding reprotoxic effects has to be assumed if the content in tattoo inks or PMU products leads to a RCR > 1. The RCR for a substance is defined as the ratio between exposure level and DNEL (ECHA, 2016). Using this concept, the respective concentration limit in the ink can be derived using the total amount of tattoo ink injected into the skin in the relevant exposure scenario (see section B.9.) and the overall $DNEL_{general\ population,\ reproductive\ effects}$. The $DNEL_{general\ population,\ reproductive\ effects}$ of 0.001 mg/kg bw/d was derived and discussed in section B 5.11. The risk is considered to be controlled if the concentration of reprotoxic substances (Cat. 1A/1B) is lower than the concentration calculated in the table:

Table 24. Calculation of concentration limit for substances classified as Repr. 1A/1B

Exposure Scenario	
Tattoo Size	$300\ cm^2$
Amount of ink per cm^2	$14.36\ \frac{mg_{ink}}{cm^2}$
Amount of ink per kg bw (60 kg/person)	$72.00\ \frac{mg_{ink}}{kg_{bw} \times d}$
Amount of substance per kg bw	$72.00\ \frac{mg_{ink}}{kg_{bw} \times d} \times c_{substance}$
Concentration limit	
$c_{substance}$ shall result in RCR < 1	$RCR = \frac{Exposure}{DNEL} = \frac{72.00\ \frac{mg_{ink}}{kg_{bw} \times d}}{DNEL\left[\frac{mg_{substance}}{kg_{bw} \times d}\right]} \times c_{substance} < \mathbf{1}$
concentration limit ($c_{substance}$)	$c_{substance} < \frac{DNEL\left[\frac{mg_{substance}}{kg_{bw} \times d}\right]}{72.00\ \frac{mg_{ink}}{kg_{bw} \times d}}$
concentration limit ($c_{substance}$) for DNEL = 0.001 mg/kg bw /d	$\boldsymbol{c_{substance}} < \frac{0.001\ \frac{mg_{substance}}{kg_{bw} \times d}}{72.00\ \frac{mg_{ink}}{kg_{bw} \times d}} = 0.0000139 \frac{mg_{substance}}{mg_{ink}} = \mathbf{13.9}\ \boldsymbol{\frac{mg_{subst.}}{kg_{ink}}}$

According to calculations shown in the table, the proposed concentration limit for reprotoxic "only" substances (classified as Repr. 1 A/B without being simultaneously classified as carcinogen, mutagen or skin sensitiser) is 13.9 ppm (rounded off to 0.0014% w/w).

Reprotoxic substances classified in Category 2

It is proposed to extend the concept of 'one concentration for all reprotoxic substances classified as category 1A/B to include also reprotoxic substances of category 2 assuming that the most sensitive DNEL of 0.001 mg/kg and the concentration limit of 13.9 ppm will be conservative enough to cover also the risks from category 2 reprotoxins. Based on the fact that the generic concentration limit for Category 2 reprotoxic substances in mixtures is tenfold higher than for Category 1A/B reprotoxic substances, a pragmatic approach to include Category 2 substances and to consider the potentially lower/uncertain potency may be to apply a factor of 10 to the concentration limit of 13.9 ppm. Then the proposal for the concentration limit for Category 2 reprotoxicants would be 139 ppm (rounded off to 0.014% w/w).

Description of RO1

As restriction option 1 a quantitative approach is applied in which one concentration limit for all reprotoxic "only" substances classified as Repr. 1A/B and 2 (without being simultaneously classified as carcinogen, mutagen or skin sensitizer) is proposed. The proposed concentration limit for reprotoxic "only" substances under RO1 is 0.0014% w/w. The proposed concentration limit for Category 2 reprotoxicants under RO1 is 0.014% w/w.

Discussion of RO1

The proposed concentration limit for all reprotoxic substances based on the reprotoxic "only" substances Cat 1A/B is 0.0014% w/w (mg substance/kg ink). A potential risk regarding reprotoxic effects has to be assumed if the content in tattoo inks or PMU products exceeds this concentration limit as the RCR would be > 1. For RCR calculation the exposure scenario was applied as described in section B.9. Regarding the hazard assessment an overall $DNEL_{general\ population,\ reproductive\ effects}$ has been derived. This DNEL is based on individual hazard assessment of effects to reproduction of all classified Repr. 1 A/B "only" substances in Annex VI of the CLP regulation which do not have a simultaneous classification as carcinogen or mutagen or sensitizer. The lowest relevant DNEL derived (0.001 mg/kg bw/d) was considered as the overall $DNEL_{general\ population,\ reproductive\ effects}$ and was selected as DNEL for risk characterisation within restriction option 1 (RO1). The selected DNEL is considered to be conservative as the risk may be overestimated for most of the reprotoxic substances assessed (if DNEL > 0.001 mg/kg bw/d; see Table 14). However, a similar value was published in Bernauer et al. (2008) using a TTC for effects on reproduction based on 91 chemicals. Thus, applying this DNEL in the risk characterization of reprotoxic substances in tattoo inks is believed to protect for effects of most, if not all of the reprotoxic substances. The risk for TBT could be underestimated using RO1 as the DNEL (with some uncertainties) for this substance derived was lower than the $DNEL_{general\ population,\ reproductive\ effects}$.

This general approach using 'one concentration limit for all reprotoxic substances' is assumed to ensure adequate treatment of reprotoxic substances which will be classified in the future. If, instead, individual concentration limits for each reprotoxic "only" substances were estimated and included in the restriction options, this would cause the need for a continuous update of the restriction entries in the future based on substance-specific assessments for all newly classified substances. This does not seem to be feasible for practical reasons. Therefore, one concentration limit for all Repr. 1A/B is considered as the most appropriate risk management option for substances toxic to reproduction.

It is further proposed to extend the concentration limit for reprotoxic substances classified as Repro. 1A/B to include also reprotoxic substances of category 2.

The concentration limit of 0.0014% w/w will be conservative enough to cover also the risks from category 2 reprotoxicants. These should be included in the scope of the restriction either with the same concentration limit or (alternatively) with a 10 times higher concentration limit of 0.014% w/w based on the fact that the generic concentration limit for Category 2 in the CLP Regulation is 10 time higher than for Category 1A/1B.

Description of RO2

For restriction option 2 (RO2) a quantitative approach based on the generic concentration limit of 0.3% (3000 ppm, 3000 mg substance/kg ink) or, where available, the specific concentration limit set for the substance in Annex VI of the CLP Regulation is proposed. Hereby, two substances, dibutyl phthalate and bis(2-ethylhexyl)phthalate are proposed to be included with an individual limit concentration, as those substances have been found in tattoo inks and a risk

RCR ≥1 is expected at 0.3%. The individual limits are shown in Table 24 and were calculated using DNELs derived individually for each substance (see B.5.11).

Table 25: Individual concentration limits for dibutyl phthalate and bis(2-ethylhexyl) phthalate

Substance	**CAS**	**DNEL [mg/ kg bw/d)**	**Specific concentration limit (ppm) in CLP regulation**	**Individual limit concentration [ppm] with RCR 1**	**Individual limit concentration [% w/w] with RCR 1**
dibutyl phthalate	84-74-2	0.0067	no	93.1	0.0093
bis(2-ethylhexyl) phthalate	117-81-7	0.048	no	666.7	0.0667

Discussion of RO2

For practical reasons, the generic concentration limit of 0.3%, unless a specific concentration limit exists, as laid down in the CLP Regulation for reprotoxic substances (Cat 1 A/B and 2) is proposed as RO2 as concentration limit for reprotoxic substances in tattoo inks and PMU. However, the Dossier Submitter found that the risk will not be controlled for all substances toxic to reproduction in tattoo inks and PMU by applying this limit. Based on the exposure scenario described in section B.9., the GCL of 0.3% would result in a "limit" DNEL of 0.216 mg/kg bw/d, which, if exceeded, lead to a RCR > 1. Thus, for substances with DNELs < 0.216 mg/kg bw/d, the risk would not be controlled given the GCL of 0.3% in RO2. For the 14 reprotoxic "only" substances in Table 25 with DNELs < 0.216 mg/kg bw/d an individual limit concentration has been calculated.

Table 26: Substances for which risk is not adequately controlled using the GCL/SCL values (RCR>1)

Substance	**CAS**	**DNEL [mg/ kg bw/d)**	**Specific concentration limit (ppm) in CLP regulation**	**Individual limit concentration [ppm] with RCR 1**	**Individual limit concentration [% w/w] with RCR 1**
tributyltin chloride	-	0.000000053 - 0.00000031	no	0.001	0.0000001
(R)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone	5543-58-8 + 5543-57-7	0.001	≥30	13.9	0.0014
4-tert-butylbenzoic acid	98-73-7	0.0027	no-	37.5	0.0038
salts and esters of dinoseb	88-85-7	0.0033	no	45.8	0.0046
dibutyl phthalate	**84-74-2**	**0.0067**	**no**	**93.1**	**0.0093**
1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear	68515-50-4	0.0067	no	93.1	0.0093
(2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol	94361-06-5	0.0139	no	193.1	0.0193
trixylyl phosphate	25155-23-1	0.014	no	194.5	0.0195
2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate	15571-58-1	0.03	no	416.7	0.0417
bis(2-ethylhexyl) phthalate	**117-81-7**	**0.048**	**no**	**666.7**	**0.0667**
dihexyl phthalate	84-75-3	0.067	no	930.6	0.0931
ammonium 2-amino-4-(hydroxymethylphosphinyl) butyrate	77182-82-2	0.0175	no	243.1	0.0243
ketoconazole	65277-42-1	0.11	no	1527.8	0.1528

Using this approach for all currently known reprotoxic substances, including TBT, the risk would only be adequately controlled if the individual concentration limits were not exceeded. However, practicability is limited for reprotoxic "only" substances which will be classified in future. These will be automatically restricted in tattoo inks and PMU with proposed GCL of 0.3%. However, risks for substances with low DNELs may not adequately be controlled. The resulting RCR has to be checked for each substance, and if above 1, the entry should consider a substance-specific limit concentration. Thus, to ensure that for those substances the risk is controlled using the GCL a specific assessment would become necessary for each newly classified substance. This approach is not feasible according to RO2 as only those substances which have already been

found in tattoo inks, namely dibutyl phthalate and bis(2-ethylhexyl)phthalate (see Table 24) were foreseen to be included with individual limit values. For all additionally mentioned substances in Table 25, if occurring in tattoo inks at concentrations higher than their individual concentration limit, the risks are not adequately controlled. Once if a substance is known to be present in tattoo inks, the Annex XVII entry would need to be updated.

The generic concentration limit for Category 2 reprotoxic substances (3%) in mixtures is ten times higher than for Category 1A/B reprotoxic substances (0.3%). In case a Category 2 reprotoxic substance has been found in tattoo inks, the same problem holds true for these substances. If the RCR was above 1 at concentration lower than 3%, action would be needed to estimate an individual concentration limit that needs implementation in the entry.

Furthermore, a continuous follow-up on newly classified substances is required. These impracticabilities support that the proposal RO2 might not be feasible or, if the GCL is applied for future Cat 1A/B reprotoxic substances, RO2 may result in inadequately controlled risks for a number of substances.

Substances on Table 3 of the CoE ResAP(2008)1, impurities in tattoo inks and PMU

Industry consultations conducted during the development of the second CoE resolution (ResAP(2008)1) led to the recommendation to limit the concentration of selected impurities. The limits are demonstrated to be technically achievable as a large share of tattoo inks and PMU currently on the market in Member States with national legislation are compliant with them. For selected impurities – arsenic, barium, copper, lead, and zinc – the Dossier Submitter's risk assessment has suggested the need for different concentration limits than those recommended by ResAP(2008)1 (see Appendix B.6. Risk assessment of arsenic (As), Appendix B.7. Risk assessment of barium (Ba), Appendix B.8. Risk assessment of copper (Cu), Appendix B.10. Risk assessment of lead (Pb) and Appendix B.11. Risk assessment of zinc (Zn)). The general approach for derivation of risk-based concentration limits described above was used to derive concentration limits for these substances. For PAHs and BaP the CL in Annex XVII entry 50(6) is used (see above). For practicality purposes, the Dossier Submitter proposes to carry forward the limits in the CoE ResAP(2008)1 for the remaining substances on Table 3, except for nickel (Ni). In the case of Ni, surveillance/monitoring data from three member states (IT, DK , DE) indicate that the majority of the inks in which Ni was measurable contains Ni as impurity in an amount of 5 mg/kg or less. Due to the limited number of samples which were analysed in the monitoring programs, a concentration limit of 0.001 % w/w or less seems appropriate for Ni. This value does not take into account the sensitizing properties of Ni for which no threshold in the context of tattooing can be established due to lack of data.

The proposed and existing concentration limits are shown in Table 26. The proposed limits are the same for both RO1 and RO2.

Table 27 Proposed and existing concentration limits for substances on Table 3 of the CoE ResAP(2008)1

Substance	**Maximum allowed concentration of impurities in products for tattoos and PMU according to CoE ResAP(2008)1 Table 3**	**Concentration limit (RO1 & RO2)**
Element or compound	**ppm (% w/w)**	**% w/w**
Arsenic	2 (0.0002)	0.0000008
Barium	50 (0.005)	0.84*
Cadmium	0.2 (0.00002)	0.00002
Cobalt	25 (0.0025)	0.0025
Chromium (VI)	0.2 (0.00002)	0.00002
Copper (soluble)	25 (0.0025)	0.05*
Mercury	0.2 (0.00002)	0.00002
Nickel	As low as technically possible	0.001
Lead	2 (0.0002)	0.00007
Selenium	2 (0.0002)	0.0002
Antimony	2 (0.0002)	0.0002
Tin	50 (0.005)	0.005
Zinc	50 (0.005)	0.23*
Polycyclic aromatic hydrocarbons (PAH)	0.5 (0.00005)	0.00005[#]
Benzene-a-pyrene (BaP)	5 ppb (0.0000005)	0.00005[#]

* Soluble

[#]Based on a qualitative approach, the Dossier Submitter proposes the same concentration limit as in REACH Annex XVII, entry #50 (6), for toys and childcare articles, for all PAH substances with harmonised classification as CM

No quantitative or qualitative risk assessment has been carried out for selenium, antimony and tin (or their compounds) and no assessment is available to explain why these substances were originally included in the resolution. However, as national legislation in the relevant Member States has included these substances with the same concentration limits and the substances only appear as impurities then it was considered to be appropriate to include them[11].

[11] If stakeholders can justify different limits in the Public Consultation for these substances, then these can be taken into account in the discussions.

Overview of the proposed concentration limits for RO1 and RO2

Table 28. Concentration limits in RO1 and RO2

Substance group	**Concentration limit (% w/w)**	
	RO 1	**RO 2**
CPR Annex II	Shall not contain	0.1
CLP Carcinogenic 1a/b	Shall not contain	0.1
CLP Carcinogenic 2	Shall not contain	1
CLP Mutagenic 1/ab	Shall not contain	0.1
CLP Mutagenic 2	Shall not contain	1
CLP Reprotoxic 1a/b	0.0014	0.3¤
CLP Reprotoxic 2	0.014	3
CPR Annex IV (column g)	Shall not contain	0.1
CPR Annex IV (column h)	See Supplementary Table E	See Supplementary Table E
PAH with harmonised classifications as CM	0.00005	0.00005
PAA (dissolved fraction)	0.00003[#]	0.00003[#]
Azo dyes	0.1	0.1
CLP Skin sensitisers 1a	0.1	0.1
CLP Skin sensitisers 1, 1b	0.1	1
CLP Skin irritant & corrosive 1a/b/c, 2	0.1	1, 3, 5 or 10
CLP Eye irritant & damaging 1, 2	0.1	1, 3, 5 or 10
Methanol	10.9	10.9
Impurities (ResAP(2008)1 Table 3)		
- Cadmium	0.00002	0.00002
- Chromium**	0.00002	0.00002
- Mercury	0.00002	0.00002
- Copper*	0.05	0.05
- Zinc	0.23	0.23
- Barium*	0.84	0.84
- Nickel	0.001	0.001
- Selenium	0.0002	0.0002
- Antimony	0.0002	0.0002
- Lead	0.00007	0.00007
- Cobalt	0.0025	0.0025
- Arsenic	0.00000082	0.00000082
- Tin	0.005	0.005

*Soluble, **Chromium VI compounds, [#]A CL of 0.00005 % is proposed due to socio-economic reasons (see Annex D), ¤For certain Repr 1A/B specific CL are proposed, see Supplementary Table A.

Risk characterisation based on the measured content of selected substances in tattoo inks reported by JRC (JRC 2015b)

The source for data on content of substances in tattoo inks results from national surveys and market surveillance activities compiled by JRC (JRC, 2015b):

Table 29 Content of selected substances in tattoo inks (facsimile from JRC 2015b)

Table 4.38: PAAs presence in tattoo and PMU inks.

Substance	CAS nr	Number of analysed samples	% non compliant samples	ResAP (2008)1 limit (mg/kg)	Range (min-max) (mg/kg)
PAA (total)		3283	14 (468)		0.1-68
4-Aminoazobenzene	60-09-3			0	>0
Aniline	62-53-3			0	5-61
o-Anisidine	90-04-0	3655	10 (347)	0	0.52-2197

Table 4.39: Metals present in tattoo and PMU inks.

Substance	CAS nr	Number of analysed samples	% non compliant samples	ResAP (2008)1 limit (mg/kg)	Range (min-max) (mg/kg)
Antimony (Sb)	7440-36-0	932	7 (70)	2	0.02 - 147
Arsenic (As)	7440-38-2	1164	5 (62)	2	0.2-60
Barium (Ba)	7440-39-3	886	20 (180)	50	50-17737
Cadmium (Cd)	7440-43-9	1863	5 (93)	0.2	0.01-7.84
Cr (VI)	7440-47-4			0.2	0.3-147
Cobalt (Co)	7440-48-4	350	4 (14)	25	0.003-31310
Copper (Cu) soluble	7440-50-8	283	32 (90)	25	2.5-45000
Lead (Pb)	7439-92-1	2175	8.5 (195)	2	0.015-401.5
Mercury (Hg)	7439-97-6	809	2.5 (20)	0.2	0.2-0.253
Nickel (Ni)	7440-02-0	886		ALTA	0.03-78
Selenium (Se)	7782-49-2	166	17 (28)	2	2.0-290
Tin (Sn)	7440-31-5	277	1.4 (4)	50	0.5-101
Zinc (Zn)	7440-66-6	459	21 (99)	50	0.3-1690

Table 4.40: Preservatives, nitrosamines and phthalates presence in tattoo and PMU inks.

Chemical class	Substance	CAS nr	Number of analysed samples	% non compliant samples	Range (min-max) (mg/kg)
Phthalates	Dibutyl phthalate (DBP)	84-74-3	25		0.12-691.2
Phthalates	Di-(2-ethylhexyl) phthalate (DEHP)	117-81-7	11		0.2-19.3

The RCRs given in Table 29 were calculated from DNELs and information on the content of substances in tattoo ink. The risk levels given in Table 30 were calculated from DMELs and information on the content of substances in tattoo ink.

To calculate the risk characterisation ratio (RCR) for methanol, data on the ethanol concentration of 48% reported by JRC 2015b was used. As the maximum content of methanol used as denaturing agent of ethanol (see CPR Annex III) is 5%, the maximum concentration of methanol in tattoo ink is estimated to be 2.4%. This results in an RCR for methanol of 0.22. These calculations demonstrate that the currently known use of methanol in tattoo inks does not pose a risk. No risk is demonstrated for the use of methanol as impurity in tattoo inks.

It was not possible to calculate the RCR or a lifetime cancer risk comparison for azo colourants as such since no DN(M)EL were derived for these, but these may contain PAAs as impurities

from production or decomposition and are usually analysed for content of PAAs (see the table below).

For reprotoxic "only" substances classified as Repr. 1A/B, the DNEL was derived in a group approach. The content range of a single reprotoxic substance (dibutyl phthalate, DBP) reported in JRC was compared to this group DNEL and risk was demonstrated as the RCR could be as high as 50 (RO1).

The RCR for soluble barium was found to be in the range of 0.006-2.11. This could indicate a risk. However, it should be noted that most/all analytical methods cannot differentiate between soluble and insoluble barium (see further details in Appendix B.7. Risk assessment of barium (Ba)

For soluble copper, the RCR was calculated from a DNEL and the content range resulting in an RCR in the range of 0.005-90. A high risk could be demonstrated, but is questioned by the fact that not all analytical methods distinguish between soluble and solid copper.

No risk could be demonstrated for soluble zinc with RCR in the range of 0.018-0.73.

Table 30. RCRs for substances at various content ranges in tattoo inks

Substance	**Concentration limit (% w/w) (RO1 & RO2)**	**RCR**	**Content range (min-max) (mg/kg) (JRC 2015b)**	**Content range (min-max) (% w/w) (JRC 2015b)**	**RCR range (min – max)**
Methanol	10.9	1	-	2.4[a]	0.22
Azo colourants	0.1	N/A[b]	N/A	-	-
PAHs with harmonised classification as CM	0.00005	N/A	0.5 - 55000	0.00005 – 5.5	-
Reprotoxic substances 1A/B	0.0014 (RO1)	1	0.12 – 691.2 (DBP)	0.000012 – 0.07	0.009 - 50
Reprotoxic substances 2	0.014 (RO1)	1[c]	N/A	-	-
Barium	0.84	1	50 - 17737	0.005- 1.77	0.006 - 2.11
Cadmium	0.00002	N/A	0.01 – 7.84	0.000001 – 0.00078	-
Cobalt	0.0025	N/A	0.003 – 31310	0.0000003 – 3.13	-
Chromium (VI)	0.00002	N/A	0.3 – 147	0.00003 – 0.015	-
Copper (soluble)	0.05	1	2.5 – 45000	0.00025 – 4.5	0.005 - 90
Mercury	0.00002	N/A	0.2 – 0.253	0.00002 – 0.000025	-
Nickel	0.001	N/A	0.03 -78	0.000003 – 0.0078	-
Selenium	0.0002	N/A	2.0 - 290	0.0002 – 0.029	-
Antimony	0.0002	N/A	0.02 - 147	0.000002 – 0.015	-
Tin	0.005	N/A	0.5 – 101	0.00005 – 0.01	-
Zinc	0.23	1	0.3 - 1690	0.00003 – 0.17	0.018 - 0.73

[a]Estimated from ethanol concentration in JRC 2015b

[b]N/A = non applicable

[c]Estimated from RCR for Repr. 1A/B (10x)

RCRs could not be calculated for some of the substances in the table above because no DN(M)EL have been derived for these (PAHs, cadmium, cobalt, chromium (VI), mercury, nickel, selenium, antimony and tin). However the content range is given and can be compared to the proposed concentration limits. These substances are included in the CoE Table 3.

For PAAs, arsenic and lead, concentration limits were derived based on DMELs. In the table below, there is a comparison of risks based on the proposed CLs and measured content in tattoo inks reported by JRC (JRC, 2015b).

In a group approach, the lifetime cancer risk < 10^{-6} for PAAs is based on the DMEL for aniline expressed as a concentration limit of 0.00003%. The content range for aniline and total PAA is in the same order of magnitude; 5-61 and 0.1–68 mg/kg, respectively. This results in a high risk up to 2.27 x 10^{-4}.

For arsenic, the lifetime cancer risk < 10^{-6} was calculated from a DMEL and the content range resulting in a high risk up to 7.5 x 10^{-3}.

For lead, the extra risk of developmental toxicity was calculated from a DMEL and the content range resulting in risk >> RO1 and RO2, i.e. a high risk was demonstrated.

Table 31 Risk from exposure to PAAs, arsenic and lead at various content ranges in tattoo inks

Substance	**Concentration limit (% w/w) (RO1 & RO2)**	**Risk from RO1 and RO2**	**Content range (min-max) (mg/kg) (JRC 2015b)**	**Content range (min-max) (% w/w) (JRC 2015b)**	**Risk from content range (min – max)**
PAAs	0.00003	Cancer risk <10^{-6}	0.1 – 68 (total PAA)[a]	0.00001 – 0.0068	0.33 x 10^{-6} – 2.27 x 10^{-4}
Arsenic	0.0000008	Cancer risk <10^{-6}	0.2 - 60	0.00002 – 0.006	2.5 x 10^{-5} – 7.5 x 10^{-3}
Lead	0.00007	0.1% extra risk of developmental neurotoxicity at 0.05 µg Pb/kg bw per day ($BMDL_{01}$/10)	0.015 – 401.5	0.0000015 – 0.04	Up to >> Risk from RO1 and RO2

[a]5-61 mg/kg aniline

In conclusion, although no full quantitative analysis of the risks of all substances that are currently used in tattoo inks is possible, the available measured values for certain hazardous substances indicate that risks for human health cannot be excluded.

B.10.3. Indirect exposure of humans via the environment

Not relevant for this Dossier.

B.10.4. Combined exposure

Not undertaken for this Dossier although there might be other exposures to these substances.

B.10.5. Environment

Not relevant for this Dossier.

Appendix B.1. List of substances in the scope of the restriction

Appendix B.1. indicates the substances included in the restriction due to a qualitative or quantitative assessment. In addition, information on substance identity (CAS/EC number) is given along with the classification, if it is included in Annex II or IV of the Cosmetic Products Regulation, and if it has been found in tattoo inks. The information is presented in a separate Excel sheet named Appendix B.1. for ease of searching and analysis.

Appendix B.2. PAAs and azo colourants

Justification for the restriction of Primary Aromatic Amines and Azo colourants in tattoo inks

Introduction to azo colourants and PAAs

Chemically, a primary aromatic amine (PAA) consists of a nitrogen group (-NH_2) attached to an aromatic backbone (DEPA, 2017c). PAAs are used in the production of azo colourants. Azo colourants are widely used since in general they possess a high degree of chemical and photolytic stability. Azo colourants for tattoo inks consist of both pigments, which are synthesised from PAA's and other substances and lake pigments, where azo dyes are precipitated with appropriate cat- and anions.

Approximately 54% (67 in number) of the colourants used in tattoo inks and ink for permanent make-up (PMU) are azo colourants (JRC, 2015b). The other colourants are inorganic colourants, phthalocyanine etc. Azo colourants can provide almost all colours, but mainly red, yellow and orange azo colourants are used in tattoo inks (JRC, 2015b).

For comparison in relation to the significance of the various colours of the ink, it can be noted, that a survey performed by the Danish EPA showed, that among the Danish tattooed population, the following colours were most used in the tattoos: black (91%), red (29%), green (22%), blue (21%) and yellow (17%).

Further, for example, in total, 44 and 42 red colourants find application in tattoo and PMU inks, respectively. 66% and 60% of all red colourants in use in tattoo and PMU inks, respectively, belong to the class of monoazo colourants. The other classes being diazo, indigoid, xanthene, antraquinone, aminoketone, heterocycle and natural (JRC, 2015b).

Since the PAAs are used in the production of azo colourants, the PAAs might be present in the final colourant as non-reacted impurities. In some cases, additional PAAs may be added to an azo colourant for achieving a specific nuance of a colour (JRC, 2015b) (DEPA, 2012). However, the addition of PAAs to achieve a specific nuance of a colour would probably require a higher concentration than 0.1-0.2%, which is the highest concentration level observed in tattoo inks.

Degradation of azo colourants can generate PAAs. Azo colourants can be degraded by irradiation: sunlight or laser (JRC, 2015b). Enzymatic degradation or bacterial degradation has also been shown (Sudha, et al., 2014) (Chacko & Subramaniam K, 2011).

Even though the azo colourants used in tattoo inks are considered to be insoluble in water and deposited in the derma as microcrystalline grains, equilibrium will always to some extent exist

between the solid phase and small amounts of colourants dissolved in the lymph fluid constantly circulating in the body. Therefore, it cannot be excluded that to a certain degree dissolved azo colourant molecules may be available for metabolic decomposition when situated in the skin – or in the liver after release into the bloodstream (JRC, 2015b).

In general, colourants used in tattoo inks are not produced for the purpose of tattooing, but normally by the chemical industry for outdoor applications in products like textiles, paints for cars and plastics, because they show good light resistance (general resistant to fading when exposed to light). For these applications, sterility is not necessarily required and biological degradation can happen during storage, which may result in the formation of PAAs in the ink before use (DEPA, 2017c).

PAAs

PAAs identified in tattoo inks:

According to the review performed by (JRC, 2015b), 13 primary aromatic amines (PAAs) have been identified in tattoo inks (before application to the skin) placed on the market in several Member States. These are listed in Table 31.

Table 32. PAAs found in tattoo and PMU inks in the last years in surveillance campaigns in Europe (JRC, 2015b). The concentrations in a significant number of these samples were above the recommended levels in the CoE ResAP(2008)1 which is the technical zero level (detection limit). The analytical method was not described in the reference.

CAS no.	Primary Aromatic Amine	No. of analysis	Percentage of samples with concentrations above the detection limit (%)
90-04-0	o-Anisidine*	3655	10
95-53-4	o-toluidine*	3675	5
91-94-1	3,3'-dichlorobenzidine*/**	3647	2.4
95-80-7	4-methyl-m-phenylendiamine*/**	3516	2.5
106-47-8	4-chloroaniline*/**	2958	2
99-55-8	5-nitro-o-toluidine*	2129	1.2
119-90-4	3,3'-dimethoxybenzidine*	827	0.5
139-65-1	4,4'-thiodianiline*	100	1
95-68-1	2,4-xylidine	120	1
106-50-3	p-Phenylenediamine*/**	29	3
119-93-7	4,4-bi-o-toluidine*	829	0.1
95-69-2	4-chloro-o-toluidine*	43	1
91-59-8	2-naphthylamine*	19	1.2

Explanations to the table: * = Harmonised classification as carcinogenic and ** = Harmonised classification as skin sensitiser. The harmonized classifications for the PAAs are summarized in Table 4 and Table 36.

Furthermore, relative high amounts of aniline, another PAA, have been detected in another more limited study in 19 out of 32 samples, i.e. 60% (DEPA, 2012). Aniline has a harmonised classification as a carcinogen. Thus, in total 14 PAAs have been identified in tattoo and PMU inks on the European market.

The PAAs have been identified in tattoo and PMU inks with test methods both with and without an attempt to perform a reductive cleavage of any parent azo colourant in the tattoo ink.

13 of the 14 PAAs found in tattoo and PMU inks have a harmonised classification as carcinogenic. 3,3'-dichlorobenzidine, 4-Methyl-m-phenylendiamine, 4-chloroaniline and P-Phenylenediamine (PPD) also have harmonised classifications as skin sensitiser.

It is suggested that the 13 carcinogenic PAAs detected in tattoo and PMU inks are included in the scope of the restriction proposal (see Table 36).

PAAs from decomposition of azo colourants used in tattoo inks

JRC (JRC, 2015b) has identified 67 azo colourants that are used in tattoo inks and inks for PMU. These 67 azo colourants have - based on their chemical structures - been analysed with respect to a theoretical decomposition by cleavage of the azo bond and amide hydrolysis (DEPA, 2017c). The result of the analysis shows that 24 PAAs may theoretically occur in tattoo and PMU inks due to cleavage of the azo bond, as an impurity from the production of the azo colourant, or due to amide hydrolysis (DEPA, 2017c). The 24 PAAs are listed in Table 32.

Table 33. The 24 PAAs that theoretically may occur in tattoo inks, due to the 67 azo colourants applied in tattoo inks and inks for PMU. The light grey colour indicates the decomposition both via amide hydrolysis and reductive cleavage and dark grey colour indicate only decomposition by amide hydrolysis (DEPA, 2017c).

CAS number	Primary Aromatic Amine	Number of samples where the substances have been detected	Number of inks that may decompose to the PAA via reductive cleavage	Number of inks that may decompose to the PAA via amide hydrolysis
91-94-1	3,3'-dichlorobenzidine*/**[F]	88 (2.4%)	8	
92-87-5	Benzidine*	0	1	
119-90-4	3,3'-dimethoxybenzidine*[F]	4 (0.5%)	2	
2835-68-9	4-aminobenzamide	0	2	
95-69-2	4-chloro-o-toluidine*[F]	1 (0.4%)	1	1
99-55-8	5-nitro-o-toluidine*[F]	0	3	
90-04-0	o-anisidine*[F]	366 (10%)	2	6
95-53-4	o-toluidine*[F]	184 (5%)	0	5
106-49-0	p-toluidine*/**	0	0	1
95-82-9	2,5-dichloroaniline	0	3	
99-59-2	2-amino-4-nitroanisole	0	4	
636-30-6	2,4,5-trichloroaniline	0	1	
62-53-3	Aniline*/**[F]	19 (60%)	0	8
118-92-3	anthralinic acid	0	2	
95-74-9	3-chloro-p-toluidine	0	1	
88-17-5	α,α,α-trifluoro-o-toluidine	0	1	
121-50-6	6-chloro-α,α,α-trifluoro-m-toluidine	0	1	
89-63-4	4-chloro-2-nitroaniline	0	6	
2834-92-6	1-amino-2-naphthol	0	7	
88-44-8	4-Aminotoluene-3-sulfonic acid	0	2	

81-16-3	2-Amino-1-naphthalenesulfonic acid	0	1	
84-86-6	4-Amino-1-naphthalenesulfonic acid	0	2	
121-57-3	4-Aminobenzenesulfonic acid**	0	6	
7248-98-8	5-amino-6-hydroxy-2-naphthalenesulfonic acid	0	1	

Explanations to the table: * = Compounds classified as carcinogenic; ** = Compounds classified as skin sensitiser and [F] = Found in tattoo inks on the market. The harmonized classifications for the PAAs are summarized in Table 4 and Table 36.

Nine of the 24 PAAs in Table 32 have a harmonised classification as carcinogenic and four have harmonised classifications as skin sensitiser of which 3 have both harmonised classification as carcinogenic and skin sensitiser.

Seven of the 24 PAAs have been found in tattoo inks and inks for PMU on the market (marked with F in the table). Thus, 50% (7 out of 14) of the PAAs found in tattoo inks can be explained by decomposition of azo colourants known to be used in tattoo inks and inks for PMU. All seven have a harmonized classification as carcinogenic.

It is suggested that the sensitising 4-Aminobenzenesulfonic acid and the 9 carcinogenic PAAs in Table 32 are included in the scope of the restriction proposal (see also Table 36).

PAAs from azo colourants in the CoE ResAP(2008)1

In the CoE ResAP(2008)1, 35 colourants have been included in table 2 in the resolution. The resolution recommends not using these colourants. The relevant background documents for the inclusion of these colourants in the resolution are not available. However, it is assumed that previously, these colourants were used in tattoo inks. Since the CoE ResAP(2008)1 has not been legally binding unless implemented in the legislation by a few Member States - and despite that some producers in other countries may have chosen voluntarily to follow the resolution, it is possible that there may still be inks on the market that contain these colourants. Seventeen of these colourants are azo colourants (DEPA, 2017c). Thus, an assessment of the decomposition products of the 17 azo colourants is relevant as the colourants may still be used in some tattoo inks and inks for PMU.

The results from the assessment show that to the extent the 17 azo colourants decompose, the 13 PAAs listed in Table 33, 8 may be formed (DEPA, 2017c). The investigation also concludes that the azo colourants in table 2 of the CoE ResAP(2008)1 do not decompose via amide hydrolysis (DEPA, 2017c).

Table 34. PAAs formed from decomposition of the azo colourants from the negative list in the CoE ResAP(2008)1.

CAS no.	Primary Aromatic Amine
95-68-1	2,4-dimethylaniline[F]
100-01-6	4-nitroaniline
88-53-9	2-Amino-5-chloro-4-methylbenzenesulfonic acid
121-47-1	3-Aminobenzenesulfonic acid
95-84-1	2-amino-p-cresol
106-50-3	p-Phenylenediamine*/**[F]
95-70-5	2,5-Diaminotoluene, 2-Methyl-benzene-1,4-diamine**
99-30-9	2,6-dichloro-4-nitroaniline
97-02-9	2,4-Dinitroaniline
95-53-4	o-toluidine*[F]
62-53-3	Aniline*/**[F]
2834-92-6	2-Amino-5-chloro-4-methylbenzenesulfonic acid

Explanations to the table: * = Compounds classified as carcinogenic, ** = Compounds classified as skin sensitiser and [F]= Found in tattoo inks on the market. The harmonized classifications for the PAAs are summarized in Table 4 and Table 36.

Of the 13 PAAs listed in Table 33, 3 PAAs have a harmonised classification as carcinogenic. Two of these, aniline and o-toluidine, have been found in tattoo inks and/or in inks for PMU. Both aniline and o-toluidine may also be formed due to amide hydrolysis of other azo colourants theoretically occurring in tattoo inks and inks for PMU (see Table 32).

It is suggested that the three PAAs identified as decomposition products from the azo colourants listed in the CoE ResAP(2008)1 with a harmonised classification as carcinogenic are included in the scope of the restriction proposal (see Table 36).

PAAs restricted in REACH

REACH Annex XVII (entry 43, appendix 8) lists 22 PAAs. All 22 PAAs have a harmonised classification as carcinogenic. If an azo colourant decomposes to one of these PAAs, the azo colourant is restricted in textiles and leather. These 22 PAAs are also suggested to be included in the scope of this restriction. Even though these substances have not all been found in tattoo inks and inks for PMU on the market, or could be expected to be found due to the structure of known azo colourants currently used, they are relevant to include to prevent substitution to other azo colourants that may also decompose to these PAAs.

Table 35. REACH Annex XVII entry 43 appendix 8.

CAS no.	Primary Aromatic Amine
92-67-1	Biphenyl-4-ylamine*
92-87-5	Benzidine*
95-69-2	4-chloro-o-toluidine*[F]
91-59-8	2-naphthylamine*[F]
97-56-3	4-o-tolylazo-o-toluidine*
99-55-8	5-nitro-o-toluidine*[F]
106-47-8	4-chloroaniline*[F]
615-05-4	4-methoxy-m-phenylenediamne*
101-77-9	4,4'-methylenedianiline*
91-94-1	3,3'-dichlorobenzidine*[F]
119-90-4	3,3'-dimethoxybenzidine*[F]
119-93-7	3,3'-dimethylbenzidine*[F]
838-88-0	4,4'-methylenedi-o-toluidine*
120-71-8	6-methoxy-m-toluidine*
101-14-4	4,4'-methylenebis[2-chloroaniline] (MOCA)*
101-80-4	4,4'-oxydianiline*
139-65-1	4,4'-thiodianiline*[F]
95-53-4	o-toluidine*[F]
95-80-7	4-methyl-m-phenylenediamine (toluene-2,4-diamine)*[F]
137-17-7	2,4,5-trimethylaniline*
90-04-0	o-Anisidine* [F]
60-09-3	4-Aminoazobenzene*

Explanations to the table: * = Compounds classified as carcinogenic and [F]= Found in tattoo inks on the market. In this table skin sensitisation has not been marked. The harmonized classifications for the PAAs are summarized in Table 4 and Table 36.

Ten of the 22 PAAs listed in REACH Annex XVII (entry 43 appendix 8) have been identified in tattoo inks and inks for PMU (see Table 34).

It is suggested that the 22 PAAs in Table 32 are included in the scope of the restriction proposal (see Table 36).

PAAs in the CoE ResAP(2008)1

The CoE ResAP(2008)1 has listed 27 PAAs in Table 1 of the resolution, which is recommended neither should be present in tattoo inks or PMU products nor released from azo colourants.

The relevant background documents for the inclusion of these PAAs in the resolution are not available. However, it is assumed that previously, there must have been a reason for assuming that these PAAs might have been present in tattoo inks. Since the CoE ResAP(2008)1 has not been legally binding unless implemented in the legislation by a few Member States - and despite that some producers in other countries may have chosen voluntarily to follow the resolution, it is possible that there may still be inks on the market that contain the PAAs.

Except for 4 PAAs there is an overlap with those PAAs from Table 31 to Table 34 that have been identified for the scope of this restriction. The 4 substances are listed in the table below.

Table 36. PAAs in the table 1 of the CoE ResAP(2008)1 not already identified for the scope of this proposal (Table 31 to Table 34).

CAS no.	Primary Aromatic Amine	Description
293733-21-8	6-amino-2-ethoxynaphthaline	Neither found in inks on the market nor harmonised classification
399-95-1 (EC no. 402-230-0)	4-amino-3-fluorophenol* and **	Harmonised classification as Carc 1B and skin sens
95-68-1	2,4-xylidine[F]	Found in inks on the market, but no harmonised classification
87-62-7	2,6-xylidine*	Harmonised classification as Carc 2

Explanations to the table: [F]= Found in tattoo inks on the market. * = Compounds classified as carcinogenic; ** = Compounds classified as skin sensitiser. The harmonized classifications for the PAAs are summarized in Table 4 and Table 36.

Based on the lack of harmonised classification, only 4-amino-3-fluorophenol and 2,6-xylidine qualifies to be in the scope of the restriction due to it classification as both carcinogenic and sensitiser and carcinogenic respectively.

Scope of restriction proposal for the PAAs

Table 36 provides an overview of the identified PAAs with a harmonised classification as carcinogenic or as skin sensitising, that:

- have been found in tattoo inks or inks for PMU on the market (13 PAAs);
- may be present in tattoo inks due to either cleavage of azo bond or amide hydrolysis of an azo colourant used in tattoo inks or originate from the production of the azocolourants used in tattoo inks (10 PAAs);
- may be present in tattoo inks due to reductive cleavage of azo bond of one of the azo colourants listed in the CoE ResAP(2008)1 (3 PAAs); or
- may be present in tattoo inks either due to reductive cleavage of azo bond or due to Amide hydrolysis of one of the azo colourants restricted in Annex XVII entry 43 of REACH in various textiles (22 PAAs).
- may be present in tattoo inks due to the listing in the CoE ResAP(2008)1 (25 PAA)

This leads to in total 29 different PAAs that are listed in Table 36 and suggested to be considered within the scope of the current restriction proposal.

Table 37. The PAA in the scope of the restriction and harmonised classifications

	CAS no.	**Primary Aromatic Amine**	**Carc.**	**Muta.**	**Skin sens.**
1	90-04-0	o-Anisidine	1B	2	
2	95-53-4	o-toluidine	1B		
3	91-94-1	3,3'-dichlorobenzidine	1B		1
4	95-80-7	4-methyl-m-phenylendiamine	1B	2	1
5	106-47-8	4-chloroaniline	1B		
6	99-55-8	5-nitro-o-toluidine	2		
7	119-90-4	3,3'-dimethoxybenzidine	1B		
9	119-93-7	4,4’-bi-o-toluidine	1B		
8	139-65-1	4,4'-Thiodianiline	1B		
10	95-69-2	4-chloro-o-toluidine	1B	2	
11	91-59-8	2-naphthylamine	1A		
12	62-53-3	Aniline	2	2	1
13	92-87-5	Benzidine	1A		
14	106-49-0	p-toluidine	2		1
15	95-70-5	2-methyl-p-phenylenediamine			1
16	92-67-1	Biphenyl-4-ylamine	1A		
17	97-56-3	4-o-tolylazo-o-toluidine	1B		1
18	615-05-4	4-methoxy-m-phenylenediamne	1B	2	
19	101-77-9	4,4'-methylenedianiline	1B	2	1
20	838-88-0	4,4'-methylenedi-o-toluidine	1B		1
21	120-71-8	6-methoxy-m-toluidine	1B		
22	101-14-4	4,4'-methylenebis[2-chloroaniline]	1B		
23	101-80-4	4,4'-oxydianiline	1B	1B	
24	137-17-7	2,4,5-trimethylaniline	1B		
25	60-09-3	4-Aminoazobenzene	1B		
26	106-50-3	p-Phenylenediamine			1
27	121-57-3	Sulphanilic acid			1
28	399-95-1	4-amino-3-fluorophenol	1B		1
29	87-62-7	2,6-xylidine	2		

Also note that some of these PAAs are listed in Annex II of the CPR.

Azo colourants

Since azo colourants may decompose to PAAs, it is relevant to investigate if the release of carcinogenic PAAs from specific azo colourants can be predicted as these would be relevant to include in the scope of the restriction proposal.

Two main decomposition routes are proposed here, either biologically (amide hydrolysis) or by photo-decomposition.

Further, scientific evaluations and harmonized classification have been taken into account.

Azo colourants decomposing to carcinogenic PAAs

O-anisidine, o-toluidine and aniline are the most frequently observed PAAs in tattoo and PMU inks, as can be seen from Table 32 (extracted from (DEPA, 2017c) and which was also observed in the survey by the Danish EPA (2012). Furthermore, these 3 PAAs are observed in relative high concentrations. Since these 3 PAAs are all expected to be formed either partly or only from amide hydrolysis, the data in Table 32 strongly suggest that amide hydrolysis of azo colourants is the major source of PAAs. It should also be noted that amide hydrolysis most likely is biologically mediated.

Furthermore, 68% of the theoretical predicted PAAs from reductive cleavage of the azo bond, which are expected to be in tattoo and PMU inks based on the used azo colourants (JRC, 2015b), are clearly absent in investigated inks on the market. Whereas, for the amide hydrolysis only 5% of the theoretical predicted PAAs are absent. This also supports the presumption of amide hydrolysis as being the dominant decomposition mechanism.

Additionally, as noted previously 50% of the observed PAAs cannot be explained by either azo cleavage or amide hydrolysis. This indicates that besides the amide hydrolyses another mechanism may be involved.

It should be noted that if other azo colourants are in use than those identified by (JRC, 2015b), this might also explain the presence of the PAAs. However, the lack of explanation for 50% of the observed PAAs shows that there is still room for further research/analysis. Nevertheless, the azo colourants giving rise to these PAAs will be restricted via the restriction on the PAAs.

In general the azo bond is the weakest (most reactive) bond in the azo colourant molecule (DEPA, 2017c). However, it is unlikely that cleavage of the azo bond - as a default - can account for the PAAs observed in the inks. And since it is likely that the cleavage of the azo bond doesn’t take place for many of the colourants, it does not seem justified to include specific azo colourants based on the argumentation of this decomposition route.

Due to these findings, it is proposed that for the moment only the specific azo colourants used in tattoo and PMU ink, which can decompose via amide hydrolysis to form PAAs classified as carcinogenic, are included in the scope of the restriction.

In general, azo colourants with simple structures and low molecular weight exhibit higher rates of degradation and decomposition than high molecular weight compounds. Further, mono azo colourants are less stable than di azo colourants. Electron withdrawing groups such as SO_3H or SO_2NH_2 attached to the phenyl ring also increases the stability of the azo bond and azo colourants with hydroxyl groups are less stable compared to methyl, methoxy, sulpho or nitro groups attached to the phenyl ring (Environment Canada, 2012). However, possible correlations based on this information have not been investigated.

Thus, the 21 azo colourants in the table below that based on amide hydrolysis can decompose to form PAAs classified as carcinogenic are suggested to be included in the scope of the restriction (see Table 41).

Table 38. Specific azo colourants in the scope of the restriction based on possible amide hydrolysis.

CAS No	**CI no.**	**CI name**
6471-51-8	12420	Pigment Red 7 (PR7)
6410-38-4	12460	Pigment Red 9 (PR9)
6410-39-5	12465	Pigment Red 15 (PR15)
61932-63-6	12477	Pigment Red 210 (PR210)
85776-14-3	No CI no.	Pigment Orange 74 (PO74)
6528-34-3	11740	Pigment Yellow 65 (PY65)
6358-31-2	11741	Pigment Yellow 74 (PY74)
6410-32-8	12385	Pigment Red 12 (PR12)
6471-50-7	12380	Pigment Red 14 (PR14)
6655-84-1	12390	Pigment Red 17 (PR17)
6535-46-2	12370	Pigment Red 112 (PR112)
5468-75-7	21095	Pigment Yellow 14 (PY14)
6358-37-8	21096	Pigment Yellow 55 (PY55)
6041-94-7	12310	Pigment Red 2 (PR2)
6448-95-9	12315	Pigment Red 22 (PR22)
5280-68-2	12485	Pigment Red 146 (PR146)
67990-05-0	12466	Pigment Red 269 (PR269)
6505-28-8	21160	Pigment Orange 16 (PO16)
2512-29-0	11680	Pigment Yellow 1 (PY1)
6358-85-6	21090	Pigment Yellow 12 (PY12)
12225-18-2	11767	Pigment Yellow 97 (PY97)

Note that none of the 17 azo colourants from table 2 in the CoE ResAP(2008)1 have shown to form PAAs by amide hydrolysis.

Photo-decomposition of azo colourants

Only limited research has been performed on the photo-decomposition of azo colourants. Hauri and Hohl (Hauri & Hohl, 2015) investigated the photo-decomposition of azo colourants in tattoo inks. An in vitro method simulating solar irradiation of a pigment in the skin was developed. The exposed colourants and the results are listed in the table below.

Table 39. Simulation of solar irradiation of pigment in the skin (Hauri & Hohl, 2015).

CAS No	CI no.	CI name	Decomposition product with different light sources	Decomposition product with laser
6410-38-4	12460	Pigment Red 9 (PR9)	Not investigated	2,5-dichloroaniline1,4-dichlorobenzene Methoxy-naphthol AS
6358-31-2	11741	Pigment Yellow 74 (PY74)	o-acetoacetanisidide (CAS no. 92-15-9) 2-(hydroxyimine)-N-(2-methoxyphenyl)-3-oxobutanamide (CAS not identified) N,NO-bis(2-methoxyphenyl)urea (CAS no.1226-63-7) 2-methoxyacetanilide (CAS not identified)	None identified
6535-46-2	12370	Pigment Red 112 (PR112)	2-toluidine (CAS no. 95-53-4) 2,4,5-trichloroaniline (CAS no. 636-30-6) 2-methyl formanilide (CAS no. 94-69-9) 2-methylacetanilide (CAS no. 120-66-1)	2-toluidine (CAS no. 95-53-4) 2,4,5-trichloroaniline (CAS no. 636-30-6)
5468-75-7	21095	Pigment Yellow 14 (PY14)	2'-methyl formanilide (CAS no. 94-69-9) 2-methylacetanilide (CAS no. 120-66-1) 3,3'-dichlorodiphenyl (CAS no. 2050-67-1 or 55600-34-5) 3,3'-dichlorobenzidine (CAS no. 91-94-1)*	3,3'-dichlorobenzidine (CAS no. 91-94-1)*
6448-95-9	12315	Pigment Red 22 (PR22)	2-amino-4-nitrotoluene (CAS no. 99-55-8) 4-nitrotoluene (CAS no. 99-99-0)	2-amino-4-nitrotoluene (CAS no. 99-55-8) 4-nitrotoluene (CAS no. 99-99-0) Naphtanol AS
6505-28-8	21160	Pigment Orange16 (PO16)	Formanilide (CAS no. 103-70-8) Acetanilide (CAS no. 103-84-4) 3,3'-dichlorodiphenyl (CAS no. 2050-67-1 or 55600-34-5)	None identified
12225-18-2	11767	Pigment Yellow 97 (PY97)	None identified	Aniline CAS no. 62-53-3)*
	12475	Pigment Red 120 and 170 (PR120 and PR 170)	Benzamide (CAS no. 55-21-0) 4-hydroxybenzamide (CAS no. 619-57-8) 4-aminobenzamide (CAS no. 2835-68-9)	Not investigated
5567-15-7	21108	Pigment Yellow 83 (PY83)	Not investigated	3,3'-dichlorobenzidine (CAS no. 91-94-1)*
3520-72-7	21110	Pigment Orange 13 (PO13)	3,3'-dichlorobenzidine (CAS no. 91-94-1)* 3,3'-dichlorodiphenyl(CAS no. 2050-67-1 or 55600-34-5)	3,3'-dichlorobenzidine (CAS no. 91-94-1)* Aniline CAS no. 62-53-3)*

15793-73-4	21115	Pigment Orange 34 (PO34)	3,3'-dichlorobenzidine (CAS no. 91-94-1)*	Not investigated

* PAAs in the scope of the restriction

Looking only at UV radiation, the study shows that 6 out of 9 decompose into the PAA expected based on the structural analysis. Three of the azo colourants investigated with UV radiation (PY14, PO13 and PO34) are all based on 3,3'-dichlorobenzidine and all decompose by cleavage of the azo bond. It is thus likely that all azo colourants based on 3,3'-dichlorobenzidine may decompose to 3,3'-dichlorobenzidine when exposed to UV radiation. To support this assumption PY83, which is also based on 3,3'-dichlorobenzidine, also decomposes to 3,3'-dichlorobenzidine when exposed to laser.

In addition, tests with Pigment Orange 13 and Pigment Orange 34 showed that already after 0.5 hour of irradiation under daylight containing UVA, both colourants underwent strong photo degradation to the 3,3'-dichlorobenzidine, which have a harmonized classification as carcinogenic.

However, the study showed that 3,3'-dichlorobenzidine and aniline could not be detected in the in-vivo studies, which indicated that the photodecomposition does not take place in the skin.

Despite the lack of decomposition in the in-vivo study, it is proposed that all azo colourants based on 3,3'-dichlorobenzidine are restricted. For the other azo colourants it is difficult to draw general conclusions based on the study presented by Hauri and Hohl (Hauri & Hohl, 2015). Thus, the study by Hauri and Hohl (Hauri & Hohl, 2015) does not in general justify the inclusion of all azo colourants into the scope of the restriction due to possible photo degradation.

Thus, these 7 azo colourants indicated in the table below are suggested to be included in the scope of the restriction (see Table 41). Some of them are already included in the scope due to amide hydrolysis.

Table 40. Azo colourants suggested to be included in the scope of the restriction proposal due to photo degradation into carcinogenic PAAs.

CAS No	CI no.	CI name
3520-72-7	21110	Pigment Orange 13 (PO13)
15793-73-4	21115	Pigment Orange 34 (PO34)
5468-75-7	21095	Pigment Yellow 14 (PY14)
6358-85-6	21090	Pigment Yellow 12 (PY12)
6358-37-8	21096	Pigment Yellow 55 (PY55)
5567-15-7	21108	Pigment Yellow 83 (PY83)
15110-84-6	21107:1	Pigment Yellow 87 (PY87)

Review by SCCP on Azo colourants in Cosmetic Products

In 2002, the SCCP was asked to review four azo colourants, CI 12150, CI 20170, CI 26100 and CI 27290 (SCCNFP/0495/01, final).

The SCCP concluded on the use of these 4 colourants in cosmetic products: The colourants CI12150, CI20170, CI27290 and CI 26100 and other azo colourants which may release one or more carcinogenic aromatic amines, pose a risk to the health of consumers.

Of the four specific mentioned azo colourants, only CI 12150 (Solvent Red 1 (SR1)) is used in tattoo and PMU inks according to (JRC, 2015b). However, all four colourants are suggested to be restricted in tattoo inks and PMU to avoid unwanted substitution (see Table 41).

Azo colourants in table 2 of the CoE ResAP(2008)1

In order to avoid unwanted substitution, the harmonized classification of the azo colourants in the CoE ResAP(2008)1 is taken into account. This classification is summarized in the table below.

Table 41. The harmonised classification of the azo colourants restricted in the CoE ResAP(2008)1.

CI Name	CAS no.	CI no.	Chemical class	Harm. Class.	In Annex II of the CPR
Acid Red 26 (AR 26)	3761-53-3	16150	monoazo	no	no
Disperse Red 1 (DiR 1)	2872-52-8	11110	monoazo	no	no
Disperse Red 17 (DiR 17)	3179-89-3	11210	monoazo	no	no
Pigment Red 53 (PR 53)	2092-56-0	15585	monoazo	no	yes
Solvent Red 24 (SR 24)	85-83-6	26105	diazo	no	yes
Acid Yellow 36 (AY 36)	587-98-4	13065	monoazo	no	yes
Disperse Yellow 3 (DiY 3)	2832-40-8	11855	monoazo	Skin Sens. 1 and Carc. 2	no
Solvent Yellow 1 (SY 1)[PAA]	60-09-3	11000	monoazo	Carc. 1B	yes
Solvent Yellow 2 (SY 2)	60-11-7	11020	monoazo	no	no
Solvent Yellow 3 (SY 3)[PAA]	97-56-3	11160	monoazo	Skin Sens. 1 and Carc. 1B	yes
Disperse Blue 106 (DiB 106)	12223-01-7	111935	monoazo	no	no
Disperse Blue 124 (DiB 124)	61951-51-7	111938	monoazo	no	no
Disperse Orange 3 (DiO 3)	730-40-5	11005	monoazo	no	yes
Disperse Orange 37 (DiO 37)	12223-33-5	11132	monoazo	no	no
Pigment Orange 5 (PO 5)	3468-63-1	12075	monoazo	no	yes
Solvent Orange 7 (SO 7)	3118-97-6	12140	monoazo	no	yes

[PAA] these azo colourants are also PAAs – see table

Disperse Yellow 3 (DiY3), Solvent Yellow 1 (SY1) and Solvent Yellow 3 (SY3) all have a harmonised classification as carcinogenic and are suggested to be restricted in tattoo inks and PMU. However, Solvent Yellow 1 (SY1) and Solvent Yellow 3 (SY3) are also PAAs already suggested restricted (see Table 36 and Table 41).

For most of the other substances, the classification provided by companies to ECHA in REACH registrations or in CLP notifications identifies these substances as substances that may cause cancer and allergic skin reaction. However, this restriction proposal is not based on the self-classifications by companies and thus cannot include the colourant without further investigation. If more resources had been available these substances could have been further investigated. However, many of these substances (PR 53, SR 24, AY 36, DiY 3, SY 1, SY 3, DiO 3, PO 5 and SO 7) are also listed in CPR Annex II and will be restricted in tattoo inks and PMU based on that.

Scope of restriction proposal for the azo colourants

The table below provides a summary of the azo colourants suggested to be in the scope of the restriction.

The azo colourants are proposed to be restricted in tattoo inks and PMU due to either:

- decomposition by amide hydrolysis
- photodecomposition forming 3,3'-dichlorobenzidine
- scientific evaluation by SCCP
- listed in the CoE ResAP(2008)1 and harmonised classification as skin sensitiser, category 1 or as carcinogenic, category 1B

Table 42. Azo colourants suggested to be included in the scope of the restriction proposal.

	CAS No	**CI no.**	**CI name**
1	6471-51-8	12420	Pigment Red 7 (PR7)
2	6410-38-4	12460	Pigment Red 9 (PR9)
3	6410-39-5	12465	Pigment Red 15 (PR15)
4	61932-63-6	12477	Pigment Red 210 (PR210)
5	85776-14-3	No CI no.	Pigment Orange 74 (PO74)
6	6528-34-3	11740	Pigment Yellow 65 (PY65)
7	6358-31-2	11741	Pigment Yellow 74 (PY74)
8	6410-32-8	12385	Pigment Red 12 (PR12)
9	6471-50-7	12380	Pigment Red 14 (PR14)
10	6655-84-1	12390[#]	Pigment Red 17 (PR17)
11	6535-46-2	12370	Pigment Red 112 (PR112)
12	5468-75-7	21095[(*)]	Pigment Yellow 14 (PY14)
13	6358-37-8	21096	Pigment Yellow 55 (PY55)
14	6041-94-7	12310	Pigment Red 2 (PR2)
15	6448-95-9	12315	Pigment Red 22 (PR22)
16	5280-68-2	12485	Pigment Red 146 (PR146)
17	67990-05-0	12466	Pigment Red 269 (PR269)
18	6505-28-8	21160	Pigment Orange16 (PO16)
19	2512-29-0	11680	Pigment Yellow 1 (PY1)
20	6358-85-6	21090	Pigment Yellow 12 (PY12)
21	15110-84-6	21107:1	Pigment Yellow 87 (PY87)
22	12225-18-2	11767[(*)]	Pigment Yellow 97 (PY97)
23	3520-72-7	21110*	Pigment Orange 13 (PO13)
24	15793-73-4	21115*	Pigment Orange 34 (PO34)
25	5567-15-7	21108*	Pigment Yellow 83 (PY83)
26	1229-55-6	12150[##]	Solvent Red 1 (SR1)
27	1320-07-6	20170[##]	Acid Orange 24 (AO24)

28	85-86-9	26100[##]	Solvent Red 23 (SR23)
29	5413-75-2	27290[##]	Acid Red 73 (AR73)
30	2832-40-8	11855[HCC]	Disperse Yellow 3

Explanation to the table: * = due to photodecomposition; (*) = due to both photodecomposition and amide hydrolysis; # = restricted in the CoE ResAP(2008)1; ## = due to evaluation by SCCP and [HCC] = Harmonised classification as Carc.

Specific concentration limits for PAAs in tattoo inks

Introduction

As previously discussed, PAAs can occur in tattoo inks as impurities from the manufacture of azo colourants or as decomposition products.

Following the risk management options where the presence of all CMs are restricted (below detection limit), it should be noted that the lowest concentration of PAAs detected in an ink on the Danish market was 2 ppm (DEPA, 2012). Therefore, prohibiting the presence of PAAs in tattoo inks may well prohibit the use of any azo colourants that are not restricted themselves, as ingredients. Therefore, it is proposed to derive a specific concentration limit relevant for all PAAs based on a risk evaluation.

Following the risk management option where all carcinogenic substances are restricted using classification limits, it will be shown in this chapter that the DMEL values will lead to limit values that are much lower than the classification limit, which is then not going to protect the consumers. Thus, a specific concentration limit relevant for all PAAs based on a risk evaluation is relevant in both cases.

Hazard assessment

A hazard assessment has been performed for the ten PAAs found in significant amounts in a Danish survey of tattoo inks (DEPA, 2012) to determine a DMEL for the carcinogenic effects.

The hazard evaluation was not performed for other PAAs since they were not detected in significant amounts in tattoo inks on the Danish market (DEPA, 2012a). Further, as it was only possible to establish DMEL values for two of the ten PAAs investigated, it most likely is not worth the effort to try to establish DMELs for more PAAs.

Aniline

According to Annex VI of the CLP Regulation (EC No. 1272/2008), aniline is classified Carc. 2 (H351: Suspected of causing cancer), Muta. 2 (H341: Suspected of causing genetic defects), Acute Tox. 3 (H301+ H311 + H331: Toxic if swallowed, in contact with skin or if inhaled), STOT RE 1 (H372: Causes damage to organs through prolonged or repeated exposure), Eye Dam. 1 (H318: Causes serious eye damage) and Skin Sens. 1 (H317: May cause an allergic skin reaction).

The EU risk assessment report (RAR) for aniline under the existing substances regulation (ECB, 2004) determined that the lowest concentration causing an effect was for carcinogenicity. For skin sensitisation, no effect level could be determined. With regard to carcinogenicity the RAR concluded that aniline is probably carcinogenic for humans (tumours primarily in the spleen) and that there is no threshold for this effect. A T25 (for rats) of 46 mg/kg bw per day was estimated, and a HT25 (T25 for humans) of 4.6 mg/kg bw per day for oral exposure was calculated by applying a factor of 10 for extrapolation of the T25 for rats to HT25 for humans.

Based on the HT25 of 4.6 mg/kg bw per day and application of a HtLF (High to low dose risk extrapolation factor) of 250.000 (the 'default' for the 10^{-6} lifetime risk when T25 is used as a

PoD (ECHA Guidance chapter 8 appendix R.8-6 and 8-7) (ECHA, 2012), a DMEL of approximately 2 x 10-5 mg/kg bw per day (approximately 20 ng/kg bw per day) can be established.

Conclusion on aniline:

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

o-Anisidine

According to Annex VI of the CLP Regulation (EC No. 1272/2008), o-anisidine (CAS no. 90-04-0) is classified Carc. 1B (H350: May cause cancer), Muta. 2 (H341: Suspected of causing genetic defects), Acute Tox. 3 (H301+ H311 + H331: Toxic if swallowed, in contact with skin or if inhaled).

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

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

The critical effect of o-anisidine for the assessment of human health was concluded to be the carcinogenic effect (mainly tumours in the bladder), and that there is no threshold for this effect. A T25 (for rats) of 39.7 mg/kg bw per day was estimated, and a HT25 (T25 for humans) of 9.9 mg/kg bw per day by applying a factor of 4 for extrapolation of the T25 for rats to HT25 for humans.

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

Based on the HT25 of 9.9 mg/kg bw per day and application of a HtLF (High to low dose risk extrapolation factor) of 250,000 (the 'default' for the 10^{-6} lifetime risk when T25 is used as a

PoD (ECHA Guidance chapter 8 appendix 8-6 and 8-7)), a DMEL of approximately 4 x 10^{-5} mg/kg bw per day (approximately 40 ng/kg bw per day) can be established.

Conclusion on o-anisidine:

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

4-Chloroaniline

According to Annex VI of the CLP Regulation (EC No. 1272/2008), 4-chloroaniline (CAS no. 106-47-8) is classified Carc. 1B (H350: May cause cancer), Acute Tox. 3 (H301+ H311 + H331: Toxic if swallowed, in contact with skin or if inhaled) and Skin Sens. 1 (H317: May cause an allergic skin reaction).

IARC (IARC, 1993) has classified 4-chloroaniline in Group 2B 'possibly carcinogenic to humans' (human evidence: inadequate; evidence in experimental animals: sufficient).

4-Chloroaniline has been evaluated in the CICAD programme (an international programme sponsored by UNEP/ILO/WHO jointly with reviews on the effects on human health and the environment of chemicals or combinations of chemicals, aiming to characterize the hazard and dose-response of exposure to chemicals) (WHO, 2003).

Based on the available data, 4-chloroaniline was considered to be a skin sensitiser.

The critical effect after repeated exposure over a prolonged period of time was considered to be the damage to red blood cells, including haemolytic anaemia and methaemoglobinaemia as observed in experimental animals; a LOAEL of 2 mg/kg bw per day was established based on a 2-year study in rats. Based on the LOAEL of 2 mg/kg bw per day and application of a total uncertainty factor of 1000 (10 for interspecies variation, 10 for inter-individual variation, 10 because a LOAEL was used instead of a NOAEL), a tolerable intake of 0.002 mg/kg bw was derived.

4-Chloroaniline is carcinogenic in male rats, with the induction of unusual and rare tumours of the spleen, which is typical for aniline and related substances. Whether the mechanism for the carcinogenic effect is mediated through genotoxic or non-genotoxic events is unresolved. No PoD (e.g. T25) for the carcinogenic effect has been considered.

Conclusion on 4-chloroaniline:

Based on the above, the critical effects of 4-chloroaniline in relation to tattooing are considered to be sensitisation and carcinogenic effects. A DNEL/DMEL for the critical effects cannot be established.

4-Chloro-o-toluidine

According to Annex VI of the CLP Regulation (EC No. 1272/2008), 4-chloro-o-toluidine (CAS no. 95-69-2) is classified Carc. 1B (H350: May cause cancer), Muta. 2 (H341: Suspected of causing genetic defects), Acute Tox. 3 (H301+ H311 + H331: Toxic if swallowed, in contact with skin or if inhaled).

IARC (IARC, 2000) (IARC, 2010) has classified 4-chloro-o-toluidine in 2A 'probably carcinogenic to humans' (human evidence: limited; evidence in experimental animals: sufficient).

The U.S. National Cancer Institute (National Cancer Institute, 1979) has investigated 4-chloro-o-toluidine for carcinogenic effects after dietary administration.

Tumours (haemangiosarcomas, a rare tumour type developed from blood vessels into the surrounding tissue) were observed in mice but not in rats. No other expert opinions of 4-chloro-o-toluidine of relevance in relation to 4-chloro-o-toluidine in tattoo inks have been located.

Conclusion on 4-chloro-o-toluidine:

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

3,3'-Dichlorobenzidine

According to Annex VI of the CLP Regulation (EC No. 1272/2008), 3,3'-dichlorobenzidine (CAS no. 91-94-1) is classified Carc. 1B (H350: May cause cancer), Acute Tox. 4 (H312: Harmful in contact with skin) and Skin Sens. 1 (H317: May cause an allergic skin reaction).

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

IARC (IARC, 2010) has classified Benzidine in Group 1 'carcinogenic to humans' (human evidence: sufficient; evidence in experimental animals: sufficient). In this more recent IARC monograph, it is mentioned that the evidence for a carcinogenic effect of 3,3'-dichlorobenzidine in experimental animals is sufficient; however, an evaluation of the human evidence as well as an overall evaluation of the carcinogenic effect of 3,3'-dichlorobenzidine has not been provided.

3,3'-Dichlorobenzidine has been evaluated in the CICAD programme (an international programme sponsored by UNEP/ILO/WHO jointly) (WHO, 1998a).

Dermatitis has been reported among workers (one limited study, no further details); no data on the sensitisation in experimental animals were identified.

The available data were considered as being inadequate to assess the effects after repeated exposure over a prolonged time period.

The critical effect of 3,3'-dichlorobenzidine was considered to be the carcinogenic effect for which there is no threshold. The TD0.05 (the dose associated with a 5% increase in tumour incidence in rats) was estimated to be in the range of 0.74 to 1.4 mg/kg bw per day depending on the tumour type used as the basis for the TD0.05 (mammary tumours: 0.74; leukaemia: 1.4).

Based on the TD0.05 of 0.74 mg/kg bw per day and application of a total factor of 5000-50000, a guidance value of 1.48×10^{-4} - 1.48×10^{-5} mg/kg bw was derived. It was noted that the limitations of the critical study upon which this guidance value is based should be borne in mind in its interpretation (only a single dose level and the exposure was shorter than 2 years (up to 488 days)).

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

Conclusion on 3,3'-dichlorobenzidine:

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

4-Methyl-m-phenylenediamine

According to Annex VI of the CLP Regulation (EC No. 1272/2008), 4-methyl-m-phenylenediamine (2,4-diaminotoluene / 2,4-toluenediamine) (CAS no. 95-80-7) is classified Carc. 1B (H350: May cause cancer), Muta. 2 (H341: Suspected of causing genetic defects), Repr. 2 (H361f: Suspected of damaging fertility), STOT RE 2 (H373: May cause damage to organs through prolonged or repeated exposure), Acute Tox. 3 (H301: Toxic if swallowed), Acute Tox. 4 (H312: Harmful in contact with skin) and Skin Sens. 1 (H317: May cause an allergic skin reaction).

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

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

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

Conclusion on 4-methyl-m-phenylenediamine:

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

4-Methoxy-m-phenylenediamine

According to Annex VI of the CLP Regulation (EC No. 1272/2008), 4-methoxy-m-phenylenediamine (2.4-diaminoanisol) (CAS no. 615-05-4) is classified Carc. 1B (H350: May cause cancer), Muta. 2 (H341: Suspected of causing genetic defects), Acute tox 4 (H302: Harmful if swallowed).

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

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

Conclusion on 4-methoxy-m-phenylenediamine:

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

2-Naphthylamine

According to Annex VI of the CLP Regulation (EC No. 1272/2008), 2-naphthylamine (CAS no. 91-59-8) is classified Carc. 1A (H350: May cause cancer) and Acute Tox. 4 (H302: Harmful if swallowed).

IARC (IARC, 1987a) (IARC, 2010) has classified 2-naphthylamine in Group 1 'carcinogenic to humans' (human evidence: sufficient; evidence in experimental animals: sufficient).

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

Conclusion on 2-naphthylamine:

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

5-Nitro-o-toluidine

According to Annex VI of the CLP Regulation (EC No. 1272/2008), 5-nitro-o-toluidine (CAS no 99-55-8) is classified Carc. 2 (H351: Suspected of causing cancer) and Acute Tox. 3 (H301+ H311 + H331: Toxic if swallowed, in contact with skin or if inhaled).

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

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

Conclusion on 5-nitro-o-toluidine:

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

o-Toluidine

According to Annex VI of the CLP Regulation (EC No. 1272/2008), o-toluidine (CAS no. 95-53-4) is classified Carc. 1B (H350: May cause cancer), Acute Tox. 3 (H301 + H331: Toxic if swallowed or if inhaled) and Eye Irrit. 2 (H319: Causes serious eye irritation).

IARC (IARC, 2010) has classified o-toluidine in group 1 'carcinogenic to humans' (human evidence: sufficient; evidence in experimental animals: sufficient).

o-Toluidine has been evaluated in the CICAD programme (an international programme sponsored by UNEP/ILO/WHO jointly) (WHO, 1998b). The available data were not considered valid for an evaluation of the sensitisation potential. The critical effect of o-toluidine was considered to be the carcinogenic effects. The mechanism for the carcinogenic effect is not clear, but involvement of a genotoxic mechanism cannot be eliminated. No PoD (e.g. T25) for the carcinogenic effect has been considered.

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

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

Conclusion on o-toluidine:

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

Summary: Hazard Assessment

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

For the evaluated PAAs it is considered that there is no threshold for the carcinogenic effects and, therefore, a DNEL cannot be established. Instead, DMELs may be derived.

For two of the PAAs (aniline and o-Anisidine), a DMEL could be established. For the remaining 8 PAAs a DMEL could not be established based on the available data.

The DMEL for aniline is set at approximately 2×10^{-5} mg/kg bw per day for the carcinogenic effects. The DMEL for o-anisidine is set at approximately 4×10^{-5} mg/kg bw per day.

Since all the PAAs with a harmonised classification as carcinogenic are very similar, a grouping approach is applied and the lowest DMEL value of 2×10^{-5} mg/kg bw per day is applied for members of the group as a conservative assumption. In general there may be large variations among the cancer potency in a group of similar carcinogenic substances. However, this is considered a conservative approach.

Sensitisation was also considered as a critical effect in relation to tattooing for aniline, 4-chloroaniline, 3,3'-dichlorobenzidine and 4-methyl-m-phenylenediamine. In the EU, these substances are classified Skin Sens. 1 (H317: May cause an allergic skin reaction) according to Annex VI of the CLP Regulation (EC no. 1272/2008). The health effect assessment of chemical contact allergens can only be performed if the potency and the threshold value have been carefully examined for the specific chemical allergen (Nielsen, et al., 2005). For the selected substances, the available data is not sufficient for an evaluation of either the potency or the threshold value and, therefore, a DNEL for sensitisation cannot be established for these substances.

Calculation of the risk based concentration limit

For the derivation of limit values for the PAAs, 4308 mg ink/tattoo session, a body weight (bw) of 60 kg, and a DMEL value of 2×10^{-5} mg/kg bw per day are applied.

This gives a risk based limit value for the concentration of PAAs in the ink of 0.28 ppm for each individual PAA. This figure is rounded to 0.3 ppm.

The highest concentration of o-anisidine in ink on the Danish market where the sample had not been digested or treated with reducing agents was 15 ppm. For 5-nitro-o-toluidine it was 190 ppm and for aniline it was 79.

Implementability of the proposed inclusion of PAAs and azo colourants in scope

PAAs

Analytical methods

It has not been possible to determine an available analytical method that can reproduce the possible release of PAAs from azo colourants (DEPA, 2017c). Thus, the restriction will only be able to address PAAs dissolved in the ink due to impurities or degradation in the ink prior to use or enforcement/control of the ink. Even the methodology for the dissolved fraction will need to be harmonised.

The analytical chemical methodology available from standards relevant to measuring PAAs in finger paints and textiles, where reductive cleavage is applied to assure that all PAAs that potentially may be formed (also at a later stage when the colourant slowly decomposes) are measured, is not suitable when it comes to tattoo inks since the azo colourant (pigment or lake) is in the shape of particles. It has thus been extremely difficult to reproduce the analytical results. See also (DEPA, 2017c), where a proposal for the future development of an analytical method for measuring PAAs has been outlined.

However, previous analyses have shown that for 21 (by the Italian authorities) and 24 (by (DEPA, 2012)) of the 29 identified PAAs analytical methods are available, and have been applied (even though they are not harmonised).

In DEPA (2012), the method used for analysis was the method described in CoE ResAP (2008)1, DS/EN 14362-1 (Methods for determination of certain aromatic amines liberated from azo colourants and colourants). The method UNI EN ISO 17234-1 is being used in Italy for the detection of the PAAs in tattoo inks. The PAAs detected and their detection limits are listed in Table 42. Validation data were obtained (according to the standard) only for selected substances taken as references.

As the methodology will become fully harmonised, the compliance control of the content of all 29 PAAs is expected to be performed using a single measurement, where all the PAAs are detected in the same analysis.

In the table below the detection limits are listed for the substances found in tattoo inks.

Table 43. Detected PAAs found in tattoo inks in Italy and Denmark and corresponding detection limits.

CAS no.	**Primary Aromatic Amine**	DS/EN 14362-1: Detection limit (ppm)	UNI EN ISO 17234-1: LOQ (ppm)
90-04-0	o-Anisidine	0.5 - 1	1
106-47-8	4-Chloroaniline	1	1
95-69-2	4-Chloro-o-toluidine	2	1
91-94-1	3,3'-Dichlorobenzidine	1	-
119-90-4	3,3'-Dimethoxybenzidine	2	
119-93-7	3,3'-Dimethylbenzidine	2	-
95-80-7	4-Methyl-m-phenylendiamine	1	1
91-59-8	2-Naphthylamine	2	1
99-55-8	5-nitro-o-toluidine	5	1
139-65-1	4,4'-Thiodianiline	2	
95-53-4	o-Toluidine	1	1
92-87-5	Benzidine	2	1
62-53-3	Aniline	0.5 - 1	
87-62-7 and 95-68-1	2,6-xylidine and 2,4-xylidine	1	
95-70-5	2-methyl-p-phenylenediamine	-	
92-67-1	Biphenyl-4-ylamine	1	-
97-56-3	4-o-tolylazo-o-toluidine	10	-
615-05-4	4-methoxy-m-phenylenediamne	10	
101-77-9	4,4'-methylenedianiline	10	-
838-88-0	4,4'-methylenedi-o-toluidine	2	-
120-71-8	6-methoxy-m-toluidine	1	-
101-14-4	4,4'-methylenebis[2-chloroaniline]	2	-
101-80-4	4,4'-oxydianiline	10	-
137-17-7	2,4,5-trimethylaniline	1	-

The limit of detection of the test method applied in Italy is 1 ppm. However, in Denmark (DEPA, 2012) for some of the PAAs a limit value of up to 10 ppm is found.

Therefore, assuming a limit value of 0.3 ppm for the PAAs is not considered practical.

In some cases, the detection limit in the different laboratories, where different standards were applied, are not the same as e.g. is the case for 5-nitro-o-toluidine, where the detection limit is 5 ppm in one case and 1 ppm in another.

As the data illustrate, the detection limits are dependent on the laboratories that perform the chemical measurements.

Note that the development of the methodology is on-going.

The limit value should address the dissolved concentration of the PAAs in the tattoo inks.

Azo colourants

As the chemical structure of the azo colourants has a larger variation than the PAAs, it is not expected that one analytical method for analysing them can be developed as is the case for the PAAs. Thus, a specific methodology for each azo colourant or perhaps sub-groups of azo colourants should be developed.

For the azo colourants a practical approach is chosen. A minimum concentration of azo colourants of 5-10 percent in the tattoo ink is normally required in order to be able to colour the skin. Thus, a practical limit of 0.1% will prevent the use of the azo colourants that are in the scope of the restriction. This limit is proposed for both RO1 and RO2.

Other considerations for derivation of the concentration limit

The risk based safe concentration level for dissolved PAAs (as a group) has been derived at 0.3 ppm. Considering the detection limits described above, which depending on the specific PAA and the laboratory varies between 0.5 and 10 ppm, the risk based safe concentration level is not considered practical feasible.

Note that in table 4.38 in the JRC report (JRC, 2015b), shown in Table 45 below, it is indicated that PAA (total) and o-Anisidine have been identified in concentrations below 0.5. The laboratories, which the dossier submitter has been in contact with (as described above) have however not been able to obtain lower detection limits than 1 ppm. However, with (substantial) investments in the development of the analytical methodology it is always possible to improve detection limits.

Further, based on the below argumentation, the risk based limit is also not considered economic feasible.

Concentration ranges (min-max) for PAAs in tattoo inks has been gathered by JRC and are shown in the JRC report in table 4.38 and Table 45 in this dossier (JRC, 2015b). According to the JRC report the content of PAAs goes from 0.1 to 6900 ppm. Thus inks with a PAA content below 0.3 ppm appear to be available on the market. However, the percentage of the tattoo inks below and above 0.3 ppm is not presented in/or can be derived from the table. The colours of the inks below and above 0.3 ppm are also not available. Further, the analytical method has not been specified in the JRC report (dissolved or both dissolved and solid).

Table 44 Table 4.38 in JRC report (JRC 2015) PAAs presence in tattoo and PMU inks.

Table 4.38: PAAs presence in tattoo and PMU inks.

Substance	CAS nr	Number of analysed samples	% non compliant samples	ResAP (2008)1 limit (mg/kg)	Range (min-max) (mg/kg)
PAA (total)		3283	14 (468)		0.1-68
4-Aminoazobenzene	60-09-3			0	>0
Aniline	62-53-3			0	5-61
o-Anisidine	90-04-0	3655	10 (347)	0	0.52-2197
Benzidine	92-87-5			0	>0
Biphenyl-4-ylamine	92-67-1			0	>0
4-Chloroaniline	106-47-8	2958	2 (61)	0	1.1-691
4-Chloro-o-toluidine	95-69-2	43	1 (2)	0	5.9-15
3,3'-Dichlorobenzidine	91-94-1	3647	2.4 (91)	0	1.0-4758
3,3'-Dimethoxybenzidine	119-90-4	827	0.5 (2)	0	20-26
3,3'-Dimethylbenzidine	119-93-7	829	0.1 (1)	0	>0
4-Methoxy-m-toluidine	120-71-8			0	>0
4,4'-Methylenbis(2-chloroaniline)	101-14-4			0	>0
4,4'-Methylendianiline	101-77-9			0	>0
4,4'-Methylendi-o-toluidine	838-88-0			0	>0
4-Methyl-m-phenylendiamine	95-80-7	3516	2.5 (85)	0	10-6900
2-Naphthylamine	91-59-8	19	5 (1)	0	2.6
5-Nitro-o-toluidine	99-55-8	2129	1.2 (27)	0	9-285
4,4'-Oxydianiline	101-80-4			0	>0
p-Phenylenediamine	106-50-3	29	3 (1)	0	8
4,4'-Thiodianiline	139-65-1	100	1 (1)	0	>0
o-Toluidine	95-53-4	3675	5 (184)	0	1.1-2197
2,4,5-Trimethylaniline	137-17-7			0	>0
2,4-xylidine	95-68-1	120	1 (1)	0	>0

In contrast, in the survey by the DEPA (DEPA, 2012) the highest (max.) concentrations observed have been listed in Table 46 together with the colours of the tattoo inks and the analytical methods applied, dissolved or total (both dissolved and solid).

Since there has been thorough discussions on the reproducibility of the methods for measuring total concentrations and since it is the dissolved fraction that is bioavailable. The Dossier Submitter has proposed to focus the restriction on the dissolved fraction. The discussion here will thus focus on the 24 inks where dissolved PAAs have been analysed.

Some inks were only measured for dissolved PAAs and not for total concentrations of PAAs and vice versa. This is marked with "NM" in the respective column in Table 46. When the ink was analysed and no PAA was detected above the detection limit it is indicated with "ND".

Eight of the 30 inks investigated have been claimed to cause effects by consumers and new bottles of the inks (same brand, name and colour) were found and included in the analysis. The producers of the inks claim that it is copies of their inks which have caused the effects. Three of these eight inks have relative high concentrations of PAAs (53 red = 190 ppm 5-nitro-o-toluidine, 57 brown = 79 ppm aniline and 48 red = 9.0 ppm o-anisidine). However other ink, not being claimed to have effects also show high contents (34 red = 34 ppm o-anisidine and 49 red = 15 ppm o-anisidine). Nevertheless, in order not to create any bias to inks in general, ink 53 red and 57 brown are excluded from the analysis. Ink 48 red is included since it is below what can be observed in randomly chosen inks. Thus the sample of inks considered for the discussion here is 22.

Table 45 Max-concentrations of PAAs detected in 30 tattoo inks on the Danish Market (2012, DEPA)

Ink no. and colour	**Dissolved concentration (ppm)**	**Total concentration (after reductive cleavage) = both dissolved and solid (ppm)**
1 red	NM	10 (o-toluidine)
5 red	ND	1.1 (o-toluidine)
7 green	ND	2.6 (o-toluidine)
8 blue	ND	NM
10 gray	ND	NM
12 black	ND	NM
13 green	ND	NM
15 blue	ND	NM
18 red*	4.9 (o-anisidine)	95 (o-anisidine)
20 orange	ND	56 (aniline)
22 violet	2.0 (o-toluidine)	NM
23 black	4.9 (o-anisidine)	NM
24 red*	6.2 (5-nitro-o-toluidine)	25 (aniline)
25 blue	4.9 (o-anisidine)	NM
26 green	NM	1775 (o-anisidine)
27 yellow	NM	1150 (o-anisidine)
30 black	ND	NM
34 red	34 (o-anisidine)	NM
35 violet*	2.0 (aniline)	4.2 (aniline)
36 yellow*	4.6 (o-anisidine)	5.6 (o-anisidine)
37 violet*	1.6 (aniline)	1.6 (aniline)
43 black	ND	NM
44 green	NM	133 (o-toluidine)
45 blue	5.9 (4-chloro-o- toluidine)	15 (4-chloro-o- toluidine)
48 red*	9.0 (o-anisidine)	55 (o-anisidine)
49 red	15.0 (o-anisidine)	424 (o-anisidine)
53 red*	190 (5-nitro-o-toluidine)	400 (4-methyl-m-phenyldiamine)
57 brown*	79 (aniline)	230 (aniline)
60 green	NM	42 (o-toluidine)
65 orange	NM	110 (aniline)

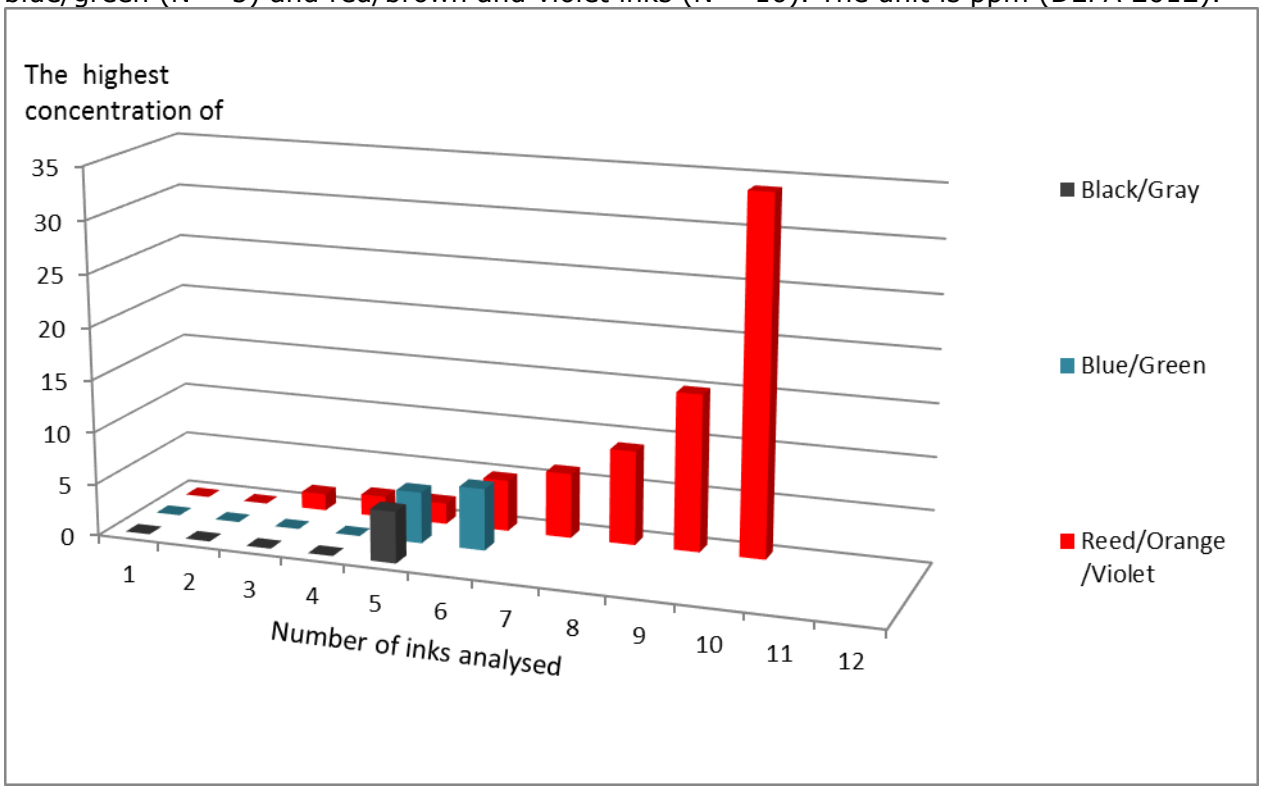
ND = Not detected, NM = Not measured, * = another batch or copy of the ink has been claimed to cause severe skin reactions.

From the data in Table 46 on dissolved PAAs it can be seen that 12 out of 22 inks (55%) contain dissolved PAAs above the detection limit. Further, 8 out of 10 (80%) of the inks with a dissolved PAA content below the detection limit are either black, gray, blue or green, which are known to be produced without the use of azo colourants. Considering the red, orange, violet

inks where red azo colourants are often applied, 8 out of 10 (80%) has a PAA content above the detection limit. If a risk based limit value is applied most likely around 80% of the red inks will be indirectly banned. Therefore the risk based limit value is also not considered economically feasible.

A visual comparison of the highest content of PAAs in black, blue/green and red/brown/violet inks have been shown in the figure below.

Figure 2 Visual comparison of the content of the highest content of PAAs in black (N = 5), blue/green (N = 5) and red/brown and violet inks (N = 10). The unit is ppm (DEPA 2012).



Since the risk based limit value is not considered practical or economically feasible, the ALARA principle (or the BAT) is applied to establish a limit value. Applying the ALARA principle the limit value has often been based on the 90 % percentile. However, considering the limited number of data and a possible bias, a 75 % percentile is applied.

Considering the data in Table 46 and applying the ALARA principle (or a BAT approach) for all inks and a 75 % percentile approach, a limit value for PAAs on 4.9 ppm would appear. This limit value would correspond to a 60 % percentile for the red, violet and orange inks.

In the CoE ResAP(2008)1, the limit value for the PAAs is referred to as being as low as technically possible, which would be in line with the ALARA principle applied here. However, the standard methods recommended for assessment of PAAs in tattoo inks in the CoE ResAP(2008)1 has a detection limit of 10 ppm.

Thus in order to assure a practical and economically feasible limit value, which is reasonable consistent with the current regulation in some Member States, a limit value of 5 ppm for PAAs is proposed.

A limit value of 5 ppm would imply a risk level of $1,7 * 10^{-5}$.

Appendix B.3. Hazard assessment for reprotoxic substances

1. Bis(2-ethylhexyl) phthalate (CAS No. 117-81-7)

The European Chemicals Agency (ECHA) identified bis(2-ethylhexyl) phthalate (DEHP) as Substance of Very High Concern (SVHC), based on the classification as reproductive toxicant category 1B. DEHP is listed in Annex XIV and XVII of REACH related to toys and childcare articles and in entry 30 of Annex XVII to REACH.

RAC recently adopted an opinion (RAC and SEAC, 2017) in support of the proposal by ECHA and Denmark to restrict the four phthalates (DEHP, DBP, DIBP and BBP) in articles. The restriction proposal (Larsen, 2011) is related to their reproductive toxicity properties. Moreover, the toxicity of DEHP has extensively been reviewed, i.a. in the EU by the European Chemicals Bureau (within the framework of the Existing Substances Regulation (EEC) 793/93), resulting in EU-Risk Assessment Reports (European Chemicals Bureau, 2008), and by the European Food Safety Authority (EFSA, 2005b).

The key study and respective NOAEL value for the endpoint reproductive toxicity of DEHP is shown in Table 47 and was selected based on the EU-RAR, EFSA and RAC assessment documents mentioned above. A recent literature research has been performed as well.

The EU-RAR forms the main information source in the publicly available Background Document to the above mentioned restriction proposal and the content is not repeated here. Comprehensive discussions of the selected key study and the NOAEL value can be found in this document.

DEHP is not classified for any other human health endpoint than for reproductive toxicity indicating that this is the most sensitive endpoint for this phthalate.

DEHP is classified as Repr. 1B for fertility and developmental effects (H360 FD).

1.1 Adverse effects on sexual function and fertility

The key study and respective NOAEL value for the reproductive toxicity endpoint of DEHP were selected based on the EU-RAR and EFSA assessment documents and on a recent RAC opinion. Here, the most sensitive endpoint is related to developmental effects. Thus, the endpoint fertility is not considered here in detail.

1.2 Adverse effects on development

1.2.1 Animal data

Table 47 summarises the studies considered to be key studies for adverse effects on development of DEHP by RAC and SEAC (2017)and European Chemicals Bureau (2008).

Table 46: Key animal studies on adverse effects of bis(2-ethylhexyl) phthalate (DEHP) on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Three-generation toxicity study (similar to OECD TG 416 and according to GLP) Dietary exposure Rats (Sprague-Dawley) 17 animals (m and f) /group	bis(2-ethylhexyl) phthalate (DEHP) 1.5, 10, 30, 100, 300, 1000, 7500 mg/kg food (corresponding to 0.1, 0.47, 1.4, 4.8, 14, 46, 359 mg/kg bw/d in F2 animals) Exposure: 6 week premating, during mating, gestation and lactation periods for breeding of the F1, F2 and F3 animals (after weaning)	**NOAEL(F1)*: 4.8 mg/kg bw/d** **LOAEL (F1)*: 14 mg/kg bw/d** (small testes; small and/or aplastic epididymis, seminiferous tubular atrophy in offspring) (For more detailed results please refer to the EU_RAR report)	Wolfe and Layton (2004)* (supported as key study by RAC;
Pre- and postnatal developmental toxicity study, no guideline followed, results considered to have major uncertainties (as discussed below) Oral (gavage) Rats (Wistar rats) 30 f/ group, 12 pups (6f and 6m)/ group	bis(2-ethylhexyl) phthalate (DEHP) 0, 0.25, 6.25 mg/kg bw/d Exposure: GD 0 to PND 21 Observation of pups: until week 33 after birth (sacrifice on week 3, 15, 21, 33)	**NOAEL(F1): could not be determined** **LOAEL (F1): 0.25 mg/kg bw/d** (significant decrease of glomerular number per kidney after weaning and 33 weeks after birth at 0.25 and 6.25 mg/kg bw/d (dose dependent) and in males and females, significant decrease of total glomerular volume 33 weeks after birth in males and females at 0.25 and 6.25 mg/kg bw/d (dose dependent), significantly reduced creatinine clearance after 21 weeks at 0.25 and 6 mg/kg bw/d in males and females) **Maternal toxicity:** -no effects are documented	Wei et al. (2012)

* Key Study and NOAEL concluded by European Chemicals Bureau (2008) and supported by RAC and SEAC (2017)

1.2.2 Human data

Epidemiological studies which allow determining a human "no effect level" for DEHP are currently not available.

1.2.3 Discussion of adverse effects on development

The RAC supported a **NOAEL of 4.8 mg/kg bw/d** (LOAEL of 14 mg/kg bw/d; small testes; small and/or aplastic epididymis, seminiferous tubular atrophy in offspring) for DEHP as dose descriptor for reproductive toxicity based on a three-generation study similar to OECD TG 416 and according to GLP considered reliable without restriction (Wolfe and Layton, 2004). These NOAEL and LOAEL values have been selected as dose descriptors in the EU-RAR assessment.

For the present restriction a recent literature research for literature published since 2011 has been performed. One study has been identified which resulted in a lower LOAEL of 0.25 mg/kg bw/d compared to Wolfe and Layton (2004) based on histopathological and functional kidney effects in offspring (Wei et al., 2012). However, due to incomplete data, results on only two dose levels and low animal number, the results of the study are considered to be of high uncertainty. Functional kidney impairments considered being adverse and which were measured by the creatinine clearance rate have been found in pups of 21 weeks of age. No data for this effect are available shortly after weaning and at other time points investigated. Pups have not been treated with DEHP after weaning. Thus, it is uncertain if observed functional kidney impairments were treatment related. Moreover, data were obtained from 12 pups and at two dose levels only which is considered to weaken the reliability of the results and to reduce statistical power. In the study by Wei et al. (2012) significant increase in kidney weight and in the kidney to body weight ratio have also been observed at 6.25 mg/kg bw/d for male offspring. But these findings were not confirmed in the GLP study by Wolfe and Layton (2004). Here, no (significant) positive trends in increased kidney weights or kidney to body weight ratio have been detected in the F1, F2 and F3 generations. Moreover, no dose-dependent macroscopically changes in the kidney were found in this study in offspring in the F1, F2 and F3, which does not indicate a manifestation of kidney toxicity in offspring.

1.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as DEHP has no classification related to adverse effects on or via lactation.

1.4 Information taken into account for risk assessment and uncertainties

1.4.1 POD-selection

A reliable three generation study by Wolfe and Layton (2004) was considered to be the key study for reproductive toxicity of DEHP. In this study, testicular toxicity (small testes; small and/or aplastic epididymis, seminiferous tubular atrophy in offspring) was observed in offspring exposed to 14 mg/kg bw/d as the most sensitive effect. The induced testicular effects are considered relevant for humans and the NOAEL of 4.8 mg/kg bw/d was selected as starting point (POD) for risk assessment of humans. This NOAEL was also selected in the EU-RAR (European Chemicals Bureau, 2008) and by EFSA and was supported by RAC.

1.4.2 Uncertainty

The derived POD was based on a reliable three-generation study similar to OECD TG 416 and to GLP. Thus, uncertainties are considered to be low.

1.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in the table below.

Table 47: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{Developmental\ effects}$	**NOAEL: 4.8 mg/kg bw/d**	**The NOAEL for developmental effects results from a three-generation toxicity study (similar to OECD TG 416 and according to GLP) in rats (Wolfe and Layton, 2004) and is based on small testes, small and/or aplastic epididymis and seminiferous tubular atrophy observed in offspring.**
Overall AFs	**100**	
AF for interspecies differences - remaining differences - allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL.
AF related to quality of database	1	Default value.
DNEL general population, , reproductive effects (related to developmental effects)	**0.048 mg/kg bw/d**	

2 Dibutyl phthalate (CAS No. 84-74-2)

The European Chemicals Agency (ECHA) identified dibutyl phthalate (DBP) as Substance of Very High Concern (SVHC), based on the classification as reproductive toxicant category 1B (H360 Df). DBP is listed in Annex XIV of REACH and in Annex XVII of REACH related to toys and childcare articles and in entry 30 of Annex XVII to REACH.

RAC recently adopted an opinion (RAC and SEAC, 2017) in support of the proposal by ECHA and Denmark to restrict the four phthalates (DEHP, DBP, DIBP and BBP) in articles. The restriction proposal (Larsen, 2011) is related to their reproductive toxicity properties. Moreover, the toxicity of DBP has extensively been reviewed in the recent past, i.a. in the EU by the European Chemicals Bureau (within the framework of the Existing Substances Regulation (EEC) 793/93), resulting in EU-Risk Assessment Reports (RARs)), and by the European Food Safety Authority (EFSA, 2005c).

The key study and respective NOAEL value were selected based on the EU-RAR (European Chemicals Bureau, 2004), EFSA and RAC assessment documents mentioned above. A recent

literature survey was performed since 2011.

The EU-RAR forms the main information source in the publicly available background document to the above mentioned restriction proposal and is not repeated here. Comprehensive discussions of the selected key study and the NOAEL value can be found in this document.

DBP is not classified for any other human health endpoint than for reproductive toxicity indicating that this is the most sensitive endpoint for this phthalate.

2.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as DBP has no Repr 1A/B classification related to adverse effects on sexual function and fertility.

2.2 Adverse effects on development

2.2.1 Animal data

The table below summarises the studies considered to be key studies for adverse effects on development of DBP by RAC and SEAC (2017) and European Chemicals Bureau (2008)and, if applicable, relevant studies since 2011 which could lead to a lower "point of departure" (POD) compared to RAR and RAC assessment reports.

Table 48: Key animal study for adverse effects of dibutyl phthalate (DBP) to development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Developmental toxicity study(prenatal and postnatal), (no guideline followed), considered as reliable with restriction (due to low animal number) dietary exposure Rats 6-8/group	dibutyl phthalate (DBP) dose levels: 20, 200, 2000, 10000 mg/kg food Exposure: GD15 to PND 21 (end of lactation)	**NOAEL (F1): could not be determined** **LOAEL (F1)*: 2 mg/kg bw/d (=20 mg/kg food)** (reduced testicular spermatocyte development and mammary gland changes in offspring) *Maternal toxicity:* - significant reduction in bw at 20 and 10000 mg/kg (food) at GD 15 to GD20 *Foetal toxicity:* **20 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) -mammary gland changes in both sexes of offspring (significant number of vacuolar degeneration, alveolar cells in males at PNW 11;significant hypoplasia of alveolar bud in females at PND 21) **200 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) -mammary gland changes in both sexes of offspring (PND 21) **2000 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) - significant loss of germ cell development - significant increase in scattered foci of aggregated Leydig cells - significantly decreased ductular cross sections in epididymis -mammary gland changes in both sexes of offspring (PND 21) **10000 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) -significant reductions in anogenital distance (PND 2) -significantly increased incidence of retained nipples/areolae	Lee et al. (2004)*

* Key Study and NOAEL selected by EFSA (2005b) and supported by RAC and SEAC (2017).

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		in male offspring (PND 14) - significant increase in scattered foci of aggregated Leydig cells - significantly decreased ductular cross sections in epididymis -mammary gland changes in both sexes of offspring (PND 21) -significant increase in liver cell hypertrophy	

2.2.2 Human data

Epidemiological studies which allow determining a human "no effect level" for DBP are currently not available.

2.2.3 Discussion of Adverse effects on sexual function, fertility and development of dibutyl phthalate

In the most recent opinion on DBP the RAC supported the study by Lee et al. (2004) as key study for reproductive toxicity and the **LOAEL of 2 mg/kg bw/d** as dose descriptor for DBP. This LOAEL was based on reduced testicular spermatocyte development and mammary gland changes in offspring observed in a developmental toxicity study in rats with exposure from gestation day (GD) 15 to postnatal day (PND) 21. The study was not performed similar to a standardised guideline but results were considered reliable. A NOAEL could not be derived from the study as doses below 1.5-3 mg/kg bw/d have not been tested in that study.

For the present restriction a recent literature research for literature published since 2011 has been performed. No other key study leading to a lower LOAEL value for reproductive toxicity compared to Lee et al. 2004 was found.

2.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as DBP has no classification related to adverse effects on or via lactation.

2.4 Information taken into account for risk assessment and uncertainties

2.4.1 POD-selection

The study by Lee et al. (2004) was considered to be the key study for reproductive toxicity of DBP. The observed effects are considered relevant for humans and the LOAEL of 2 mg/kg bw/d was selected as dose descriptor for risk assessment of humans. Human data which allow determining a human "no effect level" for DBP are currently not available. The RAC supported this LOAEL as dose descriptor in his latest opinion on DBP and the EFSA took the same study and LOAEL as a basis for deriving the TDI for DBP.

2.4.2 Uncertainty

There are some uncertainties for the selected LOAEL as a NOAEL could not be derived and only 6-8 female adults have been tested per dose group in the study by Lee et al. (2004).

2.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in the table below.

Table 49: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{Developmental\ effects}$	**LOAEL: 2 mg/kg bw/d**	**This LOAEL for developmental effects results from a prenatal and postnatal developmental study in rats (Lee et al. (2004); supported as key study by RAC) and is based on reduced testicular spermatocyte development and mammary gland changes in offspring.**
Overall AFs	**300**	
AF for interspecies differences - remaining differences - allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	3	An AF was applied as the POD is a LOAEL.
AF related to quality of database	1	Default value.
$DNEL_{general\ population,\ reproductive\ effects}$ (related to developmental effects)	**0.0067 mg/kg bw/d**	

3 Diisobutyl phthalate (CAS No. 84-69-5)

The European Chemicals Agency (ECHA) identified **diisobutyl phthalate (DIBP)** as Substance of Very High Concern (SVHC), based on the harmonised classification as reproductive toxicant category 1B (H360Df). DIBP is listed in Annex XIV of REACH.

RAC recently adopted an opinion (RAC and SEAC, 2017) in support of the proposal by ECHA and Denmark to restrict the four phthalates (DEHP, DBP, DIBP and BBP) in articles. The restriction proposal (Larsen, 2011) is related to their reproductive toxicity properties. Except for DIBP the points of departure (PoD) chosen are identical to those previously agreed by RAC (2012a) following an extensive evaluation of the available information related to the hazard profile of the substances. For DIBP a new DNEL was proposed by the Dossier Submitter, to better reflect its anti-androgenic potency.

3.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as DIBP has no Repr 1A/B classification related to adverse effects on sexual function and fertility.

3.2 Adverse effects on development

3.2.1 Animal data

The table below summarises the studies considered to be key studies for adverse effects on development of DIBP.

Table 50: Key animal study on adverse effects of diisobutyl phthalate (DIBP) on sexual function and development for

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Developmental toxicity study(prenatal and postnatal), (no guideline followed), considered as reliable with restriction (due to low animal number) dietary exposure Rats 6-8/group	Read across to dibutyl phthalate (DBP) dose levels: 20, 200, 2000, 10000 mg/kg food Exposure: GD15 to PND 21 (end of lactation)	**NOAEL (F1): could not be determined** **LOAEL (F1)*: 2 mg/kg bw/d (=20 mg/kg food)** (reduced testicular spermatocyte development and mammary gland changes in offspring) *Maternal toxicity:* - significant reduction in bw at 20 and 10000 mg/kg (food) at GD 15 to GD20 *Foetal toxicity:* **20 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) -mammary gland changes in both sexes of offspring (significant number of vacuolar degeneration, alveolar cells in males at PNW 11;significant hypoplasia of alveolar bud in females at PND 21) **200 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced	Lee et al. (2004)*

* Key Study and NOAEL supported by RAC and SEAC (2017).

		number of spermatocytes) -mammary gland changes in both sexes of offspring (PND 21) **2000 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) - significant loss of germ cell development - significant increase in scattered foci of aggregated Leydig cells - significantly decreased ductular cross sections in epididymis -mammary gland changes in both sexes of offspring (PND 21) **10000 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) -significant reductions in anogenital distance (PND 2) -significantly increased incidence of retained nipples/areolae in male offspring (PND 14) - significant increase in scattered foci of aggregated Leydig cells - significantly decreased ductular cross sections in epididymis -mammary gland changes in both sexes of offspring (PND 21) -significant increase in liver cell hypertrophy	
Postnatal developmental toxicity study (no guideline followed), results considered reliable Oral (gavage) Rats (Sprague-Dawley) 11-14 f /group Parameters investigated:	diisobutyl phthalate (DIBP) 125, 250, 500, 625 mg/kg bw/d Exposure: daily, GD 12-21 Observation time: litter: PND 0 to PND 76-82 or PND 111-122 (without exposure)	**NOAEL (F1): could not be determined** **LOAEL (F1)*: 125 mg/kg bw/d** (histopathological lesions in testes and epididymis in male offspring post weaning) *Maternal toxicity:* **-** no effects - no effect on implantation and litter size	Saillenfait et al. (2008)

F0: toxicity, no. implantation sites, F1 (male pups):body weights, anogenital distance (AGD) on PND 1, presence of areola and/or nipples on ventral surface of the thorax, preputial separation (PPS) at PND 40, gross abnormalities of external and internal genitalia and position of testes, weights of testes, epididymides, seminal vesicles and prostate, histopathology of testes an d epididymes		*Foetal toxicity:* - No effect on sex ratio and pup survival **125 mg/kg bw/d:** - histological lesions in testes and epididymis post weaning: degenerative features of seminiferous tubules: oligosperimia, azoospermia, and tubular degeneration-atrophy/hypoplasia (grade 2 and 5) **250 mg/kg bw/d:** - AGD male pups significantly reduced on PND1 (dose dependent p< 0.01) -retained thoracic and/or nipples in male pups at PND 12-14 and PND 76-86 or PND 111-122 (dose dependent) -underdeveloped or absent testes and/or epididymis in 2% male pups - histological lesions in testes and epididymis post weaning: degenerative features of seminiferous tubules: oligosperimia, azoospermia, and tubular degeneration-atrophy/hypoplasia (grade 1 -5), tubular necrosis **500 mg/kg bw/d** - AGD male pups significantly reduced on PND 1 (dose dependent p< 0.01) - significant later onset of PPS (no dose dependent) -retained thoracic and/or nipples in male pups at PND 12-14 and PND 76-86 or PND 111-122 (dose dependent) - severe malformations of the external and internal genitalia (e.g. hypospadias, non-scrotal testes, non-descended testes, small penis) -underdeveloped or absent testes and/or epididymis in 16% male pups (7m,5 litters) - male pup weight significantly reduced post weaning (dose dependent) - significantly reduced absolute weights of testes, epididymis, seminal vesicles and prostate post weaning (dose dependent) - histological lesions in testes and epididymis post weaning: degenerative features of seminiferous tubules: oligosperimia, azoospermia, and tubular degeneration-atrophy/hypoplasia (grade 1 - 5), tubular necrosis, interstitial cell	

		hyperplasia **625 mg/kg bw/d** - AGD male pups significantly reduced on PND 1 (dose dependent p< 0.01) - male pup weight significantly reduced on PND 1 and at PND 21 and post weaning -retained thoracic and/or nipples in male pups at PND 12-14 and PND 76-86 or PND 111-122 (dose dependent) -significant later onset of PPS (no dose dependent) - severe malformations of the external and internal genitalia (e.g. hypospadias, non-scrotal testes, non-descended testes, small penis) -underdeveloped or absent testes and/or epididymis in 13% male pups (5 m, 4 litters) - significantly reduced absolute weights of testes, epididymis, seminal vesicles and prostate post weaning (dose dependent) - histological lesions in testes and epididymis post weaning: degenerative features of seminiferous tubules: oligosperimia, azoospermia, and tubular degeneration-atrophy/hypoplasia (grade 1 - 5), tubular necrosis, interstitial cell hyperplasia	

Table 51: Results published in the study by Saillenfait et al. (2008): histopathological lesions in the testes and epididymis of male rats post weaning (PNW: 11-12)

	0	***125 mg/kg bw/d***	***250 mg/kg bw/d***	***500 mg/kg bw/d***	***625 mg/kg bw/d***
No. males/litters examines	*24/12*	*20/10*	*28/14*	*22/11*	*20/10*
Epididymides					
Oligospermia	*0*	*1*	*3*	*2*	*1*
Azoospermia	*0*	*1*	*3*	*10*	*18*
Granulomatous inflammation	*0*	*0*	*0*	*4*	*3*
Testes					
Tubular degeneration-atrophy/hypoplasia	*2*	*2*	*7*	*16*	*20*
Grade 1	*2*	*0*	*1*	*3*	*1*
Grade 2	*0*	*1*	*1*	*1*	*0*
Grade 3	*0*	*0*	*2*	*0*	*2*
Grade 4	*0*	*0*	*1*	*4*	*0*
Grade 5	*0*	*1*	*2*	*8*	*17*
Tubular necrosis	*0*	*0*	*1*	*3*	*5*
Interstitial cell hyperplasia	*0*	*0*	*0*	*1*	*9*

3.2.2 Human data

Epidemiological studies which allow determining a human "no effect level" for DIBP are currently not available.

3.2.3 Discussion of adverse effects on development

Previously, the LOAEL of 125 mg/kg bw/day from the study of Saillenfait et al. (2008) was proposed as PoD for DNEL derivation. In this study, histopathological effects in testes (degeneration of seminiferous tubules) and oligo-/azospermia in epididymes were observed in male rats perinatally (from gestation day 12 to 21) exposed by gavage to dose levels ranging from 125 to 625 mg DIBP/kg bw/day. However, as the database on DIBP is rather poor, with only very few reproductive toxicity studies published, and as DIBP has not been tested at doses below 100 mg/kg bw/day, the RAC recently supported a lower dose descriptor based on read across to DBP.

Given the similarities between DBP and DIBP in structure and potency as regards anti-androgenic effects, RAC agreed that the previous PoD of 125 mg/kg bw/day for DIBP does not appropriately reflect this potency, and therefore needed reconsideration (RAC and SEAC, 2017). From the study by Saillenfait et al. (2008) it appears that a 25% higher dose of DIBP (625 mg/kg bw/day) is needed to cause the same developmental effects as 500 mg/kg bw/day of DBP. RAC considered the extrapolation of the potency findings from the high dose to the low dose are justifiable and supported the PoD of 2.5 mg/kg bw/day for DIBP[8]

2.3.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as DIBP has no classification related to adverse effects on or via lactation (360D).

3.4 Information taken into account for risk assessment and uncertainties

3.4.1 POD-selection

RAC agreed that the LOAEL of 125 mg/kg bw/d (by Saillenfait et al. (2008)) does not adequately reflect the potency of DIBP. Based on a read across to DBP RAC supported a PoD of 2.5 mg/kg bw/d for DIBP.

3.4.2 Uncertainty

There are some uncertainties for the selected LOAEL as based on a read across.

3.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in the table below.

Table 52: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{Developmental\ effects}$	**LOAEL: 2.5 mg/kg bw/d**	**Read across from DBP**
Overall AFs	**300**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	3	An AF was applied as the POD is a LOAEL.
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to developmental effects)	**0.0083**	

4 Dihexyl phthalate (CAS No. 84-75-3)

The Member State Committee has agreed on the identification of dihexyl phthalate (DHP) as Substance of Very High Concern (SVHC), based on the harmonised classification as reproductive toxicant category 1B (H 360FD) and in entry 30 of Annex XVII to REACH.

For DHP a CLH Report (ANSES, 2010) for its classification as reproductive toxicant category 1B and a RAC opinion supporting the proposed classification and key studies exist.

The key studies for the endpoint reproductive toxicity of DHP as shown in the table below were selected based on the CLH report, the respective RAC opinion and a current literature survey (since 2011).

DHP is not classified for any other human health endpoint than for reproductive toxicity indicating that reproductive toxicity is the most sensitive endpoint.

DHP is classified as Repr. 1B for fertility and developmental effects (H360 FD) which are discussed below.

4.1 Adverse effects on sexual function and fertility

4.1.1 Animal data

Table 53: Key animal study on adverse effects of DHP on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Fertility study (no standardised guideline followed), results considered reliable Oral (dietary study) Mice (CD1-mice) 16-19 m and f /group Parameters investigated: Fertility parameters: number of fertile pairs, litter/pair, live pups/litter, proportion of pups born alive, live pup weight, necropsy only with animals of highest dose group	dihexyl phthalate (DHP) 0, 0.3, 0.6 and 1.2% in feed Corresponding to 0, 380-430, 800-880, 1670-1870 mg/kg bw/d Exposure: daily, 7 days prior to and during 98-day cohabitation period	**NOAEL: could not be determined** **LOAEL*: 380 mg/kg bw/d** (Dose dependent significant decrease in number of fertile pairs) - Dose dependent significant reduction in mean bw in m/f - Dose dependent significant (all dose levels) decrease in number of fertile pairs 380-430 mg/kg bw/d: 82%, 800-880 mg/kg bw/d: 5% and 1670-1870 mg/kg bw/d: 0%) due to reduced mating capabilities in treated males 1670-1870 mg/kg bw/d: (only dose group with necropsy) - Absolute testes weight 70% less - Relative prostate weights increased by 9% - -Relative weights of epididymis and seminal vesicles reduced by 23 and 18% - Epididymal sperm concentration reduced by 93%, motility reduced 80% - Extensive atrophy of seminiferous tubules (no histological lesions in the reproductive organs of females) - Relative liver weight increased by 34% (m), 32% (f) - Relative kidney weights reduced by 9% (m) and 6% (f) - (no histopathological lesions in liver and kidney) (Results for litter/pair, live pups/litter, pups born alive and live pup weight were considered to be	Lamb et al. (1987)*

* Key Study and LOAEL are supported by RAC (RAC, 2011c).

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		with high uncertainty as pup number too low at mid-dose and zero at highest dose level, moreover no dose-dependence and significant effects at the mid-high but the lowest dose level tested.)	

4.1.2 Human data

Epidemiological studies which allow determining a human "no effect level" for fertility effects of DHP are currently not available.

4.1.3 Discussion of adverse effects on sexual function and fertility

Regarding effects of DHP on fertility the study by Lamb et al. (1987) was considered to be the key study. In this oral continuous breeding study in mice a dose-related decrease in the proportion of fertile pairs able to produce litter was observed from 380-430 mg/kg bw/d onward in absence of parental toxicity. This effect was found to be due to reduced mating capabilities of treated males. Findings in the treated males at the highest dose level tested (1670-1870 mg/kg bw/d) included severe effects on testes weight, epididymal sperm concentration and motility, and extensive atrophy of the seminiferous tubules providing a plausible basis for the decreased mating index in the mouse study.

The study was not performed according to a standardised guideline but the results are considered to be reliable and the RAC also supported the study as key study for fertility effects of DHP. From the study a **LOAEL** for fertility effects of DHP of **380 mg/kg bw/d** could be derived.

4.2 Adverse effects on development

4.2.1 Animal data

Table 54: Key animal study on adverse effects of DHP on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity study (similar to OECD TG 414) Oral (gavage) Rat (Sprague-Dawley)	dihexyl phthalate (DHP) 0,250, 500, 750 mg/kg bw/d Exposure: daily, GD 6 to GD 20	**NOAEL (F1): could not be determined** **LOAEL (F1)*: 250 mg/kg bw/d** (significant decrease in anogenital distance of male foetuses) Maternal toxicity:	Saillenfait et al. (2009b)*

* Key Study and LOAEL are supported by RAC (RAC, 2011c)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
8-12/group		-decreased bw at 750 mg/kg bw/d -significant increase in serum ASAT activity at 750 mg/kg bw/d -increased absolute and relative liver weights at all doses -no histological lesions in liver Foetal toxicity: 250 mg/kg bw/d: -significant decrease in anogenital distance of male foetuses (dose dependent) 500 mg/kg bw/d & 750 mg/kg bw/d: - significant decreases in foetal weight -decrease in anogenital distance of male foetuses (dose dependent) - significant increase in incidence of undescended testes in male foetuses - significant increase of malformations (e.g. cleft palate, eye defects, vertebra malformations) -high incidence of post-implantation loss at 750 mg/kg bw/d	
Postnatal developmental toxicity study (no guideline followed); considered reliable Oral (gavage) Rat (Sprague-Dawley) 10-12/group	dihexyl phthalate (DHP) 0, 50, 125, 250, 500 mg/kg bw/d Exposure: daily, GD 12 to GD 21 Observation: litter until postnatal week 10-12 (necropsy on PNW 10-11	**NOAEL (F1): could not be determined** **LOAEL (F1): 50 mg/kg bw/d** (increase of males with areolas and/or nipples at PND 12-14, histopathologic lesions in testes of male offspring at PNW >10) Maternal toxicity:	Saillenfait et al. (2009a)*

* Key Study and LOAEL supported by RAC (RAC, 2011c).

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Parameters investigated:F0: bw, F1:bw, gross abnormalities of reproductive organs, anogenital distance (AGD) of males, presence of areola and/or nipples on PND12-14, preputial separation (PPS) on PND 40, weight of liver, kidneys, testes, epididymides, seminal vesicles and prostate	and 16-17)	**-** no effects offspring toxicity: 50 mg/kg bw/d: - increase of males with areolas and/or nipples at PND 12-14 (dose-dependent) -histopathologic lesions in testes at PNW >10 shown in table 14 (tubular degeneration) 125 mg/kg bw/d: - sig. decrease of relative AGD (dose-dependent - increase of males with areolas and/or nipples at PND 12-14 and PNW>10 (dose- dependent - increase in number of males with undescended testes (dose dependent.) - histopathologic lesions in testes of male rats at PNW >10 shown in Table 56 250 mg/kg bw/d: - sig. decrease of absolute and relative (bw) AGD(dose- dependent - increase of males with areolas and/or nipples at PND 12-14 and PNW>10 (dose-dependent) - increase in number of malformations of external and internal genitalia (hypospadias, cleft phallus, undescended testes) (dose dependent) - histopathologic lesions in testes of male rats at PNW >10 shown in Table 56 500 mg/kg bw/d - significant decrease of absolute and relative AGD (dose-dependent) - significantly reduced proportion of live pups (PND 1) - increase of males with areolas and/or nipples at PND 12-14 and PNW>10 (dose-dependent)	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		- increase in number of malformations of external and internal genitalia of males > PNW10 (cleft prepuce, underdeveloped testes, hypospadias, cleft phallus, undescended testes, crossed vasa deferentia) (dose dependent - histopathologic lesions in testes male rats at PNW >10 shown in Table 56	
Postnatal developmental toxicity study (no guideline followed); results considered reliable Oral (gavage) Rat (Wistar albino) 10/group Parameters investigated:F0: bw, F1: bw, weight histopathological investigations of testes, epididymis, ventral prostate and seminal vesicle, count and morphology of epididymal sperm, evaluation of seminiferous and epididymal round tubules	dihexyl phthalate (DHP) 0, 20, 100, 500 mg/kg bw/d Exposure: daily, GD 6 to GD 19 Observation: offspring at PND 20, PND 32 and PND 90	**NOAEL (F1): could not be derived** **LOAEL (F1): 20 mg/kg bw/d** (significantly increased number of malformations of reproductive tract e.g. tubular atrophy and atrophic and damaged tubules in testes) - Significant dose dependent malformations of reproductive tract in prepubertal rats at PND 20 at 20, 100 and 500 mg/kg bw/d (testes: tubular atrophy, picnotic cells, atrophic and damaged tubules, tubules without lumen; epididymis: spermatogenic cells in lumen, atrophic tubules; prostate gland: atrophic prostate gland, prostatic intraepithelial neoplasia) - Significant dose dependent malformations of reproductive tract in pubertal rats at PND 32 at 20, 100 and 500 mg/kg bw/d (testes: increase in apopitotic cells; epididymis: spermatogenic cells in lumen; prostate gland: atrophic tubules, prostatic intraepithelial neoplasia); at 100 and 500 mg/kg bw/d (testes: tubular atrophy, germinal cell debris, atrophic and damaged tubules; epididymis: atrophic tubules) - Significant dose dependent malformations of reproductive tract in adult rats at PND 90 at 20, 100 and 500 mg/kg bw/d (testes: germinal cell debris and sertoli cell vacuolisation, epididymis: spermatogenic cells in lumen;	Aydogan Ahbab and Barlas (2013)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		prostate gland: prostatic intraepithelial neoplasia, mononuclear cell infiltration); at 100 and 500 mg/kg bw/d (testes: tubular atrophy) (All observed effects on bw, organ weights of testes, epididymis, prostate and seminal vesicle are not considered treatment related due to high variations at all dose groups)	

Table 55: Results published in the study by Saillenfait et al. (2009b): histopathological lesions in the testes and epididymis of male rats post weaning (PNW: 11-12)

	0	***50 mg/kg bw/d***	***125 mg/kg bw/d***	***250 mg/kg bw/d***	***500 mg/kg bw/d***
No. males/litters examines	*24/9*	*61/11*	*41/9*	*32/10*	*33/8*
Epididymides					
Oligospermia	*1*	*3*	*0*	*2*	*3*
Azoospermia	*0*	*2*	*2*	*7*	*16*
Sloughed cells	*3*	*2*	*5*	*8*	*13*
Granulomatous inflammation	*1*	*2*	*2*	*8*	*16*
Testes					
Tubular degeneration-atrophy/hypoplasia					
Grade 1	*1*	*0*	*2*	*1*	*1*
Grade 2	*0*	*0*	*0*	*1*	*0*
Grade 3	*1*	*1*	*0*	*1*	*1*
Grade 4	*0*	*3*	*0*	*1*	*1*
Grade 5	*0*	*1*	*1*	*7*	*16*
Tubular necrosis	*0*	*0*	*0*	*0*	*5*
Interstitial cell hyperplasia	*0*	*0*	*0*	*1*	*6*

4.2.2 Human data

Epidemiological studies which allow determining a human "no effect level" for fertility effects of DHP are currently not available.

4.2.3 Discussion of adverse effects on development

Three key studies for the assessment of developmental toxicity of DHP have been identified (Aydogan Ahbab and Barlas, 2013; Saillenfait et al., 2009a; Saillenfait et al., 2009b).

The study by Saillenfait et al. (2009b) is a prenatal developmental toxicity study performed similar to OECD TG 414. Pregnant rats were orally treated with three dose levels of DHP. Significant developmental effects were detected at all dose levels and at dose levels not leading to maternal toxicity (250 and 500 mg/kg bw/d). A **LOAEL of 250 mg/kg bw/d** was derived based on significantly decreased anogenital distance in male foetuses. As lower dose levels than 250 mg/kg bw/d have not been investigated in this study the derivation of a NOAEL was not possible.

The two other oral studies (Aydogan Ahbab and Barlas, 2013; Saillenfait et al., 2009a) focused on postnatal development of untreated male offspring from DHP treated pregnant females. The two studies were not performed according to a standardised guideline but the results are considered to be reliable. Significant toxic effects in untreated offspring were observed at all dose levels tested. A **LOAEL of 50 mg/kg bw/d** was derived from the study by Saillenfait et al. (2009b) based on increased numbers of males with areolas and/or nipples at PND 12-14 and on increased number of histopathological lesions in testes at PNW >10 (tubular degeneration). Histopathological lesions in testes were also observed by Aydogan Ahbab and Barlas (2013) in prepubertal, pubertal and adult male offspring at similar dose levels. From this study a **LOAEL of 20 mg/kg bw/d** was derived. As lower dose levels than 50 mg/kg bw/d (Saillenfait et al., 2009a) and 20 mg/kg bw/d (Aydogan Ahbab and Barlas, 2013) have not been investigated in the studies the derivation of a NOAEL was not possible.

4.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as DHP has no classification related to adverse effects on or via lactation.

4.4 Information taken into account for risk assessment and uncertainties

4.4.1 POD-selection: sexual function and fertility

The study by Lamb et al. (1987)was considered to be the key study for fertility effects of DHP. The observed effects are considered relevant for humans and the LOAEL of 380 mg/kg bw/d was selected as starting point (POD) for risk assessment. Human data which allow determining a human "no effect level" for DHP are currently not available.

4.4.2 POD-selection: development

The studies by Saillenfait et al. (2009a), (2009b) and Aydogan Ahbab and Barlas (2013) were considered to be key studies for developmental toxicity of DHP. The developmental prenatal and postnatal effects are considered relevant for humans and the lowest LOAEL of 20 mg/kg bw/d derived from the study by Aydogan Ahbab and Barlas (2013) was selected as starting point (POD) for risk assessment. Human data which allow determining a human "no effect level" for DHP are currently not available.

4.4.3 Uncertainty: sexual function and fertility

There are some uncertainties for the selected LOAEL as the study by Lamb et al. (1987) was not performed according to a standardised guideline and GLP and a NOAEL could not be derived from the study.

4.4.4 Uncertainty: development

There are some uncertainties for the selected LOAEL as the study by Ahbab and Barlas (2013) was not performed according to a standardised guideline and GLP and a NOAEL could not be derived from the study.

4.4.5 DNEL derivation: sexual function and fertility

The calculated DNEL and applied assessment factors are shown in Table 57.

4.4.6 DNEL derivation: development

The calculated DNELs and applied assessment factors are shown in Table 57. DNELs were calculated for developmental and fertility effects of DHP.

Table 56: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Fertility		
$POD_{Fertility\ effects}$	**LOAEL: 380 mg/kg bw/d**	**This LOAEL for fertility effects is based on an oral fertility study in mice (Lamb et al. (1987); supported as key study by RAC) in which a dose dependent significant decrease in number of fertile pairs was observed.**
Overall AFs	**1050**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 7	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	2	The default assessment factor for extrapolation from sub-chronic to chronic exposure was applied as the key study was a sub-chronic study.
AF related to dose response relationship	3	An AF was applied as the POD is a LOAEL.
AF related to quality of database	1	Default value.
DNEL general population, , reproductive effects (related to fertility effects)	**0.36**	
Development		

$POD_{Developmental\ effects}$	**LOAEL: 20 mg/kg bw/d**	**This LOAEL for developmental effects results from an oral postnatal developmental toxicity study in rat (Aydogan Ahbab and Barlas, 2013) and is based on significantly increased malformations of the reproductive tract e.g. tubular atrophy and atrophic and damaged tubules in testes.**
Overall AFs	**300**	
AF for interspecies differences - remaining differences allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	3	An AF was applied as the POD is a LOAEL
AF related to quality of database	1	Default value.
DNEL general population, , reproductive effects (related to developmental effects)	**0.067**	

5 n-pentyl-isopentylphthalate (EC No 933-378-9)

n-pentyl-isopentylphthalate has a harmonised classification as toxic for reproduction, Repr. 1B (H360FD: "May damage fertility. May damage the unborn child."). Due to this hazardous property the substance was identified as SVHC and the Member State Committee has supported ECHA`s proposal for this substance to be included in Annex XIV (REACH) for authorization and in entry 30 of Annex XVII to REACH.

No CLH report is publically available for n-pentyl-isopentylphthalate and no study reports could be found in the ECHAs`CLH archive. The substance is not registered under REACH. Moreover, no relevant data were found in a literature research performed as shortly described in section 5.9.1.

Thus, no literature or data were identified that would enable the derivation of a DNEL for reproductive toxicity for this substance.

6 1,2-benzenedicarboxylic acid, di-C6-C8-branched alkyl esters, C7-rich (CAS No. 71888-89-6)

The Member State Committee (MSC) has agreed on the identification of 1,2-benzenedicarboxylic acid, di-C6-C8-branched alkyl esters, C7-rich **(DIHP)** as Substance of Very High Concern (SVHC), based on the classification as reproductive toxicant category 1B (H360D). Moreover, based on its reproductive toxic properties and on grouping considerations DIHP is recommended for inclusion in Annex XIV of REACH and in entry 30 of Annex XVII to REACH.

A CLH report and respective RAC opinion justifying the classification for Repr. 1B (H360D) are not available for the substance as classification was done before the CLP regulation came into force.

The key studies for the endpoint reproductive toxicity (developmental toxicity) for DIHP as shown in Table 58 were based on a protocol of a meeting of the Technical Committee for Classification and Labelling at the Ispra Meeting (2003), a protocol of ECPI (2002) and a literature survey.

DIHP is not classified for any other human health endpoint than for reproductive toxicity indicating that this is the most sensitive endpoint.

DIHP is classified for developmental toxicity (H360 D) but not for fertility effects. Thus, the present section is related to developmental toxicity only.

6.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as DIHP has no classification related to fertility effects.

6.2 Adverse effects on developmental toxicity

6.2.1 Animal data

Table 57: Key animal study on adverse effects of DIHP on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal developmental toxicity study according to OECD TG 414 Oral (gavage) Rat 25 F/group	1,2-benzenedicarboxylic acid, di-C6-C8-branched alkyl esters, C7-rich (Di Iso Heptyl Phthalate (DIHP) 0,100, 300, 750 mg/kg bw/d Exposure: GD 6 to 20	**NOAEL (F1) 300 mg/kg bw/d** **LOAEL (F1) 750 mg/kg bw/d** (significant increase in resorptions per litter and per implantation site , decrease in live foetuses, significant decrease in foetal bodyweight, increase in incidences of external, visceral and skeletal malformations) Maternal toxicity: - no overt maternal toxicity - significant increase in absolute and relative liver weights at 300 and 750 mg/kg bw/d - decreased uterine weights at 750 mg/kg bw/d (related to increased embryonic death and smaller foetuses) Toxicity in offspring: **100 and 300 mg/kg bw/d** **-** no effects observed **750 mg/kg bw/d** **-** significant increase in resorptions per litter and per implantation site - decrease in live foetuses - significant decrease in foetal bodyweight - increase in incidences of external, visceral and skeletal malformations	Exxon (1997)
Two-generation reproductive toxicity study according to OECD TG 416 and GLP Oral (dietary study) Rat (Crl:CD (SD)IGS	1,2-benzenedicarboxylic acid, di-C6-C8-branched alkyl esters, C7-rich (Di Iso Heptyl Phthalate (DIHP) 1000, 4500, 8000 ppm 100, 450, 800 mg/kg	**NOAEL (F1): could not be derived** **LOAEL (F1): 100 mg/kg bw/d** (based on reduction in sperm production rate and mean testicular sperm concentration in offspring) **Effects in F0 generation**	Exxon (2003)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
30 m,f/group	bw/d* Treatment: F0: 70 days (from 6 weeks of age); F1: 70 days following weaning (at age of 22 days) *The conversion was performed according to section 3.9.2.3.3 of the Guidance on CLP criteria.	- statistically significant increases in mean absolute and relative liver and kidney weights at 4500 and 8000 ppm - no other treatment related effects **Most sensitive effects observed in offspring:** - statistically significant reduction in mean sperm production rate and mean testicular sperm concentration at all dose levels in F1 generation	

6.2.2 Human data

Epidemiological studies which allow determining a human "no effect level" for DIHP are currently not available.

6.2.3 Discussion of adverse effects on development

For the endpoint reproductive toxicity two key studies are available; namely an oral two-generation reproductive toxicity study in rats according to OECD TG 416 and an oral prenatal developmental toxicity study in rats according to OECD TG 414. Both studies were performed according to GLP and are considered reliable.

In the two-generation reproductive toxicity study, rats (F0 and F1 generations) were treated with the food with 1000, 4500 and 8000 ppm DIHP. This is equivalent to daily doses of 100, 450 and 800 mg/kg bw/d according to conversion rules described in the Guidance on CLP criteria (section 3.9.2.3.3).

Besides statistically significant increases in mean absolute and relative liver and kidney weights which are not considered adverse, no other treatment related effects including histopathological investigations were identified in the F0 generation.

In the F1 generation several statistically significant reproductive and adverse effects were observed at dose levels of 800 mg/kg bw/d. These included reduced mating rates, reductions in fertility, external malformations of the male reproductive organs and reductions in mean absolute and relative gonadal and accessory sex organ weights. Microscopic degeneration was observed in several tissues including testes, prostate, seminal vesicles, seminiferous tubules, vas deferens, coagulating gland and epididymis. Moreover evidence of feminisation of male pups (F1) was found. In F1 (at 800 mg/kg bw/d) and F2 (at 450 and 800 mg/kg bw/d) litters significant reductions in mean offspring body weights as well as incidences of centribular hepatocellular hypertrophy, hepatocellular vacuolation and nephropathy were observed.

The most sensitive effect found in offspring (F1 generation) were statistically significant reductions in mean sperm production rate and mean testicular sperm concentrations in male pups at all dose levels (including 100 mg/kg bw/d). These effects are considered to be adverse and are assumed to trigger effects in the reproductive organs at higher dose levels (450 and

800 mg/kg bw/d) as described above. No other treatment related effects were detected at 100 mg/kg bw/d. No paternal toxicity was found at 100 mg/kg bw/d. Thus, the LOAEL for developmental effects of DIHP was considered to be 100 mg/kg bw/d.

In the prenatal developmental toxicity study pregnant rats were treated with 100, 300 and 750 mg/kg bw/d DIHP by gavage. In the dams significantly increased absolute and relative liver weights at 300 and 750 mg/kg bw/d were observed. However, as no corresponding histological abnormalities were detected these effects were not considered to be adverse. Reduced uterine weights were identified in the dams at 750 mg/kg bw/d. This effect was directly related to increased embryonic death and smaller foetuses and for this reason was not interpreted as maternal toxicity. No other treatment related toxic effect was observed in the dams.

Treatment related adverse effects were not detected in offspring at doses of 100 and 300 mg/kg bw/d. Evidence of growth retardation and increased embryo/foetal death were observed at the high dose (750 mg/kg bw/d). Additionally, there was a significant increase in incidences of external, visceral and skeletal malformations at this dose level. Most common malformations were anophthalmia, microphthalmia, ectopic testes/ovaries, abnormal origin or agenesis of the blood vessels and malformed bones of the skull, sternebrae, ribs or vertebrae. Accordingly, the developmental NOAEL was established at 300 mg/kg bw/d. Regarding the parameters tested in this study, the results are in line with the two-generation reproductive toxicity study discussed above. Sperm parameters (such as sperm production rate and testicular sperm concentration) have not been investigated in the prenatal developmental toxicity study.

Thus the overall **LOAEL** established from the two studies is **100 mg/kg bw/d** based on reduction in sperm production rate and mean testicular sperm concentration observed in the guideline conform two-generation reproductive toxicity study.

6.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as DIHP has no classification related to adverse effects on or via lactation.

6.4 Information taken into account for risk assessment and uncertainties

6.4.1 POD-selection

The reliable guideline conform two-generation reproductive toxicity study in rats (Exxon, 2003) was considered to be the key study for reproductive toxicity of DIHP. The established LOAEL is 100 mg/kg bw/d based on reduction in sperm production rate and mean testicular sperm concentration observed in offspring (F1 generation). The induced testicular effects are considered relevant for humans and the LOAEL was selected as starting point (POD) for risk assessment. Within a recent literature research no new key studies leading to a lower POD for reproductive toxicity of DIHP have been found.

6.4.2 Uncertainty

The derived POD was based on a reliable two-generation study according to OECD TG 416 and to GLP. As discussed by the CMR Working Group (2003) there are some indications that the selected POD might be too sensitive. Although the sperm counts were statistically reduced, they did not demonstrate a dose-response relationship and showed considerable numeric variation in the inter-generation comparison. F1 testicular sperm counts were reduced, but there were no effects on epididymal sperm counts in either the low or mid dose groups. There is no obvious reason why only one of these organs should be affected. There was also no difference in F1 testicular weights in either the low or mid dose groups (which should have been affected since the sperm comprises a large fraction of the testicular weight) and no pathological evidence of

aspermia or testicular atrophy in either the low or mid dose groups. There was also no difference in sperm production rates/sperm concentrations in the F0 males at comparable doses. Consequently, the reported statistical finding on sperm counts for the F_1 males could also be discussed to be an experimental artefact than a treatment-related effect. However, due to clear significant reductions at all dose levels the observed effects are interpreted as treatment-related and it was decided by the DS not to deviate from the POD of 100 mg/kg bw/d. As no data is available for dose levels below 100 mg/kg bw/d a NOAEL could not be derived.

6.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in the table below.

Table 58: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{Developmental\ effects}$	**LOAEL: 100 mg/kg bw/d**	**This LOAEL for developmental effects results from a two generation reproductive toxicity study in rats (Exxon, 2003) and is based on reduction in sperm production rate and mean testicular sperm concentration in offspring.**
Overall AFs	**300**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	3	An AF was applied as the POD is a LOAEL.
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to developmental effects)	**0.33 mg/kg bw/d**	

7 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (CAS No. 68515-50-4)

Currently, 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear has no harmonised classification for reproduction toxicity. However, there exists a recent RAC opinion (RAC, 2013b) in which RAC agreed that the substance meets the criteria for classification as toxic for reproduction category 1B (H360FD) in accordance with Regulation (EC) No 1272/2008. Based on this opinion the Member State Committee has agreed in 2014 on identification of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear as Substance of Very High Concern (SVHC) and the substance was recommended for inclusion in Annex XIV of REACH in 2015 (final document). It is restricted in entry 30 of Annex XVII to REACH.

The RAC opinion (RAC, 2013b) was based on a CLH report by the Swedish Chemicals Agency in 2012 (Swedish Chemicals Agency, 2012) justifying the classification for Repr. 1B (H360Df)).

The key studies for the endpoint reproductive toxicity for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear as shown in Table 60 were based on the CLH report and RAC opinion mentioned above and on a current literature survey (since 2012).

1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear is not classified for any other human health endpoint than for reproductive toxicity indicating that this is the most sensitive endpoint.

There are no mammalian reproductive toxicity and developmental studies available for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear. Therefore, in the Swedish CLH report (Swedish Chemicals Agency, 2012), a substance grouping based on the structural similarity of seven ortho-phthalates (DBP, DIPP, DPP, DIHP, DnHP, DEHP and 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear) with a carbon backbone of 3-6 carbon atoms was constructed to fill in data gaps. RAC considered this grouping approach as justified and supported the classification as Repr. 1B. RAC further supported the specific hazard statement of H360FD for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear as the read-across data included endpoints for both fertility and developmental toxicity.

7.1 Adverse effects on sexual function and fertility

Classification of this substance was based on a read-across approach to seven ortho-phthalates. The key study which supported the derivation of the lowest LOAEL/NOAEL was related to developmental effects. Thus, fertility effects are not considered here.

7.2 Adverse effects on developmental toxicity

7.2.1 Animal data

The key study for adverse effects on development as shown in Table 60 was selected based on the study with the lowest LOAEL/NOAEL found within the grouping approach for the seven *ortho*-phthalates as explained above.

In a current literature research no study has been identified where a lower LOAEL/NOAEL could be derived for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear compared to the study by Lee et al. (2004).

Table 59: Key animal study on adverse effects of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Developmental toxicity study, prenatal and postnatal, (no guideline followed), considered as reliable with restriction (due to low animal number) dietary exposure Rats (female adults and female and male offspring) 6-8/group	Read across from dibutyl phthalate (DBP) dose levels: 20, 200, 2000, 10000 mg/kg food Exposure: GD 15 to PND 21 (end of lactation)	**NOAEL (F1): could not be determined** **LOAEL (F1)*: 2 mg/kg bw/day** (reduced testicular spermatocyte development and mammary gland changes in offspring) Matenal toxicity - significant reduction in bw at 20 and 10000 mg/kg (food) at GD 15 to GD 20 Toxicity in offspring: **20 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) -mammary gland changes in both sexes of offspring (significant number of vacuolar degeneration, alveolar cells in males at PNW 11;significant hypoplasia of alveolar bud in females at PND 21) **200 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) -mammary gland changes in both sexes of offspring (PND 21) **2000 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) - significant loss of germ cell development - significant increase in scattered foci of aggregated Leydig cells - significantly decreased ductular	Lee et al. (2004)*

* Key Study and NOAEL supported by RAC.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		cross sections in epididymis (indicating reduced coiling) -mammary gland changes in both sexes of offspring (PND 21) **10000 mg/kg food:** - dose dependent delay of testicular spermatocyte development (reduced number of spermatocytes) -significantly reduced anogenital distance (PND 2) -significantly increased incidence of retained nipples/areolae in male offspring (PND 14) - significant increase in scattered foci of aggregated Leydig cells - significantly decreased ductular cross sections in epididymis (indicating reduced coiling) -mammary gland changes in both sexes of offspring (PND21) -significant increase in liver cell hypertrophy	

7.2.2 Human data

Epidemiological studies which allow determining a human "no effect level" for adverse effects on reproduction of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear are currently not available.

7.2.3 Discussion of adverse effects on development

The key study for adverse effects on reproduction of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear (Lee et al., 2004) was selected as is was the study leading to the lowest LOAEL/NOAEL within the grouping approach of 7 phthalates as explained above. The study by Lee et al. 2004 was performed with dibutyl phthalate (DBP). In the most recent on DBP from 2012 the RAC supported the study by Lee et al. (2004) as key study for reproductive toxicity and the **LOAEL of 2 mg/kg bw/d** as POD for DBP. This LOAEL was based on reduced testicular spermatocyte development and mammary gland changes in offspring observed in a developmental toxicity study in rats with exposure from GD 15 to PND 21. The study was not performed according to a standardised guideline but results were considered reliable. A NOAEL could not be derived from the study as doses below 1.5-3 mg/kg bw/d have not been tested.

7.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as this substance has no classification related to adverse effects on or via lactation.

7.4 Information taken into account for risk assessment and uncertainties

7.4.1 POD-selection

The study by Lee et al. (2004) with DBP was considered to represent the most relevant study regarding reproductive toxicity of 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear using a grouping approach of seven phthalates. The observed adverse effects are considered relevant for humans and the LOAEL of 2 mg/kg bw/d was selected as starting point (POD) for risk assessment. Human data which allow determining a human "no effect level" for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear are currently not available. RAC supported this LOAEL as POD in his most recent opinion on DBP and the EFSA took the same study and LOAEL at the basis for deriving the TDI for DBP.

7.4.2 Uncertainty

There are uncertainties for the selected LOAEL as it was derived using a grouping approach. Mammalian reproductive toxicity and developmental studies or epidemiologic data is not available for 1,2-benzenedicarboxylic acid, dihexyl ester, branched and linear. Moreover, a NOAEL could not be derived and only 6-8 female adults have been tested per dose group in the study of a similar substance by Lee et al. (2004).

7.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in the table below.

Table 60: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{Developmental\ effects}$	**LOAEL: 2 mg/kg bw/d**	**This LOAEL was obtained using read-across approach from dibutyl phthalate (DBP). The LOAEL for developmental effects results from a prenatal and postnatal developmental study in rats with DBP (Lee et al. (2004); supported as key study by RAC) and is based on reduced testicular spermatocyte development and mammary gland changes in offspring.**
Overall AFs	**300**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	3	An AF was applied as the POD is a LOAEL
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to developmental effects)	**0.0067 mg/kg bw/d**	

8 Borates including boric acid (CAS No. 10043-35-3, CAS No. 11113-50-1, 13840-56-7), disodium tetraborate, anhydrous (CAS No. 1330-43-4), tetraboron disodium heptaoxide, hydrate (CAS No. 12267-73-1) and diboron trioxide (CAS No. 1303-86-2)

Boric acid and various borate compounds are considered chemically and toxicologically similar or equivalent by the IPCS (IPCS, 1998) under physiological conditions and in an opinion on a proposal for a harmonised classification and labelling (Repr. 1B, H360FD) for disodium octaborate tetrahydrate (RIVM, 2013b) RAC supported the application of a read-across approach (RAC, 2014d) between different borate compounds. After hydrolysis under neutral and acidic conditions and in the absence of compounds reacting specifically with the borate moiety (e.g. chelating agents), the monomeric 'orthoboric acid' $B(OH)_3$ is the predominant chemical species for all inorganic borates. Conversion factors are given in the table below.

Table 61: Overview of conversion factors of borates to equivalent dose of boron

Substance	Formula	Conversion factor for equivalent dose of B (multiply by)
boric acid	H3BO3	0.1748
boric oxide	B2O3	0.311
disodium tetraborate anhydrous	Na2B4O7	0.2149
disodium tetraborate pentahydrate	Na2B4O7•5H2O	0.1484
disodium tetraborate decahydrate	Na2B4O7•10H2O	0.1134
disodium octaborate tetrahydrate	Na2B8O13·4H2O	0.2096
sodium pentaborate(pentahydrate)	NaB5O8·5H2O	0.1832

Thus, for the present restriction proposal for the borate compounds addressed in this section [boric acid (CAS No. 10043-35-3, CAS No. 11113-50-1), disodium tetraborate, anhydrous (CAS No. 1330-43-4), tetraboron disodium heptaoxide, hydrate (CAS No. 12267-73-1) and diboron trioxide (CAS No. 1303-86-2)] for the endpoint reproductive toxicity a read-across approach was applied with boric acid as source substance. Conversion of thresholds (NOAELs, DNELs) for the individual borate compounds was based on the conversion factors shown in Table 62.

Due to the toxicological similarities of boron compounds (all classified as toxic to reproduction, category 1B) the following boron compounds have been included in the Candidate List following their identification as Substances of Very High Concern (SVHC): boric acid (CAS No. 10043-35-3 and CAS No. 11113-50-1), disodium tetraborate, anhydrous (CAS No. 1330-43-4), tetraboron disodium heptaoxide, hydrate (CAS No.12267-73-1) and diboron trioxide (CAS No. 1303-86-2).

The borate compounds addressed in this section are all classified as Repr. 1B related to both fertility and developmental effects (H360 FD).

8.1 Adverse effects on sexual function and fertility

8.1.1 Animal data

As described above key studies are based on data for boric acid which was considered as the source substance in the read-across approach for the borate compounds addressed in the present section.

For boric acid a recent CLH report (Biuro do spraw Substancji Chemicznych, 2013) and respective RAC opinion exists (RAC, 2014c). Further a recent literature research from 2013 to present was performed. The key study shown in the table below was selected based on the RAC opinion (RAC,

2014c) and CLH report (Biuro do spraw Substancji Chemicznych, 2013).

Table 62: Key animal study on adverse effects of boric acid on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Three-generation study (no guideline followed, but considered reliable as conforms to standard three generation studies normally used at that time) Oral (dietary study) Rat (Sprague-Dawley) 8 m and 16 f /group	boric acid 0, 670, 2000, 6700 ppm boric acid (0,34, 100, 336 mg/kg bw/d) Corresponding to: 0, 117, 350, 1170 ppm boron (0, 5.9, 17.5, 58.5 mg B/kg bw/d) Exposure: daily, from beginning of the study until sacrifice of P0 and from weaning till sacrifice of F2 and F3	**NOAEL (F1): 100 mg/kg bw/d equals to 17.5 mg/B/kg bw/d** **LOAEL (F1): 336 mg/kg bw/d equals to 58.5 mg B/kg bw/d** (based on testicular atrophy, reduced fertility)	Weir (1966)*

8.1.2 Human data

Epidemiological studies which allow determining a human "no effect level" for fertility effects of boric acid are currently not available.

8.1.3 Discussion of adverse effects on sexual function and fertility

The oral multi-generation study in rats by Weir (1966) has been identified as key study for fertility effects of boron acid. Male and female rats were treated orally with four dose levels of boric acid. At the highest dose testicular atrophy and reduced fertility were observed. For these effects a NOAEL of 100 mg/kg bw/d (corresponding to 17.5 mg B/kg bw/d) was established. Detailed discussions on fertility effects caused by boron acid can be found in the CLH report (Biuro do spraw Substancji Chemicznych, 2013) and respective RAC opinion (RAC, 2014c). The RAC concluded that "studies of reproductive toxicity and repeated dose toxicity studies in mice, rats and dogs clearly indicate that boron (B) impairs fertility through an effect on the testes. The effects observed in the different species are similar in nature. Based on data from the 2 years feeding study with boric acid in rats (Weir, 1966), the overall NOAEL for fertility is therefore 100 mg/kg bw/day, equal to 17.5 mg B/kg bw/day. […] There are no indications that the impaired fertility is secondary to other toxic effects." Within a recent literature research no studies were identified which would support a lower NOAEL/LOAEL.

* Key Study supported by RAC (2014c)

8.2 Adverse effects on development

8.2.1 Animal data

As described above, key studies are shown for boric acid which was considered as the source substance in the read across approach for the borate compounds addressed in the present section.

For boric acid a recent CLH report (Biuro do spraw Substancji Chemicznych, 2013) and respective RAC opinion exists (RAC, 2014c). Further a recent literature research from 2013 to present was performed. The key study shown in the table below was selected based on this RAC opinion (RAC, 2014c) and the CLH report (Biuro do spraw Substancji Chemicznych, 2013).

Table 63: Key animal study on adverse effects of boric acid on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal developmental toxicity study (similar to OECD TG 414) Oral (dietary study) Rat (Sprague-Dawley) 28-32 f/group	boric acid 0, 250, 500, 750, 1000, 2000 ppm boric (19, 36, 55, 76, 143 mg/kg bw/d) corresponding to 3.3, 6.3, 9.6, 13.3 and 25 mg B/kg bw/d Exposure: daily, days 0- 20 post mating	**NOAEL (P): 76 mg/kg bw/d (13.3 mg B/kg bw/d)** **LOAEL (P): 143 mg/kg bw/d(25 mg B/kg bw/d)** (Based on relative kidney weights.) **NOAEL (F1): 55 mg/kg bw/d (9.6 mg B/kg bw /d)** **LOAEL (F1): 76 mg/kg bw/d; (13.3 mg B/kg bw/d)** (reduction in the mean foetal bwt per litter (6% compared to controls), skeletal changes: increase in incidence of wavy ribs and short rib XIII, decreased incidence of rudimentary extra rib on lumbar 1	Price et al. (1994)*

8.2.2 Human data

Epidemiological studies which allow determining a human "no effect level" for developmental effects of boric acid are currently not available.

8.2.3 Discussion of adverse effects on development

The oral prenatal developmental study in rats by Price et al. (1994) was identified as key study for developmental effects of boric acid. Pregnant rats were treated orally with five dose levels of boric acid. At the dose of 76 mg/kg bw/d (corresponding to 13.3 mg B/kg bw/d) adverse toxic effects in the offspring were observed including reduction in the mean foetal body weight per litter (6% compared to controls) and skeletal changes. No treatment related maternal toxic effects were found at this dose level. For developmental effects a NOAEL of 55 mg/kg bw/d

* Key Study supported by RAC (2014c) and EFSA Opinion (2013)

(corresponding to 9.6 mg B/kg bw/d) was established. Detailed discussions on developmental effects caused by boron acid can be found in the CLH report (Biuro do spraw Substancji Chemicznych, 2013) and respective RAC opinion (RAC, 2014c). RAC concluded that developmental toxicity (malformations) was clearly observed in studies in rats and rabbits, the rat being the most sensitive species, with an overall NOAEL of 55 mg/kg bw/d (corresponding to 9.6 mg B/kg bw/day). With a recent literature research no studies were identified which would lead to a lower POD.

8.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as boric acid has no classification related to adverse effects on or via lactation.

8.4 Information taken into account for risk assessment and uncertainties

8.4.1 POD-selection

For boric acid the oral multi-generation study in rats by Weir (1966) was considered as key study for adverse fertility effects and the prenatal developmental toxicity study by Price et al. (1994) was considered as key study for adverse developmental effects. Observed effects are considered relevant to humans. Developmental toxicity was the most sensitive endpoint with an established NOAEL of 55 mg/kg bw/d (corresponding to 9.6 mg B/kg bw/d). The NOAEL for fertility effects for boric acid was found to be 100 mg/kg bw/d (corresponding to 17.5 mg/kg bw/d). These were selected as POD for risk assessment of humans for boric acid and for all borate compounds addressed in this section after conversion using conversion factors shown in Table 62.

8.4.2 Uncertainty

The uncertainties of the PODs for boric acid are considered to be low as established from reliable studies in rats and supported by RAC (2014c) and EFSA. Uncertainty for other borate compounds is higher as PODs were established based on a read across approach.

8.4.3 DNEL derivation

The calculated DNELs for all borate compounds addressed here and the applied assessment factors are shown in Table 65 to Table 68. For diboron trioxide the lowest DNEL was established from the boric compounds addressed in this chapter.

Table 64: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Fertility		
$POD_{Fertility\ effects}$ **(boric acid)**	**NOAEL: 100 mg/ kg bw/d** (corresponding to 17.5 mg B/kg bw/d; conversion factor: 0.175)	**This NOAEL for fertility effects results from a three generation study in rats (Weir (1966); supported as key study by RAC (2014c)) and is based on testicular atrophy and reduced fertility.**
Overall AFs	**200**	

AF for interspecies differences - remaining differences - allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	2	The default assessment factor for extrapolation from sub-chronic to chronic exposure duration was applied as the key study was a three-generation study (see section 4.8.5 in ECHA document "How to prepare toxicological summaries in IUCLID and how to derive DNELs").
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL
AF related to quality of database	1	Default value.
DNEL **general population, reproductive effects** (related to fertility effects)	**0.5 mg/kg bw/d**	
Development		
POD$_{\text{Developmental effects}}$ **(boric acid)**	**NOAEL: 55 mg/kg bw/d** (corresponding to 9.6 mg B/kg bw/d; conversion factor: 0.175)	**This NOAEL for developmental effects results from a prenatal developmental toxicity study in rats (Price et al. (1994); supported as key study by RAC (2014c)and EFSA (2013)) and is based on a reduction in the mean foetal bodyweight per litter and skeletal changes.**
Overall AFs	**100**	
AF for interspecies differences - remaining differences allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.

AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL
AF related to quality of database	1	Default value.
DNEL **general population, reproductive effects** (related to developmental effects)	**0.55 mg/kg bw/d**	

Table 65: Disodium tetraborate anhydrous (CAS No. 1330-43-4): Detailed Overview of the derivation of the $DNEL_{general\ population,\ reproductive\ effects}$

Description (AF=Assessment factor)	Value	Remark
Fertility		
$POD_{Fertility\ effects}$ **(disodium tetraborate anhydrous)**	**81.4 mg/ kg bw/d** (corresponding to 17.5 mg B/kg bw/d; conversion factor: 0.2149)	**This NOAEL for fertility effects results from a read across from boric acid and a three generation study in rats (Weir (1966); supported as key study by RAC (2014c)) and is based on testicular atrophy, reduced fertility.**
Overall AFs	**200**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	2	The default assessment factor for extrapolation from sub-chronic to chronic exposure duration was applied as the key study was a three-generation study (see section 4.8.5 in ECHA document "How to prepare toxicological summaries in IUCLID and how to derive DNELs").
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL
AF related to quality of database	1	Default value.
$DNEL_{general\ population,\ reproductive\ effects}$ (related to fertility effects)	**0.407 mg/kg bw/d**	
Development		
$POD_{Developmental\ effects}$ **(disodium tetraborate anhydrous)**	**NOAEL: 44.7 mg/ kg bw/d** (corresponding to 9.6 mg B/kg bw/d; conversion factor: 0.2149)	**This NOAEL for developmental effects results from a read across from boric acid and a prenatal developmental toxicity study in rats (Price et al. (1994); supported as key study by RAC (2014c) and EFSA (2013)) and is based on a reduction in the mean foetal bodyweight per litter and skeletal changes.**

Overall AFs	**100**	
AF for interspecies differences - remaining differences allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL.
AF related to quality of database	1	Default value.
DNEL **general population, reproductive effects** (related to developmental effects)	**0.447 mg/kg bw/d**	

Table 66: Tetraboron disodium heptaoxide, hydrate (CAS No. 12267-73-1): Detailed Overview of the derivation of the $DNEL_{general\ population,\ reproductive\ effects}$

Description (AF=Assessment factor)	Value	Remark
Fertility		
$POD_{Fertility\ effects}$ **(tetraboron disodium heptaoxide, hydrate)**	**117.9 – 154.3 mg/ kg bw/d** (corresponding to 17.5 mg B/kg bw/d, conversion factor: 0.1485 (pentahydrate); 0.1134 (decahydrate))	**This NOAEL for fertility effects results from a read across from boric acid and a three generation study in rats (Weir (1966); supported as key study by RAC (2014c)) and is based on testicular atrophy, reduced fertility.**
Overall AFs	**200**	
AF for interspecies differences - remaining differences - allometric scaling	2.5	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.

	4	
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	2	The default assessment factor for extrapolation from sub-chronic to chronic exposure duration was applied as the key study was a three-generation study (see section 4.8.5 in ECHA document "How to prepare toxicological summaries in IUCLID and how to derive DNELs").
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL
AF related to quality of database	1	Default value.
DNEL **general population, reproductive effects** (related to fertility effects)	**0.59 – 0.77 mg/kg bw/d**	
Development		
$POD_{Developmental\ effects}$ **(tetraboron disodium heptaoxide, hydrate)**	**NOAEL: 64.7 – 84.7 mg/ kg bw/d** (corresponding to 9.6 mg B/kg bw/d, conversion factor: 0.1484 (pentahydrate); 0.1134 (decahydrate)) (	**This NOAEL for developmental effects results from a read across from boric acid and a prenatal developmental toxicity study in rats (Price et al. (1994); supported as key study by RAC (2014c) and EFSA (2013)) and is based on a reduction in the mean foetal bodyweight per litter and skeletal changes.**
Overall AFs	**100**	
AF for interspecies differences - remaining differences allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.

AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL.
AF related to quality of database	1	Default value.
DNEL **general population, reproductive effects** (related to developmental effects)	**0.647 – 0.847 mg/kg bw/d**	

Table 67: Diboron trioxide (CAS No. 1303-86-2): Detailed Overview of the derivation of the DNELgeneral population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Fertility		
$POD_{Fertility\ effects}$ **(diboron trioxide)**	**56.3 mg/ kg bw/d** (corresponding to 17.5 mg B/kg bw/d), conversion factor: 0.311)	**This NOAEL for fertility effects results from a read across from boric acid and a three generation study in rats (Weir (1966); supported as key study by RAC (2014c)) and is based on testicular atrophy, reduced fertility.**
Overall AFs	**200**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	2	The default assessment factor for extrapolation from sub-chronic to chronic exposure duration was applied as the key study was a three-generation study (see section 4.8.5 in ECHA document "How to prepare toxicological summaries in IUCLID and how to derive DNELs").
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL.
AF related to quality of database	1	Default value.
DNEL **general population, reproductive effects** (related to fertility effects)	**0.28 mg/kg bw/d**	
Development		
$POD_{Developmental\ effects}$ **(diboron trioxide)**	**30.9 mg/kg bw/d** (corresponding to 9.6 mg B/kg bw/d, conversion factor: 0.311)	**This NOAEL for developmental effects results from a read across from boric acid and a prenatal developmental toxicity study in rats (Price et al. (1994); supported as key study by RAC (2014c) and EFSA (2013)) and is based on a reduction in the mean foetal bodyweight per litter and skeletal changes.**

Overall AFs	**100**	
AF for interspecies differences - remaining differences allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL.
AF related to quality of database	1	Default value.
DNEL **general population, reproductive effects** (related to developmental effects)	**0.309 mg/kg bw/d**	(Lowest DNEL for all borate compounds addressed here.)

9 Perboric acid, sodium salt (CAS No. 11138-47-9, 12040-72-1, 37244-98-7), sodium perborate (CAS No. 13517-20-9, 15120-21-5), sodium peroxometaborate (CAS No. 7632-04-0, 10332-33-9, 10486-00-7)

All three perborate compounds addressed in this section (perboric acid, sodium salt, sodium perborate and sodium peroxometaborate) are covered in one entry (Index 005-018-00-2 with CAS No. 13517-20-9, 37244-98-7, 10486-00-7) in Annex VI of the CLP Regulation and are classified as Repr. 1B (H360Df). CLH reports and RAC opinions are not available for these three perborates.

An EU Risk Assessment Report was published for perboric acid, sodium salt in 2007 (European Chemicals Bureau, 2007). In the report it is stated that sodium perborates are instable in water and in aqueous solutions at room temperature an equilibrium between sodium perborate and hydrogen peroxide/sodium metaborate is instantly established (see Figure 3). The hydrolysis degradation products such as hydrogen peroxide (i.e. boron) have been taken into account for classification.

Figure 3: Equilibrium between sodium perborate and hydrogen peroxide/sodium metaborate in aqueous solutions at room temperature (cited from EU RAR (European Chemicals Bureau, 2007))

Several subgroups of sodium perborates including sodium perborate monohydrate (CAS No.

15120-21-5, 10332-33-9) and sodium perborate tetrahydrate (CAS No. 10486-00-7, 13517-20-9) are assessed together in the EU risk assessment report (European Chemicals Bureau, 2007). Data is mainly available for sodium perborate tetrahydrate.

A registration dossier is available for perboric acid, sodium salt also based on data for the perborate compound sodium perborate tetrahydrate.

Based on their classifications as Repr. 1B, for all perborates addressed in this section, proposals for SVHC identifications and recommendations for inclusion in the authorization list exist. (Chemical Inspection Service, 2014) ECHA decided in 2014 to include these substances in the Candidate List for eventual inclusion in Annex XIV to REACH (Malm, 2014).

Perborate compounds addressed in this chapter are classified as Repr. 1B (H360 Df) related to developmental effects. Concerning fertility effects evidence is considered not to be sufficiently convincing to place the substance in Category 1. Thus, fertility effects of these compounds are considered to be not relevant for the present restriction proposal and are not addressed here.

9.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as perboric compounds do not possess a Repr. 1A/B classification related to adverse effects on sexual function and fertility.

9.2 Adverse effects on development

In the table below, relevant studies of perborate compounds on adverse effects on development are shown. Data query was based on the EU RAR (European Chemicals Bureau, 2007), on registration dossiers and on a recent literature search.

9.2.1 Animal data

Table 68: Key animal studies of perborate compounds on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal developmental toxicity study (according to OECD TG 414 and GLP) Oral (gavage) Rat (CRL:CD) 25 f/group	sodium perborate tetrahydrate 0, 100, 300, 1000 mg/kg bw/d Exposure: daily, GD 6 to 15	**NOAEL (P): 100 mg/kg bw/d** **LOAEL (P): 300 mg/kg bw/d** (based on reduced mean body weight gains and food consumption) **NOAEL (F1): 100 mg/kg bw/d** **LOAEL (F1): 300 mg /kg bw/d** (based on increase in resorptions, reduction in foetal body weights and placenta weights) (at 1000 mg/kg bw/d: increase in malformations related to skeletal and cardiovascular system)	Bussi (1995)

9.2.2 Human data

Epidemiological studies and other human data which allow determining a human "no effect level" for developmental effects of perborate compounds are currently not available.

9.2.3 Discussion of adverse effects of perborates on development

For the perborate compounds addressed in this section only one key study could be identified namely a developmental toxicity study with sodium perborate tetrahydrate according to OECD TG 414 (Bussi, 1995) and GLP. 25 pregnant rats were treated with sodium perborate tetrahydrate by the oral route (gavage) from day 6 to day 15 of gestation at dose levels of 0, 100, 300 and 1000 mg/kg bw/day. There were no clinical signs or behavioural changes and no deaths during the study. A statistically significant dose-related lower mean body weight gain and mean daily food consumption was observed in the >= 300 mg/kg bw/d treatment groups in the dams. At this dose level a dose-dependent increase of resorptions and lower mean foetal and placental weight was found in the offspring. At 1000 mg/kg bw/d also an increase of malformations in the offspring (mainly related to the skeleton and to the cardio-vascular system) was observed. The authors of the study established a **NOAEL of 100 mg/kg bw/d** for both dams and foetuses. In the EU RAR (European Chemicals Bureau, 2007) this NOAEL was supported. Effects in offspring were not considered to be a secondary non-specific consequence of other toxic effects.

No further studies performed with other perborate compounds than sodium perborate tetrahydrate were available.

9.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as perboric compounds do not possess a classification related to adverse effects on or via lactation.

9.4 Information taken into account for risk assessment and uncertainties

9.4.1 POD-selection

In the EU RAR (European Chemicals Bureau, 2007), the risk assessment for perborate compounds is carried out on sodium perborate tetrahydrate, as more data is available on this compound than on other perborate compounds. The chemicals differences were considered as minor, compared to other uncertainties in the evaluation of the data base. In accordance with EU RAR (European Chemicals Bureau, 2007) and as still data for other perborate compounds are lacking and due to similar hydrolysis products, it was decided to use the NOAEL found in a reliable OECD TG and GLP conform prenatal developmental toxicity study for sodium perborate tetrahydrate as POD for human risk assessment of all perborate compounds addressed in this section.

9.4.2 Uncertainty

The uncertainties of the POD for sodium perborate tetrahydrate are considered to be low. The study in rats was considered reliable which is in line with the assessments by the EU RAR (European Chemicals Bureau, 2007). Uncertainty for other perborate compounds is higher as data is lacking. However, hydrolysis is considered to lead to similar hydrolysis products (see Figure 3) and differences between perborates are considered to be minor (European Chemicals Bureau, 2007).

9.4.3 DNEL derivation

The calculated DNEL and applied assessment factors for all perborate compounds addressed

here are shown in the table below.

Table 69: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{Developmental\ effects}$ (perboric acid, sodium salt, sodium perborate, sodium peroxometaborate)	**NOAEL: 100 mg/ kg bw/d**	**The NOAEL for developmental effects results from read across approach from sodium perborate tetrahydrate and is based on a prenatal developmental toxicity study in rats (OECD TG 414, GLP;Bussi (1995); supported as key study by EU RAR (European Chemicals Bureau, 2007)) (increase in resorptions and reduction in foetal body weights).**
Overall AFs	**100**	
AF for interspecies differences - remaining differences - allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied.
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL
AF related to quality of database	1	Default value.
DNEL general population, , reproductive effects (related to developmental effects)	**1 mg/kg bw/d**	

10 (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol (CAS No. 94361-06-5)

Cyproconazole is listed in Annex VI of the CLP legislation as Repr. 2 with the hazard statement code H360d. However, there exists a recent CLH report and RAC opinion justifying classification as Repro 1B, H360D (Pesticide Control Service, 2014; RAC, 2015).

The key studies and respective NOAEL values for the endpoint reproductive toxicity of Cyproconazole as collated in the table below were selected based on the recent RAC opinion on

a CLH report mentioned above (Pesticide Control Service, 2014; RAC, 2015).

For the present restriction proposal a recent literature survey was performed from 2013, as the current CLH report on Cyproconazole was published in 2014.

Besides Repr. 1B the proposed entries related to human health for Cyproconazole in Annex VI of CLP regulation are: Acute Tox. 3 and STOT RE 2.

10.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as cyproconazole has no Repr. 1A/B classification related to adverse effects on sexual function and fertility.

10.2 Adverse effects on development

10.2.1 Animal data

Table 70: Key animal study on adverse effects of cyproconazole on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
two-generation reproduction toxicity study according to OECD TG 416, GLP oral exposure (dietary) rat (KFM-Wistar) 26/sex/group	cyproconazole 0, 4, 20, 120 ppm equiv. to: F0 (m/f): 0, 0.28/0.33, 1.39/1.67, 8.29/9.88 mg/kg bw day F1(m/f): 0.37/0.45, 1.77/2.16, 10.88/13.30 mg/kg bw/day	**LOAEL (P): 1.39/1.67 mg/kg bw/d (m/f)** : increased liver weight, liver fatty change → minimal parental toxicity **NOAEL (F1): 1.39/1.67 mg/kg bw/d** (**m/f)** LOAEL (F1): 8.29/9.88 **mg/kg bw/d** (**m/f)**(dose-related increase in pre/perinatal mortality in the high-dose groups in the F0 and F1 generation (16.3% and 12.6%, respectively). There was a corresponding slight increase in postnatal mortality (days 0 – 21 p.p) in the high-dose group of the F1 and F2 (8.1% and 7.6%, respectively))	Eschbach et al. (1987)
range-finding developmental toxicity test, GLP oral exposure (gavage), not published reliable	cyproconazole 0, 7.5, 30, 75 and 120 mg/kg bw/day treated from days 6-15 of gestation	**NOAEL (P): 7.5 mg/kg w/d** reduced maternal body weight gain in early treatment period **NOAEL (F1): 7.5 mg/kg bw/d** **LOAEL (F1) 30 mg/kg bw/d** (increased post implantation loss, reduced foetal body weight; malformations (cleft palate); however, no statistical evaluation of data has been performed) The two higher dose levels in this range finding study were considered to be excessively toxic	Becker (1985a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
rat (Wistar HAN) 5 mated f/group			
developmental toxicity study according to OECD TG 414, GLP oral exposure (gavage) not published reliable rat (Wistar HAN) 25 mated f/group	cyproconazole 0, 6, 12, 24 and 48 mg/kg bw/d treated from days 6-15 of gestation	**NOAEL (P): 12 mg/kg bw/d** reduced maternal body weight gain in early treatment period. **NOAEL (F1): 12 mg/kg bw/d** **LOAEL (F1): 24 mg/kg bw/d** (increased post-implantation loss, reduced foetal body weight; malformations (cleft palate, hydrocephali); retarded ossification)	Becker (1985b)
developmental toxicity study equivalent to OECD TG 414 oral exposure (gavage) reliable with restrictions strain not stated 20 mated f/group	cyproconazole 20, 50 and 75 mg/kg bw/d treated from days 6-16 of gestation	**NOAEL (P): could not be determined** **LOAEL (P): 20 mg/kg bw/d** reduced maternal body weight gain in early treatment period at all doses, marked at 75 mg/kg bw/d **NOAEL (F1): could not be determined** **LOAEL (F1): 20 mg/kg bw/d** reduced mean foetal weight from 20 mg/kg bw/d increased post-implantation loss, from 50 mg/kg bw/d; malformations (cleft palate, hydrocephali) from 20 mg/kg bw/d; retarded ossification from 50 mg/kg bw/d.	Machera (1995)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Limited maternal data. 75 mg/kg bw/d clearly toxic but effects at <75 mg/kg bw/d not clear.	
developmental toxicity study according to OECD TG 414, GLP oral exposure (gavage), not published reliable rabbit (Chinchilla rabbit) 16 mated f/group	cyproconazole 0, 2, 10, and 50 mg/kg bw/d treated from days 6-18 of gestation	**NOAEL (P): 10 mg/kg bw/d** **LOAEL (P): 50 mg/kg bw/d** (based on loss of maternal body weight and reduced food consumption in early treatment period) **NOAEL (F1): 10 mg/kg bw/d** **LOAEL (F1): 50 mg/kg bw/d** (based on increased post implantation loss)	Becker (1986b)
Developmental tox study according to OECD TG 414, GLP oral exposure (gavage), not published reliable rabbit (NZW) 18 mated	cyproconazole 0, 2, 10, 50 mg/kg bw/d treated from days 6-18 of gestation	**NOAEL (P): 10 mg/kg bw/d** loss of maternal body weight and reduced food consumption in early treatment period **NOAEL (F1): 2 mg/kg bw/d** **LOAEL (F1): 10 mg/kg bw/d** increased incidence of foetal malformations not available for assessment leading to current Annex VI classification	Muller (1991)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
f/group			

10.2.2 Human Data

No human data available.

10.2.3 Short discussion and overall relevance of the provided information on adverse effects on development

The information and data provided are partly extracted from the CLH report for Cyproconazole and the respective RAC opinion (Pesticide Control Service, 2014; RAC, 2015).

The selected key studies are thoroughly described in the CLH report on Cyproconazole (CLH 2014) and summarized in Table 71.

In the study by Eschbach et al. (1987) a dose-related increase in the pre-/perinatal mortality in F1 pups was reported at the mid and high dose group [13.6% and 16.3%, respectively vs. 10.7% in controls (high mortality in controls)]. Pre-/perinatal mortality is not reported as such in the study but on a general basis may include post-implantation losses, stillbirths and neonatal deaths. At the mid dose, a single female lost all her pups (12/13 on pre-/perinatal losses, 1/13 shortly after birth), which may explain the increase at this dose level according to the DS. However, one total litter loss also occurred in one F0 female in the high dose group (day 5 post-partum) as well as in one F1 female of the high dose group (day 4 post-partum). Additionally, at the high dose, this finding was also observed in F2 pups: the pre/perinatal mortality was increased (12.6% vs 11.3% in controls). An increase in post-natal mortality was also reported in both generations (dose-related in F0: 1.6, 6.6% and 8.1%, respectively vs 0.3% in controls; high dose only in F2: 5.9%, 2.9% and 7.6%, respectively, vs 2.2% in controls).

Increased post-implantation loss is a treatment-related effect frequently observed in most studies with rats and rabbits (Becker, 1985a; Becker, 1985b; Becker, 1986b; Machera, 1995). Other developmental effects after maternal exposure to Cyproconazole encompass reduced foetal body weight (Becker, 1985a; Becker, 1985b; Machera, 1995), malformations (e.g. cleft palate, hydrocephali) (Becker, 1985b; Machera, 1995; Muller, 1991)and retarded ossification (Becker, 1985b; Machera, 1995). The lowest NOAEL for developmental effects (1.39 mg/kg bw/d) was derived in the study by Eschbach et al. (1987) for a dose-related increased in the pre-/perinatal mortality in F1-pups.

10.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as cyproconazole has no classification related to adverse effects on or via lactation.

10.4 Information taken into account for risk assessment and uncertainties

10.4.1 POD-selection

The NOAEL of the study by Eschbach et al. (1987) is used as point of departure (POD) for the DNEL calculation because this study is considered reliable (OECD TG 416, GLP) and delivers the

lowest NOAEL available: 1.39 mg/kg bw/d.

10.4.2 Uncertainty

No obvious uncertainties could be identified.

10.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in the table below. Assessment factors were selected based on the Guidance on information requirements and chemical safety assessment Chapter R.8 (ECHA, 2012) (Interspecies-remaining difference: 2.5, allometric scaling for rats: 4 (mouse: 7), intraspecies general population: 10, LOAEL instead of NOAEL: 3 (if applicable)).

Table 71: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
POD Developmental effects	**NOAEL: 1.39 mg/kg bw/d**	**OECD TG 416, GLP, effects: dose-related increase in pre/perinatal mortality in the high-dose groups in the F0 and F1 generation (16.3% and 12.6%, respectively)** **(Eschbach et al., 1987)**
Overall AFs	**100**	
AF for interspecies differences - remaining differences allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL.
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to developmental effects)	**0.0139 mg/kg bw/d (13.9 µg/kg bw/d)**	

11 (4-ethoxyphenyl)(3-(4-fluoro-3-phenoxphenyl)propyl)dimethylsilane (CAS No. 105024-66-6)

(4-ethoxyphenyl)(3-(4-fluoro-3-phenoxphenyl)propyl)dimethylsilane has a harmonised classification as toxic for reproduction, Repr. 1B (H360F) (Index No:014-063-00-X).

No CLH report is available for (4-ethoxyphenyl)(3-(4-fluoro-3-phenoxphenyl)propyl)dimethylsilane and no data could be found in the ECHAs`CLH archive. The substance is not registered under REACH. Moreover, no relevant data were found in a literature research performed as described in section 5.9.1.

Thus, no key study for toxicity to reproduction could be identified and it was not possible to derive a DNEL.

12 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one ((R)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone (CAS No. 5543-58-8) and (S)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-2-benzopyrone (CAS No. 5543-57-7))

Both substances are listed in Annex VI of the CLP legislation as: Repr. 1A; H360D and are R- and S-enantiomers of the anticoagulant and rodenticide warfarin (CAS# 81-81-2; EC / List no.: 201-377-6), which is also listed in Annex VI of CLP as: Repr. 1A; H360D. Warfarin consists of a racemic mixture of the R- and S-enantiomers of 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one in roughly equal proportion (Hirsh et al., 2003). Warfarin exerts anticoagulant activity via vitamin K antagonistic effects while the S-isomer is up to 3.4-times more potent than the R-isomer (Reilly, 1974). Despite the discrepancy in potency, for both isomers the racemic mixture is considered in the current report due to an identical mechanism of action (Hirsh et al., 2003). Additionally, animal studies with the separate isomers were not found and available human data is based predominantly on warfarin, too.

The key studies and respective dose levels for the endpoint reproductive toxicity of Warfarin as shown in Table 73 and Table 74 were selected based on the recent RAC opinion on a CLH report (Pesticide Registration and Control Division, 2012; RAC, 2014a).

For the present restriction proposal a recent literature survey was performed from 2011 to now, as the CLH report on Warfarin was published in 2012.

12.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as the substances do not have Repr. 1A/B classification related to adverse effects on sexual function and fertility.

12.2 Adverse effects on development

12.2.1 Animal data

The current classification for reproductive toxicity relies entirely on human clinical evidence. Thus, animal studies are not reiterated in this report but are available in the RAC opinion for warfarin (RAC, 2014a).

12.2.2 Human data

Table 72: Summary table of human data on adverse effects on development extracted from Hall et al. (Hall et al. 1980)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations in offspring	Reference
human clinical data	warfarin	patient treatment: warfarin (7.5 mg/day), digitalis, penicillin time of treatment: preconception to 31 weeks	nasal hypoplasia mental retardation brachydactyly scoliosis and other skeletal abnormalities	Kerber et al. (1968)
human clinical data	warfarin	patient treatment: warfarin sodium (av. 6.25 mg/day), penicillin, digoxin time of treatment: preconception to 36 weeks	normal female	Bloomfield and Rubinstein (1969)
human clinical data	warfarin	Warfarin sodium (2.5-5 mg/day), diazapam (briefly), furosemide (2 wks at 26 weeks)	nasal hypoplasia vertebral stippling	Shaul et al. (1975)
human clinical data	warfarin	patient treatment: 1. warfarin (-), digoxin, sulfisoxazole, erythromycin time of treatment: preconception to 26 weeks patient treatment: 2. warfarin (7.5 mg/day), digoxin time of treatment: throughout, pregnancy	1. nasal hypoplasia, optic atrophy, mental retardation, kyphoscoliosis 2. shortened proximal, extremities, nasal hypoplasia, opacification of optic lens, poorly developed ears, punctate calcification of vertebra and epiphyseal regions	Becker et al. (1975)
human clinical data	warfarin	patient treatment: warfarin sodium (5 mg/day), digoxin, furosemide, potassium isoptin time of treatment: preconception to week 36 week	nasal hypoplasia choanal stenosis short fingers, dysplastic nails chondrodysplasia punctata	Fourie and Hay (1975)
human	warfarin	patient treatment:	nasal hypoplasia	Barr and Burdi (1976)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations in offspring	Reference
clinical data		warfarin sodium (7.5 mg/day), propranolol time of treatment: preconception to 17 weeks (elective abortion)	large protuberant eyes short fingers hypertelorism	
human clinical data warfarin	warfarin	patient treatment: Warfarin (20 mg –3 mg – 4.5 mg/day) time of treatment: wk 12.5 to wk 36	microcephaly bifrontal narrowing mental retardation spastic	Carson and Reid (1976)
human clinical data warfarin	warfarin	patient treatment: warfarin (-) time of treatment: 6mths preconception to wk 12 of gestation	no abnormalities apparent at birth retarded psycomotor development at 5 mths	Holzgreve et al. (1976)
human clinical data warfarin	warfarin	patient treatment: warfarin (6-7 mg/day) time of treatment: preconception to wk 24 of gestation	nasal hypoplasia epiphyseal stippling chonrodysplasia punctata	Abbott et al. (1977)
human clinical data warfarin	warfarin	patient treatment: warfarin (-) time of treatment: throughout pregnancy	nasal hypoplasia hypertelorism tachycardia hepatomegaly generalised oedema	Smith and Cameron (1979)
human clinical data warfarin	warfarin	patient treatment: warfarin (5 mg/day) time of treatment: throughout pregnancy	nasal hypoplasia optic atrophy developmental retardation	Stevenson et al. (1980)

12.2.3 Additional data

Table 73: Compilation and analysis of literature on warfarin embryopathy in humans

Type of study/data	**Test substance,**	**Observations**			**Reference**
		no of pregnancies/ live births	no of embryopathies	% of embryopathies	
literature review	warfarin	637 472 84	28 44 2	4.4% 9.3% 2.4%	Hung and Rahimtoola (2003)
metastudy by use of 7 case series	warfarin	792 549 224	35 35 16	4.4% 6.4% 7.1%	Blickstein and Blickstein (2002)
literature review	warfarin	224	16	7.1%	Hall et al. (1980)
multi-centre (n=12), observational, prospective study	warfarin	66 356	0 2	0% 0.6%	Schaefer et al. (2006)
clinical study	warfarin	71	4	5.6%	Cotrufo et al. (2002)
study based on questionnaires sent to major cardiac centres in the UK	warfarin	11	1	9.1%	Oakley and Doherty (1976)
pregnancy study	warfarin	18	0	0%	Arnaout et al. (1998)
literature review	warfarin	30	3	10%	Srivastava et al. (2002)
prospective pregnancy study	warfarin	150	0	0%	Geelani et al. (2005)
retrospective pregnancy study	warfarin	142	7	4.9%	Khamooshi et al. (2007)
pregnancy study	warfarin	43	0	0%	Akhtar et al. (2007)
pregnancy study based on questionnaires sent to	warfarin	11	1	9.1%	Shannon et al. (2008)

Type of study/data	**Test substance,**	**Observations**			**Reference**
		no of pregnancies/ live births	no of embryopathies	% of embryopathies	
women treated with warfarin					
summary of the cited literature reviews		**2279**	**97**	**4.3%**	

12.2.4 Short discussion and overall relevance of the provided information on adverse effects on development

The information and data provided are partly extracted from the CLH report for warfarin and the RAC opinion (Pesticide Registration and Control Division, 2012; RAC, 2014a).

In the review by Hall et al. (1980), retrospective summaries of case reports in which the administration of warfarin during pregnancy induced birth defects were presented, together with a description of the encountered malformations or other effects, and the dosage of warfarin involved. The duration of exposure in most of the 22 cases reviewed in detail by Hall et al. (1980) extends far beyond the first trimester (> week 30 of gestation). The daily dose of Warfarin was usually between 5-10 mg/day, only in one case at 2.5-5 mg/day. Table 73 summarizes case reports collated in Hall et al. (1980) and represent a selection from the published literature of warfarin-associated adverse developmental outcomes.

The administration of warfarin to women during pregnancy has been shown to cause a well-defined complex of malformations in some of the offspring. This occurs as a result of exposure during the first trimester. This syndrome has been designated as warfarin embryopathy or foetal warfarin syndrome (FWS). The most consistent feature of FWS is a hypoplastic nose, caused by underdeveloped nasal cartilage. The degree of severity is varied from mild abnormality to severe breathing and feeding difficulties. Bone abnormalities of the axial and appendicular skeleton (radiological stippling of the vertebral column) often also occur. Punctate calcification of other bone sites may also be present. Kyphoscoliosis, abnormal skull development, and brachydactyly have been observed as associated skeletal effects. It is believed that avoidance of exposure to anticoagulants during weeks 6-12 of gestation should avoid warfarin embryopathy. It should be noted that exposure to coumarins during the first trimester was associated with a high rate of spontaneous abortions, in addition to the incidences of specific embryopathy. Likewise, exposure during the first and second trimester was also associated with a high rate of spontaneous abortion, stillbirths and warfarin-related complications (developmental abnormality) (Hall et al., 1980). Exposure after this time interval (first trimester) is associated with an apparently separate series of warfarin-related adverse effects, not related to warfarin embryopathy, per se. Adverse effects on the central nervous system predominate and include hydrocephaly or microcephaly, microphthalmia, various eye abnormalities, Dandy-Walker malformation and other CNS malformations often associated with degrees of mental retardation (Kaplan, 1985; Pati and Helmbrecht, 1994).

The risk of adverse foetal effects due to warfarin treatment in humans is difficult to estimate, due to the inhomogeneous data base: Some review articles evaluate complication rates in

relation to pregnancies, others to live births, and this cannot always be resolved, due to incomplete information given in some articles. Nevertheless, since the number of pregnancies is predominantly referred to, this approach is adopted for the current overall evaluation. In case of significant overlap between review articles only the most comprehensive and reliable one was considered for deriving an overall foetal complication rate based on most recent data, resulting in the selection presented in Table 74. Furthermore, the data base has been restricted to Warfarin exposures only (ignoring other anticoagulants, e.g. Acenocoumarol) where possible.

Accordingly, based on the available data the risk for embryopathy due to Warfarin treatment in sensitive periods of gestation is 4.3%, relative to the number of pregnancies. This is in agreement with other authors, estimating the malformation risk to be "probably below 5%" (de Swiet, 1987), or otherwise frequently in the range of 4–7% (Table 74).

Other significant risks to the foetus or the newborn are associated with Warfarin treatment: Spontaneous abortion (27.3%, aggregated figure based on (Arnaout et al., 1998; Blickstein and Blickstein, 2002; Khamooshi et al., 2007; Oakley and Doherty, 1976; Shannon et al., 2008)), stillbirth (27.1%, based on the same articles except (Oakley and Doherty, 1976)), neonatal death (3.1%; (Arnaout et al., 1998; Blickstein and Blickstein, 2002; Khamooshi et al., 2007; Oakley and Doherty, 1976), CNS defect (4.33%; (Hall et al. 1980, Oakley and Doherty 1976)), premature delivery (66.2%; (Blickstein and Blickstein, 2002; Hall et al., 1980)), haemorrhage (2.2%; (Hall et al., 1980)), and ocular atrophy (Hall et al., 1980).

12.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as the substances do not have a classification related to adverse effects on or via lactation.

12.4 Information taken into account for risk assessment and uncertainties

12.4.1 POD-selection

Doses of 2.5 mg/day (0.04 mg/kg bw/day, human female bodyweight of 60kg) have been reported to result in nasal hypoplasia and vertebral stippling. Higher doses have resulted in a high percentage of embryofoetal mortality (A NOAEL cannot be set and the value of 0.04 mg/kg bw/day represents a LOAEL which in turn approximates to an ED10 value). This value has been used in setting the specific concentration limits for reproductive toxicity of warfarin: 0.003% (RAC 2014a). Therefore, this dose level (0.04 mg/kg bw/day) is used as point of departure (POD) in the current restriction proposal to derive the DNEL.

12.4.2 Uncertainty

It has been clearly demonstrated that Warfarin is both teratogenic and causes developmental toxicity when administered to pregnant women but there are some uncertainties regarding the selected dose level. The dose range reported in the submitted literature is from 2.5 to 20 mg/day, with 5.0-7.5 mg/day being the most frequently used dose level. The dose prescribed relates to the prothrombin clotting times in individual patients and cannot exactly be related to mg/kg/day dose level. The dose levels were not reported in some papers submitted. It is noted that the exact nature of the prescribed drug, e.g., chemical identity and purity, is not reported in all cases.

12.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in Table 75. Assessment factors were selected based on the Guidance on information requirements and chemical safety assessment Chapter R.8 (interspecies-remaining difference: 1, allometric scaling: 1, intraspecies

general population: 10, LOAEL instead of NOAEL: 3).

Table 74: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{Developmental\ effects}$ (Warfarin)	**LOAEL: 0.04 mg/kg bw/d**	**Clinical observation, nasal hypoplasia and vertebral stippling in offspring after warfarin application during pregnancy (Shaul et al., 1975)**
Overall AFs	**30**	
AF for interspecies differences - remaining differences allometric scaling	1 1	No AF for interspecies differences was applied because the LOAEL is based on clinical observations in humans.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	3	AF of 3 was applied as the POD is a LOAEL
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to developmental effects)	**0.001 mg/kg bw/d**	

13 1-methyl-2-pyrrolidone (CAS No. 872-50-4)

1-methyl-2-pyrrolidone (NMP) is listed in Annex VI of the CLP legislation as Repr. 1B (H360D) and is included in the candidate list for substances of very high concern and restricted in entry 30 of Annex XVII to REACH. Most information is extracted from the CLH report and RAC opinion for NMP (RAC, 2014b; RIVM, 2013a).

The key studies and respective NOAEL value for the endpoint reproductive toxicity of NMP as shown in Table 76 were selected based on the recent RAC opinion on a CLH report mentioned above (RAC, 2014b; RIVM, 2013a).

For the present restriction proposal a recent literature survey was performed from 2012, as the current RAC opinion on NMP was published in 2013.

Besides Repr. 1B NMP is classified as Skin Irrit. 2, Eye Irrit. 2 and STOT SE 3.

13.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as NMP has no Repr 1A/B classification related to adverse effects on sexual function and fertility.

13.2 Adverse effects on development

13.2.1 Animal data

Table 75: Summary table of animal studies on adverse effects of 1-methyl-2-pyrrolidone on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal developmental toxicity study,OECD TG 414 oral (gavage) rat (Sprague-Dawley) 25–27 time-mated females (21–25 pregnant)/group	1-methyl-2-pyrrolidone (NMP) 0, 125, 250, 500, 750 mg/kg bw/day exposure on GD 6–20	**NOAEL (P) 250 mg/kg bw/day** **NOAEL (F1) 125 mg/kg bw/day** **LOAEL (F1): 250 mg/kg bw/d**(reduced foetal body weight) *maternal effects:* ≥500 mg/kg bw/d: significantly reduced maternal body weight + maternal food consumption *foetal effects:* ≥500 mg/kg bw/d: significantly increased incidence of (litters with) malformed foetuses ≥250 mg/kg: significantly reduced foetal body weight	Saillenfait et al. (2002)
Prenatal developmental toxicity study oral (gavage) rabbit (New Zealand White) 20 inseminated rabbits/group	1-methyl-2-pyrrolidone (NMP) 0, 55, 175 and 540 mg/kg bw/day daily exposure on GD 6-18	**NOAEL (P): 55 mg/kg bw/d** **NOAEL (F1): 175 mg/kg bw/d** **LOAEL (F1) 540 mg/kg bw/d(**significantly increased post-implantation loss, reduced live litter size and reduced mean uterine weight, significantly increased cardiovascular + skeletal malformations) maternal effects: 175 mg/kg bw/d: significantly reduced body weight gain (GD 6-12) 540 mg/kg bw/d: significantly reduced food consumption (GD 6-19) foetal effects: 540 mg/kg bw/d: one abortion, significantly increased post-implantation loss, reduced live litter size and reduced mean uterine weight, significantly increased cardiovascular + skeletal malformations	IRDC (1991)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
two-generation reproduction toxicity study oral: feed rat (Wistar) 25 pairs/group	2-pyrrolidone (NMP) 0, 50, 160,350 mg/kg bw/day (nominal in diet) (high dose reduce from 500 to 350 mg/kg bw/d due to severe pup mortality in the first litter (F1a pups)) exposure: F0: 10 weeks premating, mating, gestation/lactation and rest period of F1a and F1b offspring F1: after weaning during 10 weeks premating, mating, gestation /lactation and rest period F2a/F2b offspring F2: until weaning Duration of test: approx. 54 weeks	**NOAEL:** **developmental toxicity: 160 mg/kg bw/day (male/female, F1 and F2** **generation: reduced body weight gain, increased pup mortality)** high dose level reduced from 500 to 350 mg/kg bw/day due to severe pup mortality in the first litter (F1a pups) no adverse effects on fertility and reproduction in all groups no substance-related adverse effects at 50 and 160 mg/kg bw/day (F0, F1a/b, F2, F2a/b males/females) 500/350 mg/kg bw/day: significantly reduced body weight gain and food intake (P0, F1); renal toxicity (organ weights and histopathology); increased pup mortality, reduced body weight gain in pups	BASF (1999)
modification of one-generation reproduction toxicity study (OECD TG 415) reliable study rat (Wistar) 24-28 females/group	2-pyrrolidone (NMP) 0, 150, 450, 1000 mg/kg bw/d exposure: rats were exposed 5 days/week for about 9 weeks (2 weeks before mating (only females) and 1 week of mating, 3 weeks of gestation, and 3 weeks of lactation	**NOAEL (F1): could not be determined** **LOAEL (F1): 150 mg/kg bw/d (reduced survival of pups, reduced body weight)** **NOAEL (P): could not be determined** **LOAEL (F1): 150 mg/kg bw/d: significantly reduced body weight** maternal effects -1000 mg/kg bw/d: reduced food and water consumption during the first week of mating (water only) and on days 0, 13 (food only) and 20 of gestation foetal effects: -1000 mg/kg bw/d: significantly reduced number of	Sitarek et al. (2012)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		live pups ≥450 mg/kg bw/d: significantly reduced fertility index ≥150 mg/kg bw/d: significantly reduced survival of pups, reduced bodyweight at day 4	

13.2.2 Human data

Table 76: Summary table of human data on adverse effects on development

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
case report	NMP	a 23-year old pregnant woman, 9 weeks pregnant with her first child, sustained both occupational dermal contact and repeated inhalational exposure to NMP throughout her first trimester of pregnancy no exposure concentration available	early intrauterine growth retardation late miscarriage (stillborn child)	Solomon et al. (1996)

13.2.3 Short discussion and overall relevance of the provided information on adverse effects on development

The information and data provided are partly extracted from the CLH report for NMP (RAC, 2014b; RIVM, 2013a).

The selected key studies are thoroughly described in the CLH report for NMP (RIVM, 2013a) and summarized in Table 77. All studies clearly demonstrated developmental effects of the offspring in rats and rabbits (BASF, 1999; IRDC, 1991; Saillenfait et al., 2002; Sitarek et al., 2012). Most common effects were significantly reduced foetal body weight gain in rats (BASF, 1999; Saillenfait et al., 2002; Sitarek et al., 2012), significantly increased incidence of (litters with) malformed foetuses (IRDC, 1991; Saillenfait et al., 2002), increased post-implantation loss and pub mortality.

The Repr. 1B classification with the hazard statement code H360D is supported by a case study demonstrating a case of a late miscarriage in a woman who sustained both occupational dermal

contact and repeated inhalational exposure to NMP throughout her first trimester of pregnancy (Solomon et al., 1996) (see Table 78).

The lowest LOAEL was found in the study by Sitarek et al. (2012) in a one-generation reproduction toxicity study with Wistar rats (modified after OECD TG 415): 150 mg/kg bw/d (reduced survival of pups, reduced body weight, no NOAEL derived).

The lowest NOAEL could be derived from the OECD TG 414 (prenatal developmental toxicity) study by Saillenfait et al. (2002) with Sprague- Dawley rats: 125 mg/kg bw/d (reduced foetal body weight).

13.3 Adverse effects on lactation

Not relevant for the present restriction proposal as NMP has no classification related to adverse effects on or via lactation.

13.4 Information taken into account for risk assessment and uncertainties

13.4.1 POD-selection

The LOAEL of the study by Sitarek et al. (2012) is used as point of departure (POD) for the DNEL calculation because this study is considered reliable and delivers the lowest LOAEL available: 150 mg/kg bw/d.

13.4.2 Uncertainty

No obvious uncertainties could be identified.

13.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in the table below.

Table 77: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{Developmental\ effects}$	**LOAEL: 150 mg/kg bw/d**	**one-generation reproduction toxicity study with Wistar rats (modified after OECD TG 415): reduced survival of pups, reduced body weight** **(Sitarek et al., 2012)**
Overall AFs	**300**	
AF for interspecies differences - remaining differences allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	3	AF of 3 was applied as the POD is a LOAEL
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to developmental effects)	**0.5 mg/kg bw/d**	

14 N,N-(dimethylamino)thioacetamide hydrochloride (CAS No. 27366-72-9)

Synonyms: DMATA

DMATA is listed as a hazardous substance in Annex VI of the CLP regulation with the classification as Repr. 1B (H360D). DMATA is currently not a candidate for the SVHC list.

No CLH report is publically available for DMATA and no protocols could be found in the ECHAs`CLH archive. The substance is not registered under REACH, yet. Moreover, no relevant data were found in a literature research performed as shortly described in section 5.9.1.

No literature or data were identified that would enable the derivation of a DNEL for reproductive toxicity for this substance.

15 1,2-diethoxyethane (CAS No. 629-14-1)

Synonyms/Common names: Ethylene glycol diethyl ether, EGDEE, EGdiEE, glyme ethyl

Ethylene glycol diethyl ether (EGDEE) is a member of the ethyl ether class of industrial solvents, widely used in manufacture of protective coatings. Experimental animal studies have shown that some glycol ethers cause congenital malformations, prenatal mortality, male reproductive effects and other developmental problems whereas others have not.
EGDEE is listed as a hazardous substance in part 3 of Annex VI, Table 3.1 (list of harmonized classification and labelling of hazardous substances to CLP indicating the classification as Repr. 1A (H360Df). However, it should be noted that there is a mistake regarding this classification due to a technical error which occurred whilst adapting the previous classification scheme to the new one (ECHA, 2012). The correct classification is Repr. 1B, H360Df.

Meeting the criteria of Article 57 (c) of Regulation (EC) 1907/2006 REACH (EU 2006) owing to its afore mentioned Repr. 1B classification EGDEE has been included in the Candidate list of Substances of Very High Concern.

As no CLP report is publically available a literature search was performed using search engines e.g. PubMed, Scopus, TOXLINE, EMBASE and ChemIDplus Advanced. Additionally, data resources hosted by ECHA, NTP, EFSA and EPA have been mined for toxicological information on EGDEE.

Supportive documentation (CWG, 2004) has been provided by ECHA.

15.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as EGDEE has no Repr. 1A/B classification related to adverse effects on sexual function and fertility.

15.2 Adverse effects on development

15.2.1 Animal data

Table 78: Summary table of animal studies on adverse effects of 1,2-diethoxyethane on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
developmental toxicity test (preliminary short term reproductive testing) non-guideline conform oral (gavage) Pregnant CD 1-mice 50/group	EGDEE 0 and 2955 mg/kg/d as 10 ml/kg bw per volume Daily GD 7-14	only one dose tested 10% (5/50) maternal toxicity (no further information on maternal toxicity) Mice treated showed a significant reduction in viable litter decrease in postnatal survival, pub weight at birth and pup body gain weight on postnatal days 1-3 (sample size too small to analyse statistically)	Schuler R. L. (1984)
developmental toxicity test (preliminary reproductive testing) non-guideline conform Pregnant CD 1-mice (50/group)	EGDEE 0 and 2955 mg/kg/d as 10 ml/kg body weight per volume (in water) Daily by oral gavage GD 6-13	only one dose tested 10% (5/50) maternal toxicity (no further information on maternal toxicity) Mice treated showed a significant reduction in viable litter decrease in postnatal survival, pub weight at birth and pup body gain weight on postnatal days 1-3 (sample size too small to analyse statistically)	Hardin et al. (1987)
Prenatal developmental toxicity study OECD TG 414 (to be noted: exposure started on GD 6 instead of GD 5)	EGDEE 0, 50, 150, 500, 1000 mg/kg bw/d in distilled water daily by oral	**NOAEL (P)500 mg/kg bw/d** (reduced body weight and body weight gain) **NOAEL (F1): 50 mg/kg bw/d** **LOAEL (F1) 150 mg/kg bw7d** (increase in one or more malformed foetuses)	George et al. (1988), (1992)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Reliable CD 1 outbred Albino Swiss mice 25/26 females/dose	gavage GD 6-15 termination: GD 17	≥500 mg/kg bw/d foetal body weight was reduced, malformation incidence was increased (Exencephaly and fused ribs)	
Prenatal developmental toxicity study OECD TG 414 Reliable New Zealand white rabbits 25/26 females/dose	EGDEE 0, 25, 50, 100 mg/kg bw/d in distilled water daily by oral gavage GD 6-19 termination: GD 30	**NOAEL(P) ≥ 100 mg/kg bw/d** **NOAEL (F1) ≥ 25 mg/kg bw/d** (based on increase in malformations) *Maternal toxicity:* Minimal effect on maternal weight gain (100 mg/kg bw/d) appeared to be secondary to an increased incidence in resorptions *Developmental toxicity:* ≥50 mg/kg bw/d increase in malformations (short tail, small spleen, fused sternebrae and rib cartilage)	George et al. (1988), (1992)

15.2.2 Human data

Epidemiological studies which allow deriving a NOAEL for EGDEE for adverse health effects in humans are currently lacking. Additionally, a NOAEL cannot be inferred from structurally related glycol ethers due to the absence of appropriate human toxicokinetic studies.

Toxicological relevant information on the toxicity of EGDEE on reproductive development is scarce. A CLP report outlining the decision making process for the classification of EGDEE as Repr. 1B is unavailable.

The identification of the key study by George et al. (1988); (J., 1992) is based on information in the supportive documentation obtained from ECHA. Study details are also published on the website of the National Toxicology Program of The US Department of Health and human Services.

The prenatal developmental toxicity study was performed in good agreement with OECD TG 414 and the toxicity of EGDEE was assessed both in mice and rabbits. Pregnant mice were treated by oral gavage with increasing doses of EGDEE (0, 50, 150, 500, 1000 mg/kg bw/d) in water. No maternal deaths, morbidity, or distinctive clinical signs were observed. Specific dose-related effects on embryo/foetal development were apparent which were independent from maternal toxicity. Maternal effects were observed only at the highest dose (1000 mg/kg bw/d) whereas at lower doses reductions in the foetal body weights and malformations were observed, respectively (≥500 mg/kg bw/d; ≥150 mg/kg bw/d). A NOAEL of 50 mg/kg bw/d could be

derived from this study.

Pregnant New Zealand rabbits were exposed to increasing doses of EGDEE (0, 25, 50, 100 mg/kg bw/d) in water by oral gavage. Mild clinical signs were observed in pregnant animals at 50 mg/kg bw/d EDGEE and included transient weight loss, lacrimation and diarrhoea. The percentage litters with one or more malformed foetuses was significantly elevated over the control group at both 50 and 100 mg/kg bw/d. The 25 mg/kg bw/d dose was the NOAEL for developmental toxicity in rabbits.

15.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as EGDEE has no classification related to adverse effects on or via lactation.

15.4 Information taken into account for risk assessment and uncertainties

15.4.1 POD-selection

The study by George et al. (1988); (J., 1992) was considered to be the key study for developmental toxicity of EGDEE. The induced adverse developmental effects both in rabbits and in mice are considered to be relevant to humans. The derived NOAELs are used as a point of departure (POD) for the DNEL calculation based on the reliability of the study which has been conducted in good agreement with OECD TG 414.

15.4.2 Uncertainties

No uncertainties were identified.

15.4.3 DNEL derivation

The calculated DNELs and applied assessment factors (AF) are shown in the table below.

Table 79: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
POD **developmental effects** **(mice)**	**NOAEL:50 mg/kg bw/d**	The NOAEL for adverse effects on development was derived from a prenatal developmental study in mice which was performed in compliance with OECD TG 414 (George et al., 1988; J., 1992). Dose-related adverse effects on number of litters with malformed foetuses, foetal body weight and malformation incidence (Exencephaly, fused ribs) were observed.
Overall AFs	**175**	
AF for interspecies differences - remaining differences - allometric scaling	2.5 7	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	A default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	Not relevant
AF related to dose response relationship	1	Not relevant
AF related to quality of database	1	Default value.
$DNEL_{general\ population,\ ,\ reproductive\ effects}$ **(related to developmental effects)**	**0.29 mg/kg bw/d**	

16 1-ethylpyrrolidin-2-one (CAS No. 2687-91-4)

NEP is used as a solvent, catalyst and cationic surfactant in industry.

N-ethyl-2-pyrrolidone (NEP) is listed in ANNEX VI of the CLP Regulation (EC) 1272/2008 as Repr. 1B; H360D. NEP is currently not listed in the Candidate list.

The key studies and respective dose levels for the endpoint reproductive toxicity of NEP are listed in Table 81 and were selected based on the recent CLH report (ANSES, 2011a) and the corresponding RAC opinion (RAC, 2011d). A literature research has been performed to potentially

identify more recent scientific information on the toxicity of the substance. Search engines e.g. PubMed, Scopus, TOXLINE, EMBASE and ChemIDplus Advanced and data resources hosted by ECHA, NTP, EFSA and EPA have been mined for relevant toxicological information on NEP. However, no study was identified that impacted the calculation of the DNEL.

It should be noted, that NEP is classified for its toxicity on reproduction only indicating that this is the most sensitive endpoint.

16.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as N-ethyl-2-pyrrolidone has no Repr. 1A/B classification related to adverse effects on sexual function and fertility.

16.2 Adverse effects on development

16.2.1 Animal data

Table 80: Summary table of animal studies on adverse effects of 1-ethylpyrrolidin-2-one on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal developmental toxicity study, OECD TG 414 oral Himalayan rabbit	NEP 0, 100, 300, 1000 mg/kg bw/d GD 6-28 dermal	**NOAEL (P): 300 mg/kg bw/d** **LOAEL (P): 1000 mg/kg bw/d** (Reduction in food consumption and bw gain during administration) **NOAEL (F1): 100 mg/kg bw/d** **LOAEL (F1): 300 mg/kg bw/d (**observation of rare cardiovascular malformations above historical controls) Maternal toxicity: 1000 mg/kg bw/d Reduction in food consumption and bw gain during administration (Significant at the beginning of treatment only) No effect on maternal corrected weight Developmental Toxicity: ≥300 mg/kg bw/d observation of rare cardiovascular malformations above historical controls ≥1000 mg/kg bw/d Increase in supernumerary 13th rib (variation).	BASF SE (2010) as cited in RAC (2011d)
Prenatal developmental toxicity study, OECD TG 414	NEP 0, 20, 60,	**NOAEL (P): 60 mg/kg bw/d** **LOAEL (P): 200 mg/kg bw/d** (Reduction in food consumption	BASF AG (2007a) as cited in RAC (2011d)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
oral (gavage) Himalayan rabbit	200 mg/kg bw/d GD 6-28	and bw gain during administration) **NOAEL (F1):60 mg/kg bw/d** **LOAEL (F1): 200 mg/kg bw/d (**significant increase of skeletal malformations, above historical controls)	
Consistent with OECD TG 414 Himalayan rabbit	NEP 0, 220 mg/kg bw/d GD 6-28 oral by gavage	Only one dose tested *Maternal toxicity* Reduction in food consumption and bw gain during administration. No effect on maternal corrected weight *Developmental toxicity* Reduction of foetal weight; 2 foetuses with severe multiple malformations (significant) increase of visceral and skeletal malformations, above historical controls, in particular rare cardiovascular malformations	BASF AG (2007b) as cited in RAC (2011d)
OECD TG 414 Wistar rat	NEP 0, 200, 400, 800 mg/kg bw/d GD 6-19 dermal	**NOAEL (P): 200 mg/kg bw/d** **LOAEL (P): 400 mg/kg bw/d** (reduced bw gain during administration) **NOAEL (F1): 200 mg/kg bw/d** **LOAEL (F1) 400 mg/kg bw/d** (Reduction in foetal weight Increase of some skeletal variations.)	BASF AG (2005) as cited in RAC (2011d)
Consistent with OECD TG 414 Pregnant Sprague Dawley rat	NEP 0, 50, 250, 500, 750 mg/kg bw/d in distilled water GD 6-20 Daily by oral gavage	**NOAEL (P): 50 mg/kg bw/d** **LOAEL (P): 250 mg/kg bw/d** (Reduced bw gain during gestation) **NOAEL (F1):50 mg/kg bw/d** **LOAEL (F1): 250 mg/kg bw/d** (reduction of foetal weight) Developmental Toxicity ≥500 mg/kg bw/d - (significant) increase in post-implantation loss -(significant) increase of external and skeletal malformations, observation of rare cardiovascular malformation above historical	Saillenfait et al. (2007)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		controls 750 mg/kg bw/d (significant) increase of visceral malformations variations	

16.2.2 Human data

Epidemiological studies which allow deriving a NOAEL for adverse health effects induced by exposure against NEP are currently lacking.

16.2.3 Discussion of adverse effects of NEP exposure on development

As mentioned initially the CLH dossier (ANSES, 2011a) and the corresponding RAC (2011d) have been the source of the relevant toxicological information on adverse effects of NEP on reproduction compiled in Table 81. The presented data is either compliant or similar with OECD guidelines. The studies by BASF (BASF AG (2007a) as cited by RAC (2011d)) and Saillenfait et al. (2007) are considered to be the key studies.

Regardless of the route of exposure (oral, dermal) treatment with NEP of pregnant rat and rabbit females caused teratogenic and foeto-toxic effects which coincided with maternal toxicity. However, since the corrected maternal weights were not altered significantly during the different treatment regimens it can be concluded that the observed adverse reproductive effects are not secondary to maternal toxicity.
Based on the animal studies carried out, it was clearly demonstrated that NEP induced adverse effects on foetal body weights and caused skeletal malformations in rabbits by oral route. Additionally, oral exposure induced rare cardiovascular malformations in this species at above historical control levels. The latter effect was also observed when NEP was applied to the skin of rabbits. This treatment also induced skeletal malformations (BASF AG (2007a), (2007b), (2010)as cited in RAC (2011d)).

In analogy to the rabbit NEP exposure by oral gavage also induced adverse effects on foetal body weights, normal bone development and produced rare cardiovascular malformations at above historical control levels in rats. Additionally, effects on post-implantation loss and in particular late resorptions were also observed. Dermal application of NEP however, led to a reduction of foetal body weights only.

Considering the observed adverse effects on skeletal development of NEP applied via the oral route to rabbits a NOAEL of 60 mg/kg bw/d could be derived (BASF AG (2007a) as cited by RAC (2011d); Table 81). Oral exposure of rats against NEP caused a reduction in foetal body weight at a dose ≥ 250mg/kg bw/d Saillenfait et al. (2007). Therefore a NOAEL of 50 mg/kg bw/d for developmental toxicity could be established.

16.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as NEP has no classification related to adverse effects on or via lactation.

16.4 Information taken into account for risk assessment and uncertainties

16.4.1 POD-selection

The NOAEL for developmental toxicity derived from the prenatal OECD TG 414 study on rats by Saillenfait et al. (2007) was used as the PoD.

16.4.2 Uncertainties

No uncertainties were identified.

16.4.3 DNEL derivation

The calculated DNELs and applied assessment factors (AF) are shown in the table below.

Table 81: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	**Value**	**Remark**
Development		
POD **developmental effects**	**NOAEL:50 mg/kg bw/d**	The NOAEL for adverse effects on development was derived from a prenatal developmental study in rats which was performed in compliance with OECD TG 414 (Saillenfait et al., 2007). Dose-related adverse effects on number of litters with malformed foetuses, foetal body weight and incidences of rare cardiovascular malformation were observed.
Overall AFs	**100**	
AF for interspecies differences - remaining differences - allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	A default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	Not relevant
AF related to dose response relationship	1	Not relevant
AF related to quality of database	1	Default value.
DNELgeneral population, reproductive effects (related to developmental effects)	**0.5 mg/kg bw/d**	

17 4-tert-butylbenzoic acid (CAS No. 98-73-7)

The Member State Committee has agreed on the identification of 4-tert-butylbenzoic acid (pTBBA) as Substance of Very High Concern (SVHC), based on the classification as reproductive toxicant category 1B (H 360F). For pTBBA, a CLH report (BauA, 2010) for its classification as reproductive toxicant category 1B, and a RAC opinion (RAC, 2011b) supporting the proposed classification and key studies exist. The key study for the endpoint reproductive toxicity of pTBBA as shown in Table 83 was selected based on the recent CLH report (BauA, 2010), the respective RAC opinion (RAC, 2011b), and a current literature research (since 2011).

In addition to its classification as reproductive toxicant category 1B (H 360F), pTBBA is also classified as acute toxicant category 4 (H302) and as specific organ toxicant category 1 (STOT RE 1, H372) (kidney, testes, brain and the spinal cords (neuronal dysfunctions), peripheral blood).

17.1 Adverse effects on sexual function and fertility

17.1.1 Animal data

Table 82: Summary table of animal studies on adverse effects of 4-tert-butylbenzoic acid on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Male fertility study (no standardized guideline followed), considered reliable Oral (dietary study) Rats (Wistar) 10 males/group	4-tert-butylbenzoic acid (pTBBA) 0, 20,100, and 500 ppm in feed Corresponding to 1.6, 7.9, 41 mg/kg/bw/d Exposure: daily, 70 days prior to mating trials	**NOAEL: 1.6 mg/kg/bw/d** **LOAEL: 7.9 mg/kg/bw/d** (Dose dependent decrease of male fertility/ability to impregnate females)	Hoechst AG (1987), cited according to BauA (2010)

17.1.2 Human data

There are no relevant data available.

17.1.3 Short discussion and overall relevance of the provided information on adverse effects on sexual function and fertility

The following information on the reproductive toxicity of 4-tert-butylbenzoic acid is a copy of the relevant chapter in the background document (RAC, 2011a):

"With regard to male fertility, several repeated dose toxicity studies with rats with different

routes of application (oral, inhalation, dermal) and one oral fertility study in rats (Hoechst AG, 1987) are available revealing a toxic potential of 4-tert-butylbenzoic acid with induction of testicular lesions, spermatotoxic effects (reversible at test dose of 41 mg/kg bw/d) and infertility already at relatively low dosages/concentrations. Consistently and independent from route of application, testes toxicity was characterised by lower absolute and relative organ weights, testes atrophy from seminiferous tubular degeneration, destruction of the germinative epithelium resulting in disturbance of spermatogenesis and in particular in loss of late spermatids."

Concern on possible spermatotoxic effects of 4-tert-butylbenzoic acid also in humans might be given but remains uncertain. A study on occupationally exposed workers (Whorton et al., 1981) provided some indication for slightly higher numbers of individuals with low sperm count (less than 20 million sperm/ml) in exposed participants compared to non-exposed participants. However the findings could be biased by other factors and uncertainty remains due to the low numbers of participants.

Hazard assessment for 4-tert-butylbenzoic acid with respect to female fertility is not possible, since there are no data available."

NOAEL/LOAEL values derived from the experimental studies and valid for use for risk assessment are provided in the table below.

Table 83: NOAEL/C and LOAEL/C values from different administration routes for fertility risk characterisation

Route of application (duration)	NOAEL/C	LOAEL/C	Reference
Oral (70 days)	1.6 mg/kg bw/d	7.9 mg/kg bw/d	Hoechst AG (1987)
Oral (90 days)	-	6 mg/kg bw/d	Hunter et al. (1965)
Dermal (7 and 13 weeks)	35 mg/kg bw/d	70 mg/kg bw/d	Cagen et al. (1989)
Dermal (28 days)	30 mg/kg bw/d	60 mg/kg bw/d	Shell (1975)
Inhalation (4 days (3 days rest) 3 days)	-	12.5 mg/m^3	Shell (1987)

17.2 Adverse effects on development

Not relevant for the present restriction proposal as 4-tert-butylbenzoic acid has no Repr. 1A/B classification related to adverse effects on development.

17.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as 4-tert-butylbenzoic acid has no classification related to adverse effects on or via lactation.

17.4 Information taken into account for risk assessment and uncertainties

17.4.1 POD-selection

The oral study by Hoechst AG (1987) is used for hazard/risk assessment regarding male fertility. The observed effects are considered relevant to humans. A **NOAEL and a LOAEL of 1.6 and 7.9 mg/kg bw/d**, respectively, were selected as dose descriptor (PoD) for risk assessment of humans.

17.4.2 Uncertainty

It should be noted that the study by Hoechst AG (1987) was not performed according to a standardized guideline but the obtained results are nevertheless considered reliable.

17.4.3 DNEL derivation

Table 84: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Fertility		
$POD_{fertility}$	**NOAEL: 1.6 mg/kg bw/d**	**This NOAEL for reproductive toxicity results from a 70 days male fertility study in rats (Hoechst AG, 1987) and is based on a dose-dependent decrease of male fertility/ability to impregnate females.**
Overall AFs	**600**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	6	The default factor (sub-acute to chronic) is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF related to dose response relationship	1	No AF was applied since the POD is a NOAEL
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to fertility effects)	**0.0027mg/kg bw/d**	

18 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate (CAS No. 15571-58-1)

2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate (DOTE) has a harmonised classification as Repr. 1B (H360D). However, no CLH report for this classification is available. A CLH report for DOTE exists proposing down-classification to Repr. 2 (ARKEMA, 2011). However, down-classification was not supported by RAC (RAC, 2012b).

Based on the classification as Repr. 1B, the MSC has agreed on the identification of DOTE as a substance of very high concern.

18.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as DOTE has no classification related to fertility effects.

18.2 Adverse effects on development

In Table 86, relevant studies on adverse effects on development for DOTE are shown. Data were queried based on the available CLH reports, RAC opinions, registration dossiers and a recent literature research for DOTE.

18.2.1 Animal data

Table 85: Summary table of animal studies on adverse effects of DOTE on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OECD TG 414 (Prenatal Developmental Toxicity Study) rabbit (New Zealand White) oral: gavage	**Test material: DOTE** **In original Study (EC name): 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate** 0, 4, 20, 80 mg/kg bw/day Vehicle: peanut oil Exposure: GD 6 to GD 28, daily	**NOAEL (P): 20 mg/kg bw/day** **LOAEL (P): 80 mg/kg bw/day**, (Biologically relevant depression (-12.8%) in thymus weight, dose-dependent: -9.6% at mid and -5.1% at low dose group) **NOAEL (F1): 20 mg/kg bw/day** **LOAEL (F1): 80 mg/kg bw/day** (biologically relevant effect on foetal weight (-11.9%) and foetal crown-rump length (-10.7%) relative to controls, statistically significant negative trend.)	Anonymous (2014a)
OECD TG 414 (Prenatal Developmental Toxicity Study) mouse (Swiss) oral: gavage 25 f/group	**Test material: DOTE** **In original Study (EC name): 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate** 0, 15, 30, 60 mg/kg bw/d Vehicle: peanut oil Exposure: GD 5 to GD 17, daily	**NOAEL (P):** 15 mg/kg bw/day **LOAEL (P):** 30 mg/kg bw/d (Statistically significant depression in thymus size, stat. sign. depression (23%) in thymus weight, 35% at 60 mg/kg bw/d, treatment-related not statistically significant reduction in corrected maternal body weight gain of -17.7% in the low, -16.7% in the mid, and -26.5% (2.34±2.41 g) in the high dose mice relative to controls) **NOAEL (F1): could not be determined** **LOAEL (F1): 15 mg/kg bw/d** (statistically significant positive trend on percentage of post implantation loss: 0.9 ± 2.8 at low, 1.5 ± 4.9 at mid, and 2.6 ± 5.6 at high dose, respectively)	Anonymous (2014b)

18.2.2 Human data

Epidemiological studies and other human data which allow determining a human "no effect level" for developmental effects of DOTE are currently not available.

18.2.3 Discussion of Adverse effects on development

Two key animal studies have been identified in which developmental effects of DOTE were investigated (Anonymous, 2014a; Anonymous, 2014b). The studies are prenatal developmental toxicity studies according to OECD TG 414 and GLP, one performed with mice and the other with rabbits. Both studies are considered reliable. Based on these studies there is evidence that DOTE interferes with prenatal development in mice and rabbits. In mice, study results show a statistically significant positive trend on percentages of post implantation loss with increased

values already at the lowest dose tested (15 mg/kg bw/d). Therefore, 15 mg/kg bw/d was established as the LOAEL for developmental effects of DOTE in mice. In rabbits, at the high dose (80 mg/kg bw/d) the mean foetal body weight decreased about 12% relative to controls, suggesting a marginal but biologically relevant effect on foetal maturation. Furthermore, a statistically significant reduction in the mean foetal crown-rump length was noted in this study (-10.7% relative to the controls), again suggesting a marginal but biologically relevant effect on foetal maturation which moreover correlated to the degree of skeletal ossification. The NOAEL for developmental effects in rabbits was established to be 20 mg/kg bw/d.

Harmonised classification of DOTE was based on other studies. These studies were not considered relevant here as recent guideline conform developmental toxicity studies with (pure) DOTE exist in which adverse developmental effects have been observed.

18.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as DOTE has no classification related to adverse effects on or via lactation.

18.4 Information taken into account for risk assessment and uncertainties

18.4.1 POD-selection

The LOAEL of 15 mg/kg bw/d established in the prenatal developmental study in mice (Anonymous, 2014b) is lower than the NOAEL established for rabbits (Anonymous, 2014a). Thus, this LOAEL was selected as PoD for the risk assessment for human health for developmental effects of DOTE.

18.4.2 Uncertainty

There is uncertainty considering the selected PoD. The LOAEL was established based on a significant positive trend for post-implantation losses. On the one hand there was no significance for this effect for the individual dose levels tested compared to the controls in a pair-wise comparison. On the other hand, as no lower dose levels than 15 mg/kg bw/d have been tested, a NOAEL could not be derived.

18.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in the table below.

Table 86: Detailed outline of the derivation of the DNEL $_{\text{general population, reproductive effects}}$

Description (AF=Assessment factor)	Value	Remark
Development		
POD$_{\text{Developmental effects}}$	**LOAEL:15 mg/kg bw/d**	**This LOAEL for developmental effects results from a prenatal developmental study in mice (Anonymous, 2014b) and is based on a statistically significant positive trend on percentage of post implantation loss.**
Overall AFs	**525**	
AF for interspecies differences - remaining differences - allometric scaling	2.5 7	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied.
AF related to dose response relationship	3	An AF was applied as the POD is a LOAEL
AF related to quality of database	1	Default value.
DNEL$_{\text{general population, , reproductive effects}}$ (related to developmental effects)	**0.03 mg/kg bw/d**	

19 Imidazole (CAS No. 288-32-4)

Imidazole is listed is classified as Repr. 1B with the hazard statement code H360D according to Regulation 1272/2008/EC. Most information of this report is extracted from the CLH report for imidazole, the RAC opinion (BASF SE, 2012; RAC, 2013c) and from the respective references.

The key studies and respective NOAEL value for the endpoint reproductive toxicity of imidazole as shown in Table 88 were selected based on the recent RAC opinion on a CLH report mentioned above.

For the present restriction proposal a recent literature survey was performed from 2011, as the current CLH report is from 2012.

Besides Repr. 1B imidazole is classified as Acute Tox. 4 and Skin Corr. 1C as only other human health endpoints.

19.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as imidazole has no Repr. 1A/B classification related to adverse effects on sexual function and fertility.

19.2 Adverse effects on development

19.2.1 Animal Data

Table 87: Summary table of animal studies on adverse effects of imidazole on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal developmental toxicity study,OECD TG414 oral (gavage) rat (Wistar) 25 f/group On day 20 post coitum, dams were sacrificed and examined for gross pathological changes (including weight determinations of the unopened uterus and the placentae), the number of corpora lutea in the ovaries, conception rate, the number of live foetuses and pre- and post-implantation losses. The foetuses were weighed, sexed and macroscopically examined for external alterations. One half of all foetuses were fixed and examined for effects on the inner organs, while the other half of foetuses were fixed and stained for skeletal and cartilage evaluation.	imidazole 0, 20, 60, 180 mg/kg bw/d (nominal conc.) Exposure:daily from implantation to one day prior to expected parturition (GD 6-19)	**NOAEL (P):60 mg/kg bw/d** **LOAEL (P): 180 mg/kg bw/d** (decreased food consumption, body weight gain and uterus weight) **NOAEL (F1): 60 mg/kg bw/d** **LOAEL (F1): 180 mg/kg bw/d** (reduced mean foetal weight and increased number of resorptions and(increased rate of variations and malformations)	BASF (2002)

19.2.2 Human data

No human data available.

19.2.3 Short discussion and overall relevance of the provided information on adverse effects on development

The information and data provided are partly extracted from the CLH report for imidazole (BASF SE, 2012; RAC, 2013c).

From the prenatal developmental toxicity study stated in Table 88 (BASF, 2002), it can be concluded that the oral administration of imidazole to pregnant Wistar rats from implantation to one day prior to the expected day of parturition elicited substance-related signs of maternal toxicity at the highest dose (180 mg/kg bw/d). A total of 6 rats of this group showed transient salivation (being most likely indicative for slight irritations of the upper digestive tract) during some days of the treatment period. Moreover, vaginal haemorrhage occurred in another high dose dam, which resorbed all of its implants, just before scheduled sacrifice. At initiation of dosing, the high dose dams showed statistically significant impairments in food consumption (about 13% below the control) and impaired body weight gains (about 45% below the control) on days 6 - 8 post coitum (p.c.). Moreover, high dose body weight gains were also statistically significantly diminished on gestation day 17 - 20 and the mean gravid uterus weight was distinctly affected (about 26% below the control) due to a high resorption rate and a markedly lower mean foetal body weight at 180 mg/kg bw/d. According to the scope of parameters examined in the present prenatal developmental toxicity study, the administration of 180 mg imidazole/kg bw/d to pregnant rats induced adverse effects on the dams. Concerning gestational parameters there was a high rate of resorptions at the top dose, which led to a clearly elevated post implantation loss value, but no substance-induced effects on the gestational parameters occurred at 20 or 60 mg/kg bw/d. At the highest dose level (180 mg/kg bw/d) clear signs of developmental toxicity, including indications of teratogenicity, were obtained. The external, skeletal and consequently the overall malformation rate and the incidences for several soft tissue and certain skeletal variations were statistically significantly increased and clearly above historical control values. At 20 and 60 mg/kg bw/d, however, no substance induced signs of embryo-/foetotoxicity, especially no indications of teratogenicity, were observed. Based on these results, the no observed adverse effects level (NOAEL) for maternal and prenatal developmental toxicity is 60 mg/kg bw/d (BASF, 2002).

19.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as imidazole has no classification related to adverse effects on or via lactation.

19.4 Information taken into account for risk assessment and uncertainties

19.4.1 POD-selection

The study by BASF (2002) is considered as key study in this restriction proposal because of compliance with OECD 414. The NOAEL for foetotoxicity and teratogenicity of this study is used as point of departure (PoD) for the DNEL calculation: 60 mg/kg bw/d (LOAEL: 180 mg/kg bw/d).

19.4.2 Uncertainty

No obvious uncertainties could be identified.

19.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in the table below.

Table 88: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
POD Developmental effects	**NOAEL: 60 mg/kg bw/d**	**OECD TG 414 (prenatal developmental toxicity study), reduced mean foetal weight and increased number of resorptions and increased rate of variations and malformations at 180 mg/kg bw/d** **(BASF, 2002)**
Overall AFs	**100**	
AF for interspecies differences - remaining differences allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to developmental effects)	**0.6 mg/kg bw/d**	

20 Ketoconazole (CAS No. 65277-42-1)

Ketoconazole has a harmonised classification as Repr. 1B related to effects on sexual function and fertility (H360F).

A CLH report and respective RAC opinion are not available for ketoconazole. Thus, information on reprotoxicity was obtained by performing a literature research using data sources as indicated in section B. 5.9.

Ketoconazole is an active drug ingredient e.g. for the treatment of fungal infections or the Cushing's syndrome.

In addition to its classification as reproductive toxicant category 1B, ketoconazole is also classified as acute toxicant category 3 (H301) and as specific organ toxicant category 2 (STOT RE 2, H373).

20.1 Adverse effects on sexual function and fertility

20.1.1 Animal data

Table 89: Summary table of animal studies on adverse effects of ketokonazole on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Male fertility study (no standardized guideline followed) Oral (gavage) Rats (Sprague-Dawley) About 18 males/group	ketokonazole 0, 200, 400 mg/kg bw/d Exposure: daily, three consecutive days; Each male was paired with female immediately after administration of the third dose	**NOAEL: could not be derived** **LOAEL: 200 mg/kg bw/d (based on loss of fertility)**	Waller et al. (1990)
Repeated dose toxicity study related to male fertility (no standardized guideline followed) Oral (dietary in honey) Rat (Crl:CD BR) 32 male/ group	Ketoconazole 0, 10, 100, 200 mg/kg/d Exposure: 65 days	**NOAEL: 200 mg/kg bw/d** **LOAEL: could not be derived** - no adverse effects to male fertility at all three dose levels - no decline of number of pregnancies	Heckman et al. (1992)
Repeated dose toxicity study related to male fertility (no standardized guideline followed)	ketoconazole 0, 400 mg/kg bw/d Exposure: 60d	**NOAEL: could not be derived** **LOAEL: 400 mg/kg bw/d (based on decline in fertility and significant decline in sperm motility and density in cauda epididymis)**	Joshi et al. (1994)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Oral Mice			

20.1.2 Human data

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations in offspring	Reference
human clinical data	ketokonazole	Volunteer male treatment with single doses of 200, 400 and 600 mg	Testosterone serum concentrations fell markedly, but returned toward baseline eight to 24 hours later as ketoconazole serum concentrations waned; (A marked but transient drop in testosterone levels occurred in patients receiving long-term therapy.)	Pont et al. (1982)

20.1.3 Short discussion of the provided information on adverse effects on sexual function and fertility

Ketoconazole is a drug ingredient e.g. for the treatment of fungal infections or the Cushing's syndrome. It inhibits fungal sterol production (Millsop et al., 2013) and, as summarised in the CHMP assessment report on ketoconazole (CHMP, 2014), can also inhibit several enzymes in the human adrenal steroid biosynthesis. Indeed, in a human clinical study in which a Volunteer male was treated with three single doses of ketoconazole (200, 400, 600 mg) testosterone serum concentrations fell markedly. Moreover, a marked but transient drop in testosterone levels occurred in patients receiving long-term therapy (Pont et al., 1982). As in the human studies fertility effects have not been investigated a human NOAEL for fertility effects could not be derived.

Three rodent studies with ketoconazole have been identified in which fertility parameters in orally treated male animals were analysed (Heckman et al., 1992; Joshi et al., 1994; Waller et al., 1990). In the subacute rat study (Waller et al., 1990) two dose levels (200 and 400 mg/kg bw/d) were applied daily via gavage for three consecutive days. A concentration dependent decline of fertility leading to 0% pregnancies at the higher dose level was observed. As no dose levels below 200 mg/kg bw/d have been tested a NOAEL could not be obtained. The LOAEL derived was 200 mg/kg bw/d. In the study by Heckman and co-authors (Heckman et al., 1992) in which rats were treated for 65 days no adverse effects on male fertility were observed up to and including the highest dose tested (200 mg/kg bw/d). However, results of this study are considered not to be contrary to the LOAEL derived from the data of Waller et al. (1990) as doses are administered by food and not gavage. Data on actual intake are missing. Thus, actual ingested dose might be lower than the documented dose of 200 mg/kg bw/d. Higher dose levels have not been tested in the study. Moreover two different rat strains have been used. However, Heckman and co-authors (Heckman et al., 1992) also found that longer administration of

ketoconazole to male rats resulted in steroid levels comparable with those of controls. This could be an explanation why adverse effects on fertility were found after shorter treatment times and not after 65 days of treatment. In the 60-day study with mice (Joshi et al., 1994) only one dose level has been tested. Nevertheless, this study would support decline in fertility in rodents also after longer exposure duration as at 400 mg/kg bw/d a decline in male fertility was observed. Moreover, a significant decline in sperm motility and density in cauda epididymis was found at this dose level. As ketoconazole blocked basal and gonadotropin-stimulated testosterone production also in rodents (Heckman et al., 1992; Pont et al., 1982; Vawda and Davies, 1986) the observed impaired male fertility can be associated with a decline in testosterone levels

The overall LOAEL for male fertility was considered to be 200 mg/kg bw/d.

20.2 Adverse effects on development

Not relevant for the present restriction proposal as ketoconazole has no Repr. 1A/B classification related to adverse effects on development.

20.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as ketokonazole has no classification related to adverse effects on or via lactation.

20.4 Information taken into account for risk assessment and uncertainties

20.4.1 POD-selection

The study by Waller et al. (1990) is used for hazard/risk assessment regarding male fertility. The observed effects are considered relevant to humans. The **LOAEL of 200 mg/kg bw/d** was selected as starting point (PoOD) for risk assessment of humans.

20.4.2 Uncertainty

It should be noted that the study by Waller et al. (1990) was not performed according to a standardized guideline. Only two higher dose levels were tested and treatment period was only for 3 days. Thus, there are uncertainties considering the LOAEL derived. The AF applied to estimate the DNEL are shown in the table below.

Table 90: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Fertility		
POD Fertility effects	**200**	**This NOAEL is based on a male fertility study in rats (Waller et al. 1990) and is based on loss of fertility.**
Overall AFs	**1800**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	6	To extrapolate from sub-acute to chronic.
AF related to dose response relationship	3	An AF was applied as the POD is a LOAEL
AF related to quality of database	0	Default value.
DNEL general population, , reproductive toxicity	**0.11 mg/kg bw/d**	

21 7-methoxy-6-(3-morpholin-4-yl-propoxy)-3H-quinazolin-4-one (CAS No. 199327-61-2)

7-methoxy-6-(3-morpholin-4-yl-propoxy)-3H-quinazolin-4-one has a harmonised classification as toxic for reproduction, Repr. 1B (H360D). The substance is currently not identified as SVHC.

No CLH report is publically available for 7-methoxy-6-(3-morpholin-4-yl-propoxy)-3H-quinazolin-4-one and no protocols could be found in the ECHAs`CLH archive. The substance is registered under REACH but there are currently no data for the endpoint reproduction toxicity. Moreover, no relevant data were found in a literature research performed as described in section 5.9.1.

Thus, no literature or data were identified that would enable the derivation of a DNEL for reproductive toxicity for this substance.

22 Ammonium 2-amino-4-(hydroxymethylphosphinyl)butyrate (CAS No. 77182-82-2)

Synonyms: ammonium 2-amino-4-(hydroxymethylphosphinyl)butyrate Basta, DL-glufosinate, glufosinate, phosphinothricin

Glufosinate-ammonium (GA) is classified as Repr. 1B with the hazard statement code H360Fd according to Regulation 1272/2008/EC. The substance is currently not a candidate for the SVHC list. No CLH report has been identified for this substance.

The substance is mainly used as a non-selective herbicide for total vegetation control and as a desiccant to aid in crop harvesting. GA, a racemic mixture of the D and L-isomers, is a phosphinic acid analogue of glutamic acid. Its herbicidal action is related to the inhibition of glutamine synthetase, an enzyme that plays an important role in ammonia detoxification, amino acid metabolism and protein and nucleotide biosynthesis in plants.

The key studies and respective dose levels for the endpoint fertility are listed in Table 92 and Table 93 and were selected based on EFSAs conclusions on a peer review of the pesticide risk assessment of Glufosinate (EFSA, 2005a) and a publication by Schulte-Hermann et al. (2006) focusing on the reproductive toxicity and classification of GA.
A literature research has been performed however, no study was identified that would provide relevant toxicological data for the calculation of an alternative NOAEL.

It should be noted, that regarding human health, the substance is classified only for its toxicity on reproduction indicating that this is the most sensitive endpoint.

22.1 Adverse effects on sexual function and fertility

22.1.1 Animal data

Table 91: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Multigeneration study (main study) * Rat Wistar/Han F0: 30/sex F1: 26/sex	0; 40; 120; 360 ppm in the Feed 80 days prior to mating of F0 until termination End of lactation period of F2B	Reduced litter size at 360 ppm NOAEL: 120 ppm (9.6 mg/kg bw/d) LOAEL: 27.8 mg/kg bw/d	Becker (1986a) (as cited in (EFSA, 2005a))
embryo toxicity study rabbit Himalayan 15 pregnant females per group*	GA 0, 2.0, 6.3, 20.0 mg/kg bw/d (gavage) Exposure: d7-19* termination: d29 - delivery by Caesarean section*	Premature deliveries, abortions and dead foetuses at 20 mg/kg bw/d Maternal toxicity observed at 20 mg/kg bw/d No causal connection to observed adverse effects NOAEL: 6.3 mg /kg bw/d	Baeder et al. (1983) (as cited in EFSA (2005a))

22.1.2 Human data

No human data available.

22.1.3 Short discussion on adverse effects on sexual function and fertility

In the public domain, data on toxicity of GA relevant to humans is scarce. The EFSA report on GA (EFSA, 2005a) does not provide detailed experimental information but rather summarizes study results thereby making conclusions on toxicity less transparent. Nevertheless what appears to transpire is that GA causes detrimental effects during gestation, blocks proper

* as cited in Schulte-Hermann et al. (2006)

implantation and causes foetotoxicity, both in rats and rabbits. Impaired fertility appeared to be independent from maternal toxicity. The observed adverse effects warranted the EFSA expert panel to propose to classify GA as Repr. 1B H360F.

22.2 Adverse effects on development

This specific endpoint was not in the scope for this substance in the present restriction proposal.

22.3 Adverse effects on or via lactation

This specific endpoint was not in the scope for this substance in the present restriction proposal.

22.4 Information is taken into account for risk assessment and uncertainties

22.4.1 POD-selection

Both the rat and the rabbit studies (Baeder et al., 1983; Becker, 1986a) are considered as key studies because the observed adverse effects overlap and the derived NOAEL and LOAEL are in the same range. Although experimental detail is lacking in the EFSA document, it is assumed that the studies were of adequate quality because they formed the basis for EFSAs conclusion on classification.

The respective NOAELs for fertility are used as points of departure for DNEL calculation.

22.4.2 Uncertainty

As mentioned above, the EFSA report contains very little experimental detail making conclusions on the toxicity of GA less transparent.

22.4.3 DNEL derivation

The calculated DNELs and applied assessment factors (AF) are shown in the table below.

Table 92 (key study and NOAEL):

Substance	**Cas Number**	**Reproduction Toxicity**			
		Type	**Key study (Reference)**	**Effects**	**NOAEL/ LOAEL**
Glufosinate-ammonium	77182-82-2	F	multigeneration study, oral (in the feed), rat with GA (Becker (1986a), as cited in EFSA (2005a))	Reduced litter size	**Overall NOAEL 9.6 mg/kg bw/d**
Glufosinate-ammonium	77182-82-2	F	Embryotoxicity study, oral (by gavage), rabbit with GA (Baeder et al. (1983), as cited in EFSA (2005a))	Premature deliveries, abortions and dead foetuses	**Overall NOAEL 6.3 mg/kg bw/d**

F: fertility effects

D: developmental effects

Table 93: (DNELs)

DETAILED OVERVIEW OF THE DERIVATION OF THE DNEL GENERAL POPULATION, LONG-TERM, SYSTEMIC EFFECTS		
Description (AF=Assessment factor)	**Value**	**Remark**
POD **fertility** **(rat)**	**NOAEL:9.6 mg/kg bw/d**	The NOAEL was derived from a multigeneration study in rats (Becker (1986a), as cited in EFSA (2005a)) and is based on reduced litter size.
Overall AFs	**300**	
AF for interspecies differences - remaining differences - allometric scaling	2.5 4	For interspecies differences a default factor is applied to account for: a) for remaining differences according to the REACH guidance R. 8 b) difference between the experimental animal and humans
AF for intraspecies differences	10	A default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	3	To account for extrapolation from sub-chronic to chronic exposure
AF related to dose response relationship	1	Not relevant
AF related to quality of database	1	Default value.
DNELgeneral population, , long-term, systemic effects	**0.032 mg/kg bw/d**	

DETAILED OVERVIEW OF THE DERIVATION OF THE DNEL GENERAL POPULATION, LONG-TERM, SYSTEMIC EFFECTS		
Description (AF=Assessment factor)	**Value**	**Remark**
POD **fertility** **(rabbit)**	**NOAEL:6.3 mg/kg bw/d**	The NOAEL was derived from a study in rabbits (Baeder et al. (1983), as cited in EFSA (2005a)) and is based on increases in premature deliveries, abortions and dead foetuses.
Overall AFs	**360**	
AF for interspecies differences		For interspecies differences a default factor is applied to account for: a) for remaining differences according to the REACH

- remaining differences - allometric scaling	2.5 2.4	guidance R. 8 b) difference between the experimental animal and humans
AF for intraspecies differences	10	A default factor is applied according to the REACH guidance R.8. Substance-specific information is absent for an adjustment.
AF for differences in exposure duration	6	To account for extrapolation from subacute to chronic exposure.
AF related to dose response relationship	1	Not relevant.
AF related to quality of database	1	Default value.
$DNEL_{general\ population, , reproductive\ toxicity}$	**0.0175 mg/kg bw/d**	

23 Chloro-N,N-dimethylformiminium chloride (CAS No. 3724-43-4)

Synonyms: (Chlormethylen)-dimethylammoniumchlorid, Arnold's reagent; Vilsmeier reagent

Chloro-N,N-dimethylformiminium chloride (Vilsmeier reagent) is listed as a hazardous substance in Annex VI of the CLP regulation with the classification as Repr. 1B (H360D). Vilsmeier reagent is currently not a candidate for the SVHC list.

No CLH report is publically available for Vilsmeier reagent and no protocols could be found in the ECHAs`CLH archive. The substance is registered under REACH but there are no data for the endpoint reproduction toxicity available. Moreover, no relevant data were found in a literature research performed as described in section 5.9.1.

Thus, no literature or data were identified that would enable the derivation of a DNEL for reproductive toxicity for this substance.

24 cyclic 3-(1,2-ethanediylacetale)-estra-5(10),9(11)-diene-3,17-dione (CAS No. 5571-36-8)

Cyclic 3-(1,2-ethanediylacetale)-estra-5(10),9(11)-diene-3,17-dione has a harmonised classification as toxic for reproduction, Repr. 1B (H360F). The substance is currently not identified as candidate for SVHC list.

No CLH report is publically available for cyclic 3-(1,2-ethanediylacetale)-estra-5(10),9(11)-diene-3,17-dione and no protocols could be found in the ECHAs`CLH archive. The substance is not registered under REACH. Moreover, no relevant data were found in a literature research performed as described in section 5.9.1.

Thus, no literature or data were identified that would enable the derivation of a DNEL for reproductive toxicity for this substance.

25 salts and esters of dinoseb (CAS No. 88-85-7, 35040-03-0, 6365-83-9, 2813-95-8)

Dinoseb was identified as Substance of Very High Concern (SVHC) based on the classification as reproductive toxicant category 1B (H 360Df)

The key studies for the endpoint developmental toxicity of dinoseb as shown in table 54 were selected based on information from the Integrated Risk Information System (IRIS, 2003) of US-EPA and a current literature survey as no CLH dossier has been identified for this substance.

25.1 Adverse effects on sexual function and fertility

Not relevant for the present restriction proposal as salts and esters of dinoseb do not have a Repr. 1A/B classification related to adverse effects on sexual function and fertility.

25.2 Adverse effects on development

25.2.1 Animal data

Table 94: Summary table of animal studies on adverse effects of salts and esthers of dinoseb on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
3-generation rat reproductive study comparable to OECD TG 416 oral (dietary study) Rat (CD(SD)) 25/sex/group (2 littering groups per generation)	dinoseb (6-sec-butyl-2,4-dinitrophenol) 0, 1, 3, and 10 mg/kg bw/d	**NOAEL (P): 3 mg/kg bw/d** **LOAEL (P): 10 mg/kg bw/d** (Consistent depression of parental body weight gain) NOAEL (F1): could not be determined **LOAEL (F1): 1mg/kg bw/d** (reduced pub weights) (Reduced pub weights in F0 to F1b littering groups at all dose levels. Since pub weights were similar to control at birth, the subsequently decreased pub weight gains indicate a reproductive effect during the lactation period.)	Dow Chemical Company (1981) cited according to (IRIS, 2003)
Equivalent or similar to OECD TG 414 (Prenatal Developmental Toxicity Study) dermal Rabbit (NZ white rabbit) 16/group, 17 for the highest dose	dinoseb (6-sec-butyl-2,4-dinitrophenol) 0, 1, 3 or 9 mg/kg bw/d For 6 h daily from days 7 to 19 of pregnancy	**NOAEL (F1): 1mg/kg bw/d** **LOAEL (F1): 3mg/kg bw/d** (Increased anophthalamia and hydrocephaly at 3 and 9 mg/kg bw/d) Increase of dead and resorbed foetuses, cleft palate, microcephaly, microphthalamia at 9 mg/kg bw/d *Maternal toxicity* at 3 mg/kg bw/d and higher: hyperthermia and reduced body weight in maternal rabbits; maternal mortality at 3 and 9 mg/kg bw/d: 71% and 19%, respectively	Johnson (1988)

25.2.2 Human data

There are no relevant data available.

25.2.3 Short discussion and overall relevance of the provided information on adverse effects on development

Developmental toxicity of dinoseb has been reported in a number of studies although manifestation of effects seems to be particularly dependent on the animal species used in experiments and the route of administration (see review by Matsumoto et al. (2008)). For developmental effects, a **LOAEL of 1 mg/kg bw/d** was derived from the study by Dow Chemical Company (1981).

25.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as salts and esters of dinoseb do not have a classification related to adverse effects on or via lactation.

25.4 Information taken into account for risk assessment and uncertainties

25.4.1 POD-selection

The study by Dow Chemical Company (1981) is used for hazard/risk assessment regarding developmental toxicity. The observed effects are considered relevant to humans and a **LOAEL of 1 mg/ kg bw/d** was selected as starting point (POD) for risk assessment of humans.

25.4.2 Uncertainty

The 3-generation rat reproductive study performed by Dow Chemical Company (1981) is considered equivalent to OECD TG 416 and is taken into account as principal study by the US-EPA (IRIS, 2003). However, developmental toxicity is particularly evident at doses also inducing maternal toxicity.

25.4.3 DNEL derivation

Table 95: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{developmental\ effects}$	**LOAEL: 1 mg/kg bw/d**	**This LOAEL for developmental toxicity results from a 3-generation rat reproductive study comparable to OECD guideline 416 (Dow Chemical Company, 1981), and is based on reduced pub weight in F0 to F1b littering groups.**
Overall AFs	**300**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF was applied due to developmental effects.
AF related to dose response relationship	3	An AF was applied as the POD is a LOAEL
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to developmental effects)	**0.0033 mg/kg bw/d**	

26 salts and esters of dinoterb (CAS No. 1420-07-1, 2487-01-6)

Salts and esters of dinoterb have a harmonised classification as toxic for reproduction, Repr. 1B (H360D).

No CLH report is publically available for salts and esters of dinoterb and no protocols could be found in the ECHAs`CLH archive. The substance group is not registered under REACH, yet. Moreover, no relevant data were found in a literature research performed as described in section 5.9.1.

Thus, no literature or data were identified that would enable the derivation of a DNEL for reproductive toxicity for this substance.

27 Tetrahydrofurfuryl alcohol (CAS No. 97-99-4)

Tetrahydrofurfuryl alcohol (THFA) is listed in Annex VI of the CLP legislation as Repr. 1B with

the hazard statement code H360Df. Most information of this report is extracted from the CLH report for THFA (ANSES, 2011b; RAC, 2012c).

The key studies and respective NOAEL value for the endpoint reproductive toxicity of THFA as shown in Table 97 were selected based on the recent RAC opinion on a CLH report mentioned above (ANSES, 2011b; RAC, 2012c).

For the present restriction proposal a recent literature survey was performed from 2011, as the current RAC opinion on THFA was published in 2012.

Besides Repr. 1B THFA is classified as eye Irrit. 2 as only other human health endpoint indicating that reproductive toxicity is the most sensitive endpoint for oral exposure of this substance.

27.1 Adverse effects on sexual function and fertility

This specific endpoint was not in the scope for this substance in the present restriction proposal due to Repr. 2 classification for effects on sexual function and fertility.

27.2 Adverse effects on development

27.2.1 Animal data

Table 96: Summary table of animal studies on adverse effects of THFA on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
reproduction/developmental toxicity screening test according to OECD 421, GLP oral (gavage) rat (Crj:CD(SD)IGS; 8 weeks old) 12 pairs/group parameters investigated: body weight, weight or organs, histopathology in reproductive organs, reproductive examination (e.g., oestrous cycle, copulation index, fertility, gestation length, no. of live and dead pups, sex ratio of live pups)	THFA 0, 15, 50, 150 and 500 mg/kg bw/day Males were dosed once/d for 47 days, beginning 14 days before mating and throughout the mating period and females were dosed once/d from 14 days prior to mating and throughout the mating and gestation periods, to day 4 of lactation (total administration period: 42-52 days).	**NOAEL: 50 mg/kg bw/d** LOAEL : 150 mg/kg bw/d (significantly reduced body weight gain; females: increased gestation length, sign. increased gestation index (no. of dams with live pups/no. of pregnant females x 100), reduced no of dams delivering live pups, increased no of dead pups, reduced no of live pups)dep 500 mg/kg bw/d: males: significant reduction of testes and epididymides weights, atrophy of thymus and seminiferous tubules, reduced sperm count, hyperplasia of interstitial cells; females: significantly prolonged oestrous cycle	Hirata-Koizumi et al. (2008)
range finding developmental study oral exposure study not accessible, used as supporting information only	THFA 0, 10, 50, 100, 500 and 1000 mg/kg bw/day exposure during	**NOAEL (F1): 50 mg/kg bw/d** **LOAEL (F1): 100 mg/kg bw/d** (decreased foetal body weight) *maternal effects:* 500 mg/kg bw/d: significantly decreased body weight gain	TSCA (1992)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
rat (strain not stated) 8 pregnant f/dose group	gestation day 6 to 15	*embryo toxicity:* 500 mg/kg bw/d: 100% early resorptions	

27.2.2 Human data

No human data available.

27.2.3 Short discussion and overall relevance of the provided information on adverse effects on development

The information and data provided are partly extracted from the CLH report and RAC opinion for THFA (ANSES, 2011b; RAC, 2012c).

The OECD 421 study (Hirata-Koizumi et al., 2008) was considered as key study and is summarized in Table 97. The target organs reported in the parental generation are the thymus, the spleen, the testes and/or the epididymides. In the testes, seminiferous tubular atrophy and hyperplasia of interstitial cells were observed. Despite effects on reproductive organs, no effects on reproductive parameters were noted. This could be explained by the fact that rodent males produce sperm in numbers that greatly exceed the minimum requirements for fertility (sperm production could be reduced up to 90% without affecting fertility in Sprague-Dawley and Wistar rats). Besides, in the OECD 421 study, exposure of males was limited to two weeks before mating, which is probably not sufficient to affect spermatogenesis and fertility. Testicular effects seen in rats were regarded as not severe and occurred at doses that also caused general toxicity (evident as reduced body weight gain (RAC, 2012c)). Thus, these effects were not considered sufficient for Repr. 1B classification for fertility effects. Total early resorptions and foetotoxicity is considered for classification as Repr. 1B for developmental toxicity (RAC, 2012c). Both considered studies result in a NOAEL of 50 mg/kg bw/d for developmental effects.

27.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as THFA has no classification related to adverse effects on or via lactation.

27.4 Information taken into account for risk assessment and uncertainties

27.4.1 POD-selection

The study by Hirata-Koizumi et al. (2008) is considered as key study in this restriction proposal because of compliance with OECD 421 and because it was performed according to the GLP principles. The NOAEL for foetotoxicity of this study is used as point of departure (PoOD) for the DNEL calculation: 50 mg/kg bw/d (LOAEL: 150 mg/kg bw/d).

27.4.2 Uncertainty

No obvious uncertainties could be identified.

27.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in Table 98.

Table 97: Detailed Overview of the derivation of the $DNEL_{general\ population,\ reproductive\ effects}$

Description (AF=Assessment factor)	Value	Remark
Development		
$POD_{Developmental\ effects}$	**NOAEL: 50 mg/kg bw/d**	**OECD 421, foetotoxicity (Hirata-Koizumi et al., 2008)**
Overall AFs	**100**	
AF for interspecies differences - remaining differences allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to developmental effects)	**0.5 mg/kg bw/d**	

28 tributyltin compounds (CAS No. 56-35-9, 1461-22-9, 56-36-0)

Current harmonized classification and labelling: Repr. 1B (H360FD) according to the CLP regulation. This entry includes the anionic substituents of tri-n-butyltin (TBT) compounds such as halides, alkoxylates or carboxylates. As all of them have a common feature of metabolic hydroxylation and dealkylation, the rationale for the assessment of reproductive toxicity is based on the existing toxicity data for bis(tri-n-butyltin) oxide, tri-n-butyltin chloride, and tri-n-butyltin acetate (RAC, 2013a). Currently, there are no exceptions to this compound group specified in annex VI to Regulation (EC) number 1272/2008 nor in the seventh adaptation to technical progress (Commission Regulation (EU) number 2015/1221 of 24 July 2015).

Most of the studies are extracted from the CLH report and a RAC opinion on tributyltin compounds (RAC, 2013a).

For the present restriction proposal a recent literature research was performed from 2012, as the CLH report on tributyltin compounds was published in 2013. From the recent literature survey no study would have changed the derived no effect level (DNEL) but three additional studies were included to support the findings of low-dose spermatotoxic effects in mice (Ananie and Huang, 2001; Si et al., 2012; Si et al., 2015).

28.1 Adverse effects on sexual function and fertility

28.1.1 Animal data

Table 98: Summary table of animal studies on adverse effects of tributyltin compounds on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results**	**Reference**
20 day female fertility study (no guideline followed, no GLP) reliable with restrictions: shorter exposure time and lower numbers of dams as compared to OECD TG 414 oral (gavage, vehicle: olive oil) rat (Jcl:Wistar, 12 weeks old) 10-14 f/group	tributyltin chloride 0, 8.1, 12.2, 16.3 mg/kg bw/d mated females treated from gestation day (GD) 0-7, sacrifice on GD 20	**NOAEL: 8.1 mg/kg bw/d** **LOAEL: 12.2 mg/kg bw/d (pregnancy failure)** increase of pregnancy failure dose dependent : 0% (control), 18% (8.1 mg/kg bw/d), 71% (12.2 mg/kg bw/d), 77% (16.3 mg/kg bw/d) (no foetuses with external, skeletal and internal malformations in treated or control groups)	Harazono et al. (1996)
two-generation study (no guideline followed, no GLP) reliable with restrictions: lower numbers of dams as compared to OECD TG 416 oral (diet) rat (Kud:Wistar,	tributyltin chloride 0, 0.4, 2.0, 10.0 mg/kg bw/d mated females treated from GD 0 until weaning of F1, F1 reduced to 4 m and 4 f and exposed by diet from weaning until sacrifice on PND 119 (m) or PND 148 (f), F1	**NOAEL: 0.4 mg/kg bw/d** **LOAEL: 2 mg/kg bw/d** (significant decrease of absolute organ weights of testes and epididymis)) 10 mg/kg bw/d: reduced numbers of pups/litter in both of the generations 2 mg/kg bw/d:	Ogata et al. (2001), Omura et al. (2001), Omura et al. (2004)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results**	**Reference**
11 weeks old) 10-12 f/group	males/females (n=13-18 per group) mated on PND 92 to produce the next generation F2, F2 m/f exposed from weaning until sacrifice on PND 91 (m) or PND 92 (f)		
4 week repeated dose study focused on male fertility (testicular weight, sperm head counts, histology, no guideline followed, no GLP) reliable with restrictions because of low animal numbers as compared to OECD guidelines for reproductive toxicity (e.g., 421) oral (gavage), vehicle: 0.2% ethanol in distilled water mice (ICR, 5weeks old) 6 m/group	bis tributyltin oxide 0.4, 2.0, 10.0 mg/kg bw/ twice a week exposure for 4 weeks	**$NOAEL_{corrected}$: 0.11 mg/kg bw/d (**0.4 mg/kg bw/twice a week) **LOAEL: 2 mg/kg bw/twice a week**(significantly reduced sperm head counts in two independent experiments) 10 mg/kg/d: failure of seminiferous tubules to organise as well as in vacuolisation of Sertoli cells, no test on statistical differences was applied for histological findings (convertion to a daily dose: $NOAEL_{corrected}$=NOAEL×(doses per week/7	Kumasaka et al. (2002)
30 d repeated dose toxicity study with focus on male fertility (no guideline followed, no GLP), parameters investigated: sperm parameters with histopathology of epididymis and	tributyltin chloride 0.5, 5 and 50 µg/kg bw/ once every 3 days exposure for 30 days	NOAEL: could not be determined LOAEL: 0.17 µg/kg bw/d (0.5 µg/kg bw/every 3 days) (significant decrease of sperm count, sperm viability, testicular testosterone and proliferating cell nuclear antigen (all dose-dependent))) 5 µg/kg bw: significant increase of sperm abnormality (dose-dependent)	Chen et al. (2008)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results**	**Reference**
evaluation of spermatozoa reliable with restrictions because of low animal numbers as compared to OECD guidelines for reproductive toxicity (e.g., 421) oral (gavage), vehicle: 0.1% ethanol in 0.85% sodium chloride in water mice (Kun Ming, 21 d old) 8 m/group		50 µg/kg bw: significant decrease of testes weights (dose-dependent) conversion to a daily dose: $NOAEL_{corrected}$=NOAEL×(doses per week/7	
45 day repeated dose toxicity study with focus on male fertility (no guideline followed, no GLP), parameters investigated: histological examination of epididymis, biochemical effects on epididymis, sperm parameters oral (gavage), vehicle: 0.1% ethanol in 0.85% sodium chloride in water reliable with restrictions because of low animal numbers as compared to OECD	tributyltin chloride 0.5, 5 and 50 µg/kg bw/ once every 3 days exposure for 45 days	**NOAEL: could not be determined** **LOAEL: 0.17 µg/kg bw/d** (0.5 µg/kg bw/every 3 days) (significant decrease of sperm count and viability (dose dependent), significant increase of sperm abnormality (dose dependent)) conversion to a daily dose: $NOAEL_{corrected}$=NOAEL×(doses per week/7	Yan et al. (2009)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results**	**Reference**
guidelines for reproductive toxicity (e.g., 421) mice (Kun Ming, 21 days old) 6 m/group			
repeated dose toxicity study (no guideline followed, no GLP), exposure of pregnant females and in F1 assessment of female puberty (vaginal opening, first estrus), ovarian morphology assessment, sperm parameters, hormone assays, gross necropsy and organ weights reliable with restrictions because no guideline followed, age of P0 not stated oral (gavage), vehicle: corn oil mice (Kun Ming, outbred) P0: 10-11 f/group, F1: 5 m, 5 f/litter where possible	tributyltin chloride 1, 10, 100 µg/kg bw/day exposure of pregnant females (P0) beginning on GD 6 until weaning of F1; on PND 4, all litters were standardized to 10 pups, maintaining an equal number of males and females per litter when possible; weaning on PND 21	**NOAEL (F1): could not be determined** **LOAEL (F1): 1 µg/kg bw/d** (sperm count and motility) F1 male: significant decrease of sperm count and motility at 1 and 10 µg/kg bw/d (dose dependent) F1 female, 1 µg/kg: significantly earlier onset of puberty (age of vaginal opening and first estrus)	Si et al. (2012), Si et al. (2015)
male fertility experiments with focus on testicular development and sperm parameters	dibutyltin dichloride 0.025, 0.05, 0.10,	**NOAEL: 0.025 µg DBTCl/kg bw/d (intraperitoneal injection)** 0.05 µg /kg bw/d: significantly reduced testes weight, reduced sperm viability and density, increased sperm abnormality (e.g., no hook, excessive hook, amorphous,	Ananie and Huang (2001)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
(no guideline followed, no GLP) reliable with restrictions, not considered for DNEL calculation because of different exposure route but used as supporting information because it confirms low-dose effects of a TBT metabolite intraperitoneal injection mice (Kun Ming, 7-8 weeks old) 7-8 m/group	0.20, and 0.40 µg/kg bw/d exposure for 7 days	short head, twin heads, coiled flagelium, bent flagelium, twisted flagelium); all parameter change dose-dependent 0.1 µg /kg bw/d: significantly reduced body weight	

28.1.2 Human data

No human data available.

28.1.3 Short discussion and overall relevance of the provided information on adverse effects on sexual function and fertility

Adverse effects on sexual function and fertility after repeated exposure to TBT compounds are well documented in several studies with rats and mice. The used studies are considered reliable with restrictions because they were not conducted according to international guidelines or no information on guideline compliance is available. However, they are still regarded as scientifically sound. In a 2-generation study with rats, adverse effects on fertility involved decreased organ weights of testes in F1, decreased organ weights of ventral prostate in F2, decreased homogenization-resistant spermatid counts in F2 at a daily intake of 2 mg/kg bw/d (Ogata et al., 2001; Omura et al., 2001; Omura et al., 2004) resulting in a NOAEL of 0.4 mg/kg bw/d. A male fertility study with 5 weeks old ICR mice resulted in significantly reduced sperm head counts from 2 mg/kg/twice a week (NOAEL of 0.4 mg/kg bw/twice a week or NOAELcorrected: 0.11 mg/kg bw/d). Three studies with the mouse strain KM (Kun Ming) resulted in LOAELs for tributyltin chloride from 0.17 – 1 µg/kg bw/d for spermatotoxic effects (i.e., dose dependent decrease of sperm count and motility and increase in sperm abnormality) and hence resulting in a >3 orders of magnitude discrepancy compared to other studies with rats or different mouse strains (i.e., ICR mice) (Chen et al., 2008; Si et al., 2012; Yan et al., 2009). Low-dose effects

of butyltin compounds on sperm quality and quantity were also demonstrated by Ananie and Huang (2001) with male KM mice and daily intraperitoneal injection of dibutyltin (DBT) dichloride for 7 days. Since dibutyltin derivatives are the first common metabolites of TBT compounds (RAC, 2013a), this study supports the low-dose effects after TBT exposure. The effect of TBT compounds on the rate of insemination was not assessed in Kun Ming mice. However, according to Mangelsdorf et al. (2003) mating experiments with model animals might not be suitable for predicting effects on fertility in humans because rats and rabbits are still fertile when the sperm count is reduced by 90 and 99%, respectively. In contrast, human fertility may already be affected by small reductions in sperm count (Mangelsdorf et al., 2003). Amongst male fertility endpoints, sperm motility was found to be the most sensitive endpoint in some cases (Mangelsdorf et al., 2003). In fact, sperm concentration, motility and morphology are important descriptors of male fertility (Guzick et al., 2001; Mangelsdorf et al., 2003) and hence spermatotoxic effects are of high concern.

The studies by Chen et al. (2008), Yan et al. (2009), and Si et al. (2012, 2015) are regarded as reliable with restrictions, despite the lack of guideline compliance, non-continuous exposure (Chen et al., 2008; Yan et al., 2009), low animal numbers (6 – 11/group) compared to certain guidelines and higher NOAELs in studies with other model organisms.

Unfortunately, all studies considered in this report for adverse effects on sexual function and fertility do not comply with international guidelines.

Low animal numbers reduce the statistical power and hamper the detection of statistical differences, thus statistically significant differences obtained with low animal numbers should be taken seriously. Additionally, in all three independent studies, which found low-dose effects (Chen et al., 2008; Si et al., 2012; Si et al., 2015; Yan et al., 2009), the overserved effects are dose-dependent and occur in a similar dose range. Thus, a coincidental occurrence is highly implausible. Furthermore, OECD guidelines for repeated dose toxicity studies (407, 408) recommend even lower minimum animal numbers (4-5 females, males).

Non-continuous exposure (i.e., once every three days; Chen et al. (2008); Yan et al. (2009)) reduces absolute exposure compared to continuous exposure (i.e., daily), and thus positive results under non-continuous exposure conditions are more concerning. Additionally, different vehicles have been used in this studies (i.e., 0.1% ethanol in 0.85% sodium chloride in water or corn oil) for the gavage application.

The fact that low-dose effects were not observed in other studies can either imply that the exposure conditions in the other studies are not adequate to induce effects (e.g., low-dose exposure) or that the animal models used in the other studies (rats or ICR mice) are less sensitive with respect to the observed effects. The latter is supported by a study by Farmakalidis and Murphy (1984), which demonstrated the low sensitivity to an oestrogenically active compound of the mouse strain used in the only other mouse study with higher effect concentrations (Kumasaka et al., 2002). Furthermore, high-dose effects of endocrine disrupting compounds cannot necessarily predict low-dose effects and low-dose effects of numerous compounds are well documented (Vandenberg et al., 2012). Also, dibutyltin, the first TBT metabolite (RAC, 2013a), shows similar low-dose spermatotoxic effects (Ananie and Huang, 2001) albeit with a different exposure route (intraperitoneal injection). Si et al. (2012) also demonstrated that low-dose maternal TBT exposure induced a significantly earlier onset of F1 female puberty (LOAEL: 1 µg/kg bw/d) emphasizing the endocrine disrupting effects of TBT.

28.2 Adverse effects on development

28.2.1 Animal data

Table 99: Summary table of animal studies on adverse effects of tributyl compounds on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
two-generation reproduction study (according to OECD 416, no GLP compliance stated) reliable oral (diet) rat (Sprague Dawley) 30 m, 30 f/group in P0 and F1 generation	bis(tri-n-butyltin) oxide 0, 0.5, 5.0, 50 ppm (approximately: 0, 0.03, 0.3, 3 mg/kg bw/d exposure: P0: 10 weeks prior to mating, during cohabitation with exposure of females continuing during gestation and lactation F1: 15 weeks prior to mating and during cohabitation with exposure of females continuing during gestation and up to weaning	NOAEL (F1): 0.3 mg/kg bw/day (reduced body weight of offspring) *effects on P0:* 3 mg/kg bw/d: significantly reduced thymus weights *effects on F1:* 3 mg/kg bw/d: significantly reduced body weight gain *effects on F2:* 3 mg/kg bw/d: significantly reduced body weight gain	Schroeder (1990)
18 day prenatal developmental toxicity test (no guideline followed, no GLP) reliable with restrictions because no guideline followed and exposure not	bis(tri-n-butyltin) oxide 0, 1.2, 3.5, 5.8, 11.7, 23.4, 35 mg/kg bw/d exposure GD 6-15, dams sacrificed on GD 18	**NOAEL: 5.8 mg/kg bw/day** **LOAEL: 11.7 mg/kg bw/d** (significantly increased rates of cleft palates (7% versus 0.7% in control, increase dose-dependent)) 23.4 mg/kg bw/d: slightly reduced foetal body weight, increased frequency of variations (dislocated sternum with ossification centres, 41% vs. 6% in controls); increased frequency of malformations (fused basis of the os occipitalis 27% vs. 0.4% in controls); not stated if significant	Davis et al. (1987)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
over the whole gestation period oral (gavage), vehicle: olive oil mouse (NMRI) 100 (control), 10, 9, 20, 18, 10 and 6 pregnant dams/dose (corresponds to the order of exposure groups in the next column)			
developmental toxicity study (no guideline followed, no GLP) reliable with restrictions because no guideline followed and exposure not over the whole gestation period oral (gavage) mouse (Swiss albino) 17 (control), 26, 25, 36 and 8 dams/dose (corresponds to the order of exposure groups in the next column)	bis(tri-n-butyltin) oxide 0, 5, 10, 20, 30 mg/kg bw/d exposure GD 6 – 15, dams were allowed to litter, litters were normalised at birth to 8 pups, offspring terminated at PND 7, 14 or 21	**NOAEL: Could not be determined** **LOAEL: 5 mg/kg bw/day (**significantly ($p<0.05$) decreased ratio of pups/implantation sites) 10 mg/kg bw/d: reduced nest-building activity of dams, postnatal survival decreased on PND 7 (66% vs 95% in controls, $p< 0.01$), postnatal pup body weight gain decreased on PND 7 ($p<0.01$)	Baroncelli et al. (1995)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
prenatal developmental toxicity test (similar to OECD 414 but no guideline followed, no GLP) reliable with restrictions because no guideline followed and exposure not over the whole gestation period oral (gavage), vehicle: corn oil rat (Sprague-Dawley) 24 mated f/group	bis (tri-n-butyltin) oxide 0, 5, 9, 18 mg/kg bw/d exposure GD 6-19, dams sacrificed on GD 20	**NOAEL (F1): could not be determined** LOAEL (F1): **5 mg/kg bw/day** (increased incidences of ossification variations in exposed foetuses (asymmetric sternebrae, rudimentary structures, 14th rib pair)) *maternal effects:* 18 mg/kg bw/d: significantly reduced weight gain developmental effects: at 9 mg/kg bw/d significantly ($p<0.01$) increased percentage of foetuses with at least 1 skeletal ossification variation	Schroeder (1981)
prenatal developmental toxicity test (similar to OECD 414 but no guideline followed, no GLP) reliable with restrictions because no guideline followed, low animal numbers compared to comparable guidelines, exposure not over the whole gestation period	tributyltin chloride 0, 5, 9, 15, 25 mg/kg bw/d exposure GD 7-15, dams sacrificed on GD 20	NOAEL: (could not be determined) **LOAEL: 5 mg/kg bw/day** significantly decreased female foetal (f) body weight, significantly reduced number of ossified sternebrae (variation) 25 mg/kg bw/d: significantly reduced number of live foetuses	Itami et al. (1990)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
oral (gavage), vehicle: olive oil rat (Wistar) 10-12 mated f/group			
developmental toxicity, (no guideline followed, no GLP), focus on histopathological changes of several organs, no skeletal effects examined reliable with restrictions because no guideline followed, low animal numbers compared to comparable guidelines, exposure not over the whole gestation period oral (gavage), vehicle: olive oil rats (Sprague-Dawley) 16 dams/dose group, 12 randomly selected pups/dose group/time point?	tributyltin chloride 0, 0.025, 0.25, 2.5 mg/kg bw/d dams treated from GD 8 until birth and throughout lactation, dams sacrificed post weaning, pups treated with the same dose as dams from weaning onwards and sacrificed on PND 30 (males and females), PND 60 (females only) and PND 90 (males only)	**NOAEL (F1): 0.025 mg/kg bw/d** **LOAEL (F1): 0.25 mg/kg bw/d** significant reduction of thymus weights in female offspring (day 60; decrease dose-dependent) *parental effects:* no effects observed *developmental effects:* 2.5 mg/kg bw/d: significant reduction of pup liver weights (15-20%; p<0.05; decrease dose-dependent); significant reduction of pup spleen weights (20%; p<0.05; decrease dose-dependent); significant reduction of thymus weights in male offspring (day 30)	Cooke et al. (2004)

28.2.2 Human data

No human data.

28.2.3 Short discussion and overall relevance of the provided information on adverse effects on development

Numerous studies reported developmental effects of foetuses after maternal exposure. Most common effect is a decreased pup body weight gain (Baroncelli et al., 1995; Davis et al., 1987; Itami et al., 1990; Schroeder, 1990) with a lowest NOAEL for this endpoint of 0.3 mg/kg bw/d (Schroeder, 1990). Cooke et al. (2004) detected significantly reduced thymus weights in a developmental toxicity study with Sprague-Dawley rats (dams treated from GD 8 until birth and throughout lactation, dams sacrificed post weaning, pups treated from weaning onwards and sacrificed PND 30 (males and females), PND 60 (females only) and PND 90 (males only)) with a LOAEL of 0.25 mg/kg bw/d (NOAEL: 0.025 mg/kg bw/d). Additionally, a dose-dependent decrease of the spleen weight was observed (2.5 mg/kg bw/d; $p \leq 0.05$). In a study performed in parallel, perinatal exposure of rat pups to tributyltin chloride was found to cause significant alterations in immune tissue morphology and immune function (Tryphonas et al., 2004). Thymus atrophy has been observed in other studies after exposure to TBT compounds and can be regarded as a serious effect potentially impairing the immune system (Snoeij et al., 1988). For example, Snoeij et al. (1988) found dose-dependent thymus atrophy in 4-5 weeks old rats after a single oral dose of tri-n-butyltin chloride (statistically significant at 10 mg/kg bw/d). Since organotin compounds cross the placenta and accumulate in foetal tissues (Cooke et al., 2008) it can be concluded that foetuses are at risk of adverse effects of organotin compounds.

28.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as tributyltin has no classification related to adverse effects on or via lactation.

28.4 Information taken into account for risk assessment and uncertainties

28.4.1 POD-selection: sexual function and fertility

Three different studies resulted in LOAELs for spermatotoxicity in the low µg/kg bw/d range by use of the mouse strain Kun Ming and were considered reliable with restrictions. The lowest LOAELs obtained in these studies were used as points of departure (POD) to calculate the DNEL range (Chen et al., 2008; Si et al., 2012; Si et al., 2015; Yan et al., 2009). The LOAEL from the studies by Chen et al. and Yan et al. were corrected from exposure once every three days to daily exposure ($LOAEL_{corrected} = LOAEL \times (\text{doses per week}/7)$): 0.17 µg/kg bw/d.

28.4.2 POD-selection: development

The lowest NOAEL found in literature for developmental effects in offspring is used as point of departure (PoD) to calculate the DNEL: 0.025 mg/kg bw/d (LOAEL: 0.25 mg/kg bw/d) (Cooke et al., 2004).

28.4.3 Uncertainty: sexual function and fertility

There are some uncertainties regarding the selected LOAEL because the respective studies were not performed according to a standardised guideline and GLP. Also, the LOAEL had to be extrapolated from an exposure once every three days to a daily exposure, which increases the uncertainty. Additionally, the effect concentrations of other studies by use of different test species are around three orders of magnitude higher. However, as discussed in the previous section, the study is still regarded as reliable with restrictions in particular because low-dose effects of tributyltin compounds with similar effect levels have been confirmed in three

independent studies (Cheng et al., 2014; Si et al., 2012; Si et al., 2015; Yan et al., 2009) plus one additional study with the main TBT metabolite dibutyltin (Ananie and Huang, 2001). To account for the uncertainties we calculated a DNEL range instead of a single DNEL.

28.4.4 Uncertainty: development

The respective study is rated as reliable with restrictions because no guideline was followed, low animal numbers compared to comparable guidelines and exposure was not over the whole gestation period.

28.4.5 DNEL derivation

Table 100: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Fertility		
$POD_{Fertility\ effects}$	**LOAEL: 0.17 - 1 µg/ kg bw/d (0.00017 - 0.001 mg/kg bw/d)**	**repeated dose toxicity study with focus on male fertility with KM mice, effects: dose dependent decrease of sperm count and viability (Chen et al., 2008; Si et al., 2012; Si et al., 2015; Yan et al., 2009)**
Overall AFs	**3150**	
AF for interspecies differences - remaining differences - allometric scaling	 2.5 7	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	6	The default assessment factor for extrapolation from sub-acute to chronic exposure duration was applied as the key study was a three-generation study.
AF related to dose response relationship	3	An AF of 3 was applied as the POD is a LOAEL
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to fertility effects)	**0.05 – 0.3 ng/kg bw/d (5.4×10^{-8} – 3.7×10^{-7} mg/kg bw/d)**	
Development		
$POD_{Developmental\ effects}$	**NOAEL: 0.025 mg/kg**	**developmental toxicity study with Sprague Dawley rats, effects: decreased thymus weights in offspring**

	bw/d	**(Cooke et al., 2004)**
Overall AFs	**100**	
AF for interspecies differences - remaining differences allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	1	No AF applied due to developmental effects.
AF related to dose response relationship	1	No AF was applied as the POD is a NOAEL
AF related to quality of database	1	Default value.
DNEL **general population, reproductive effects** (related to developmental effects)	**0.25 µg/kg bw/d (0.00025 mg/kg bw/d)**	

29 Trixylyl phosphate (CAS No. 25155-23-1)

Trixylyl phosphate is a substance of unknown or variable composition, complex reaction products or biological materials (UVCB) containing over 50 different constituents and no additives (RAC, 2010; RIVM, 2009). It is listed in Annex VI of the CLP legislation as Repr. 1B (H360F).

The key studies and respective NOAEL values for the endpoint reproductive toxicity of trixylyl phosphate as shown in Table 102 were selected based on the recent RAC opinion on a CLH report mentioned above (RAC, 2010; RIVM, 2009).

For the present restriction proposal a recent literature survey was performed from 2008, as the CLH report on trixylyl phosphate was published in 2009.

29.1 Adverse effects on sexual function and fertility

29.1.1 Animal data

Table 101: Summary table of animal studies on adverse effects of trixylyl phosphate on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
combined oral repeated dose and reproductive/developmental toxicity study reliable study, according to OECD 422, GLP oral exposure (gavage), vehicle: corn oil rat (Sprague-Dawley) 11 f and 11 m/dose group	trixylyl phosphate 0, 25, 200 and 1000 mg/kg bw/d exposure 2 weeks prior to mating throughout gestation and lactation. Males were dosed for 33 days in total, females for 48 days The control and high-dose groups included five additional animals/sex, which were used for recovery experiments (3 weeks for females and 4 weeks for males)	**NOAEL:** could not be determined **LOAEL: 25 mg/kg bw/d** (histological changes in reproductive organs) 25 mg/kg bw/d: dose-dependent degeneration of the germinal epithelium of the testes and sloughed epithelial cells lumen epididymis; significantly increased adrenal weight (females, in males significant in the high dose group only) 200 mg/kg bw/d: significant and dose-dependent reduction in implantations and a decreased number of gravid dams effects are reversible after 4 weeks	Experimur (2004)
Reproductive toxicity study: i) 98-day continuous breeding phase of the F0 generation, ii) cross over mating to determine the affected sex in the F0 animals, iii) examination of the fertility and performance of the last litter (F1) from the continuous breeding supporting study, read-across mouse (Swiss (ICR)BR outbred albino (CD1))	tricresyl phosphate (TCP; CAS No. 1330-78-5) TCP was mixed into the feed at 0, 0.05. 0.1, and 0.2% by weight exposure throughout the 98-day breeding phase and crossover mating	F0:dose-related seminiferous tubule atrophy and decreased testes and epididymal weights and changes in the adrenals of both sexes F1: males: reduced sperm motility, decreased fertility index	Chapin et al. (1988)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
control group: 40 breeding pairs; dose groups: 20 breeding pairs			
Reproductive toxicity study supporting study, read-across oral (gavage); vehicle: sesame oil rat (Fischer 344) control: 40 breeding pairs; dose groups: 20 breeding pairs	tricresyl phosphate (TCP; CAS No. 1330-78-5) 400 mg/kg bw/d exposure from 7 days prior to pairing continuing for a 63 day breeding period and through a 28 day post-breeding interval	Only one dose tested LOAEL: 400 mg/kg bw/d male rats: significantly reduced testicular and epididymal weights as well as significant reduction of fertility index and pups per litter female rats: increased ovarian weights crossover mating: 100% infertility of male rats	Latendresse et al. (1994)

29.1.2 Human Data

No human data available.

29.1.3 Short discussion and overall relevance of the provided information on adverse effects on sexual function and fertility

The information and data provided are partly extracted from the CLH report for trixylyl phosphate (RAC, 2010; RIVM, 2009).

In a combined oral repeated dose and reproductive/ developmental toxicity study with Sprague-Dawley rats (Experimur, 2004), rats were exposed by oral gavage to doses of 0, 25, 200 or 1,000 mg/kg bw/d of trixylyl phosphate from 2 weeks prior to mating throughout gestation and lactation. There was no effect on mating. Pregnancy and successful parturition was observed in all animals from the control and low dose group (25 mg/kg bw/d), but was reduced in animals from the mid dose group (200 mg/kg bw/d), where only 2/11 dams underwent parturition. In the high dose group (1000 mg/kg bw/d), none of the ten mated females underwent parturition. Analysis of the uterus revealed only 2 pregnant animals in the high dose group, and no additional pregnant animals in the mid dose group (besides the 2 that underwent parturition), indicating that the reduced pregnancy rate is mainly the result of decreased fertility and not post implantation loss.

To determine the cause for the adverse effects on pregnancy observed in the core groups, additional animals from the control and high dose group were left to recover from the trixylyl phosphate exposure. Male recovery rats from the high dose group were used for cross over mating with naive control females, and recovered rats from the high dose and control groups were used for within group mating. Following both cross over mating and within-group mating, no effects were observed on pregnancy or parturition, suggesting that the effects on fertility are reversible.

Based on reproductive outcome, a NOAEL of 25 mg/kg bw/d could be established. However, since histological changes in reproductive organs were already observed at the lowest dose level (25 mg/kg bw/d), for effects on reproductive organs, only a LOAEL could be established (25 mg/kg bw/d). The combination of partly different effects on the reproductive organs in males and females and an effect on the adrenals (increased weight and diffuse cytoplasmic vacuolation) suggests an effect on the steroid production.

Additionally, also other tri-substituted phosphates affect fertility (RAC, 2010; RIVM, 2009). However, like trixylyl phosphate, these substances are UVCBs with sometimes limited descriptions of the identity and content of the constituents, which limits the possibilities to apply a read-across approach. However, the data on analogues of trixylyl phosphate can be considered as additional evidence to demonstrate that the results found with trixylyl phosphate are not random findings.

In a feeding study with Tricresyl phosphate (TCP) in Swiss CD-1 mice (Chapin et al., 1988) using continuous breeding protocol impaired fertility in both sexes of mice in the parental animals and affected sperm motility at even the lowest dose in F1 males were revealed. A study with TCP in F344 rats (Latendresse et al., 1994) with daily oral administration for up to 135 days using a modified continuous breeding protocol resulted in impaired fertility in the male sex, increases in adrenal gland, liver and ovarian weights, decreases in testicular and epididymal weights and histopathological degeneration of the seminiferous tubules.

Based on the study by Experimur (2004) and supported by two read-across studies (Chapin et al., 1988; Latendresse et al., 1994), this substance is classified for reproductive toxicity in category 1B according to the criteria in regulation EC/2172/2008.

29.2 Adverse effects on development

Not relevant for the present restriction proposal as trixylyl phosphate has no Repr. 1A/B classification related to adverse effects on development.

29.3 Adverse effects on or via lactation

Not relevant for the present restriction proposal as trixylyl phosphate has no classification related to adverse effects on or via lactation.

29.4 Information taken into account for risk assessment and uncertainties

29.4.1 POD-selection

The study by Experimur (2004) is considered as key study in this restriction proposal because of compliance with OECD 422 and because it was performed according to the GLP principles. The LOAEL for foetotoxicity of this study is used as point of departure (PoD). The LOAEL for histological changes in reproductive organs is used as point of departure (PoD) for the DNEL calculation: 25 mg/kg bw/d.

29.4.2 Uncertainty

No obvious uncertainties could be identified.

29.4.3 DNEL derivation

The calculated DNEL and applied assessment factors are shown in Table 103.

Table 102: Detailed outline of the derivation of the DNEL general population, reproductive effects

Description (AF=Assessment factor)	Value	Remark
Fertility		
POD Fertility effects	**LOAEL: 25 mg/kg bw/d**	**combined oral repeated dose and reproductive/developmental toxicity study according to OECD 422, effects: histological changes in reproductive organs (Experimur, 2004)**
Overall AFs	**1800**	
AF for interspecies differences - remaining differences allometric scaling	2.5 4	For interspecies differences the appropriate default factors are applied to account for the differences between the experimental animals and humans and for remaining differences according to the REACH guidance R.8.
AF for intraspecies differences	10	The default factor is applied according to the REACH guidance R.8 because no substance-specific information is available for an adjustment.
AF for differences in exposure duration	6	The default assessment factor for extrapolation from sub-acute to chronic exposure duration was applied as the key study was a sub-acute study.
AF related to dose response relationship	3	AF of 3 was applied as the POD is a LOAEL
AF related to quality of database	1	Default value.
DNEL general population, reproductive effects (related to fertility effects)	**0.014 mg/kg bw/d**	

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Appendix B.4. Inclusion of Cosmetic Product Regulation Annex II substances in the proposed restriction

1.1. Background

Annex II of Directive 76/768/EEC (the Cosmetic Products Directive, CPD), later included as Annex II of the CPR (Cosmetic Products Regulation (EC) NO 1223/2009), is part of the Council of Europe Resolution ResAP(2008)1[12] and its predecessor ResAP(2003)2. Annex II of the CPR contains a list of substances prohibited in *cosmetic products* (see article 14 of CPR).

The ResAP recommends that tattoo and PMU must only be used if they do not contain substances listed in Annex II (in addition to other recommendations).

The ResAP (2008)1 and (2003)2 are the benchmark for those Member States having national legislation and for those taking restrictive measures against hazardous tattoo inks on the market based on general safety requirements.

Annex II of the CPR includes substances with various hazardous properties, including amongst others CMR and skin sensitising substances, but also various other substances which may or may not have a harmonised classification. Although CMR and skin sensitising substances are covered in separate group justifications of the restriction proposal, the following justification provides a basis for inclusion of the entire list of substances in Annex II within the scope of the proposed restriction. Given the similarities in exposure potential (prohibited in cosmetic products which by definition (article 2 of CPR) are applied, among other, on the external parts of the human body, which include the epidermis), there is merit in considering all of these substances for a comparable restriction for use in tattoo inks and PMU.

1.2. Analysis of CPR Annex II substances

1.2.1. Coverage of CPR Annex II

Cosmetic products are defined as any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours (CPR, Article 2(1a)).

The CPR (and its predecessor, the CPD) requires that a cosmetic product made available on the EU market shall be safe for human health[13] when used under normal or reasonably foreseeable conditions of use (article 3 of the CPR). It also requires that, prior to placing a cosmetic product on the market, the cosmetic product has undergone a safety assessment (article 10 of the CPR). Where the Commission has concerns about the safety of a substance,

[12] Council of Europe Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up (superseding Resolution ResAP(2003)2 on tattoos and permanent make-up), 20 February 2008.

[13] The environmental concerns that substances used in cosmetic products may raise are considered through the application of 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) and establishing a European Chemicals Agency(4) OJ L 396, 30.12.2006, p. 1. (4), which enables the assessment of environmental safety in a cross-sectoral manner (recital 5 of CPR).

an opinion can be requested of the Scientific Committee on Consumer Safety (SCCS).[14] This process informs the adaptation of Annex II to the CPR (and other annexes), and substances have therefore been progressively added to Annex II since 1976, when the CPD was introduced. Therefore, for some of the substances on Annex II, there is a specific or generic opinion of the Committee to support its restriction. However, for others there is not.

According to article 15 of the CPR, the use of substances classified as CMRs categories 1A, 1B, and 2 shall be prohibited unless:

- For category 2 CMR substances (article 15.1), if the substances have been evaluated by the SCCS and found safe for use in cosmetic products.
- For category 1A and 1B CMR substances (article 15.2), such substances can be exempted if the substances meet the following conditions:
    - They comply with the food safety requirements
    - There are no suitable alternative substances available, as documented in an analysis of alternatives
    - The application is made for a particular use of the product category with a known exposure
    - They have been evaluated and found safe by the SCCS for use in cosmetic products.

If the substances meet the above conditions, they are not added to Annex II but to Annexes III-VI depending on the type of substance and on the conditions of use specified in the SCCS opinion. If the substances do not meet these conditions, following the addition of the substances in Annex VI to Regulation (EC) No 1272/2008, the Commission periodically adds these substances to Annex II with a comitology measure. For category 1A and 1B CMR substances, this is to be done within 15 months from inclusion (date of application of the CMR classification) in Annex VI to Regulation (EC) No 1272/2008 as specified in article 15.2, while no timeframe is foreseen to provide an exemption for CMR 2 substances.

While the intentional use is prohibited, the non-intended presence of small quantities (traces) of prohibited substances stemming from impurities, the manufacturing process, storage, migration from packaging, which is technically unavoidable in good manufacturing practice, "is permitted" provided that such presence is in conformity of article 3, i.e., that they are safe for human health, taking into account conformity with Directive 87/357/EEC (products that appear to be foodstuffs), labelling, instructions for use and disposal, and other indication or information provided by the responsible person[15].

Annex II to the CPR therefore contains a range of substances with various health hazards that cannot be used in cosmetic products. Under the proposed restriction, these substances would also be prohibited from use in tattoo inks and permanent make-up inks.

1.2.2. CPR Annex II substances included within scope of the proposed restriction

This section presents an analysis of the substances included in Annex II of the CPR (also

[14] The SCCS replaced the previous Scientific Committee on Consumer Products (SCCP, 2004-2009) which in turn replaced the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP; 1997-2004) which was preceded by the Scientific Committee on Cosmetology (SCC, 1977-1997).

[15] "Responsible person" is defined in article 4 of the CPR.

summarised in the table below). Annex II of the CPR includes 1 490 individual substances that should not be contained in cosmetic products (prohibited substances). Of these, the majority are substances with harmonised classification under Annex VI of the CLP Regulation, while 624 substances are without harmonised classification.

Of the substances with harmonised classification, 795 are classified as CMR category 1A, 1B or 2 and 103 substances are classified as skin sensitising (SS). 805 are classified as either CMR or skin sensitising, or both.

Of the 61 substances with harmonised classification for effects other than CMR and skin sensitisation, 56 are classified for acute toxicity, and 17 of these 56 are also classified for specific target organ toxicity (STOT). Of the remaining five substances, one is classified for STOT (but not acute toxicity), one for skin corrosivity and three for explosive/pyrophoric/self-heating properties.

Of those substances without *harmonised* classification and labelling (624 substances), 357 have classifications *notified* to ECHA. Most of these notifications identify a concern, with 307 substances notified (by one or more company) as CMR, acute toxic, STOT, skin corrosive, skin irritant, skin sensitisation or eye irritation/damage. Most of the remainder (50 substances) were notified as "not classified", though a few had notified classifications for or for effects in the aquatic environment or respiratory sensitisation.

The remaining 267 substances without either harmonised or notified classifications include numerous medications/drugs (e.g., barbiturates), poisonous plant extracts such as hemlock and Datura stramonium, and various dyes including those that are, or are degradation products with carcinogenic/mutagenic properties (e.g., benzidine based azo dyes). According to the 2016 JRC study (JRC, 2016b), in total 31 out of the 67 azo colourants in use in tattoo and PMU products contain and might release, by simple reductive cleavage, one of the amines included in the negative lists cited in CoE ResAP(2008)1, including substances in Annex II of the CPR (JRC, 2016b).

Table 103 Summary of analysis of substances in Annex II CPR

Category of analysis	**Number of substances**
Substances in Annex II CPR	1 490
Substances with harmonised classifications	866
Substances classified as CMR	795
Substances classified as SS	103
Substances classified as irritant/corrosive	13
Substances classified as either CMR or SS	805
Substances classified as other than CMR or SS	61
- Of which with Acute Toxic classification	56
- Of which with Acute Toxic and STOT classification	17
Substances without HCL	624
Substances notified to ECHA (without harmonised classification)	357
- Of which notified as CMR, Acute Toxic, STOT or skin corrosive/irritant/ sensitising	307
Substances without harmonised classification or notification	267

1.2.3. Extent of use of CPR Annex II substances in tattoo inks

The list of substances on the CPR Annex II includes substances that have previously been used in cosmetic products, but also some that are prohibited despite (probably) never having been used in cosmetics. There is however a need to provide a safeguard to ensure that such use does not occur in the future.

The extent to which the totality of the CPR Annex II substances have historically been used in tattoo inks is unclear. However, it is clear that some have been used until relatively recently. From the list of 1 490 substances included in Annex II, 68 have been identified as having been used in tattoo inks in the past, based on (JRC, 2015b). Of these 68 substances, 37 are classified for properties other than CMR, skin sensitisation, corrosivity or irritation (which are also covered elsewhere in the restriction proposal). These substances are presented in the table below.

Table 104 Substances on Annex II CPR identified as present in tattoo inks or PMU

Substance Name(s)	**EC number**	**CAS number**	**CPR Annex II entry #**	**Skin sensitiser**	**Carcinogenic or mutagenic**	**Reprotoxic**	**Skin irritant/ corrosive, Eye irritant/ damaging**
Aniline, its salts and its halogen-nated and sulphonated derivatives	200-539-3	62-53-3	22	yes	yes		yes
Benzidine	202-199-1	92-87-5	26		yes		

Antimony (Sb) , Antimony and its compounds, Antimony (Sb)	231-146-5	7440-36-0	40				
Antimony (Sb) , Antimony and its compounds, Antimony (Sb)	231-146-5	7440-36-0	40				
Arsenic (As), Arsenic and its compounds	231-148-6	7440-38-2	43		yes		yes
Arsenic (As), Arsenic and its compounds	231-148-6	7440-38-2	43				
Cadmium (Cd) , Cadmium and its compounds, Cadmium (Cd)	231-152-8	7440-43-9	68		yes	yes	
Chromium (Cr) (VI)6 , Chromium; chromic acid and its salts, Chromium (tot)	231-157-5	7440-47-3	97				
Mercury (Hg), Mercury and its compounds, except those special cases included in Annex V	231-106-7	7439-97-6	221			yes	
2-naphtylamine , 1-and 2-Naphthylamines and their salts, 2-Naphthylamine	202-080-4	91-59-8	242		yes		
Lead (Pb) , Lead and its compounds, Lead (Pb)	231-100-4	7439-92-1	289				
Lead (Pb) , Lead and its compounds, Lead (Pb)	231-100-4	7439-92-1	289			yes	
Selenium (Se) , Selenium and its compounds with the exception of selenium disulphide under the conditions set out under reference No 49 in Annex III, Selenium (Se)	231-957-4	7782-49-2	297				
Thallium and its compounds, Thallium (Tl)	231-138-1	7440-28-0	317				
Thallium and its compounds, Thallium (Tl)	231-138-1	7440-28-0	317				
4-methyl-m-phenylenediamine , 4-Methyl-m-phenylenediamine (Toluene-2,4-diamine)	202-453-1	95-80-7	364	yes	yes	yes	

and its salts, 4-Methyl-mphenylendiamine							
4-methoxy-m-phenylenediamine, 1-Methoxy-2,4-diaminobenzene (2,4-diaminoanisole - CI 76050) and its salts, 4-Methoxy-mphenylendiamine	210-406-1	615-05-4	376		yes		
Colouring agent CI 12075 (Pigment Orange 5) and its lakes, pigments and salts, Pigment Orange 5 , Pigment orange 5	222-429-4	3468-63-1	397				
Pigment Orange 5 , Colouring agent CI 12075 (Pigment Orange 5) and its lakes, pigments and salts, Pigment orange 5	222-429-4	3468-63-1	397				
Colouring agent CI 45170 and CI 45170:1 (Basic Violet 10), Basic Violet 10, Basic violet 10	201-383-9	81-88-9	398				
Colouring agent CI 15585, Pigment Red 53, Pigment red 53	218-248-5	2092-56-0	401				
Colouring agent CI 15585, Pigment red 53:1	225-935-3	5160-02-1	401				
Nitrosamines e.g. Dimethylnitrosoamine; Nitrosodipropylamine; 2,2'-Nitrosoimino)bisethanol , Nitrosodimethylamine	200-549-8	62-75-9	410		yes		
Nitrosamines e.g. Dimethylnitrosoamine; Nitrosodipropylamine; 2,2'-Nitrosoimino)bisethanol , Nitrosodiethanolamine	214-237-4	1116-54-7	410		yes		
Benzo[def]chrysene (benzo[a]pyrene), Benzene-a-pyrene (BaP) , Benzo[a]pyrene	200-028-5	50-32-8	612	yes	yes	yes	
Dibenz[a,h]anthracene, Dibenzo(a,h)anthracene	200-181-8	53-70-3	637		yes		
Benz[a]anthracene, Benzo(a)anthracene	200-280-6	56-55-3	638		yes		

Benzo[j]fluoranthene, Benzo(j)fluoranthene	205-910-3	205-82-3	640		yes		
Benz(e)acephenanthrylene, Benzo(b)fluoranthene	205-911-9	205-99-2	641		yes		
Benzo(k)fluoranthene	205-916-6	207-08-9	642		yes		
Chrysene	205-923-4	218-01-9	643		yes		
Dibutyl phthalate, Dibutyl phthalate (DBP)	201-557-4	84-74-2	675			yes	
bis(2-Ethylhexyl) phthalate (Diethylhexyl phthalate), Di-(2-ethylhexyl) phthalate (DEHP)	204-211-0	117-81-7	677			yes	
4,4'-methylenedianiline, 4,4’-Methylenedianiline, 4,4'-Methylendianiline	202-974-4	101-77-9	705	yes	yes		
4,4'-methylenedi-o-toluidine, 4,4’-Methylenedi-o-toluidine, 4,4'-Methylendi-o-toluidine	212-658-8	838-88-0	707	yes	yes		
o-anisidine, o-Anisidine	201-963-1	90-04-4, 90-04-0	708		yes		
3,3'-dimethoxybenzidine, 3,3’-Dimethoxybenzidine (ortho-Dianisidine) and its salts, 3,3'-Dimethoxybenzidine	204-355-4	119-90-4	709		yes		
3,3'-d-dichlorobenzidine, 3,3’-Dichlorobenzidine, 3,3'-Dichlorobenzidine	202-109-0	91-94-1	712	yes	yes		
3,3'-dimethylbenzidine , 4,4’-Bi-o-toluidine (ortho-Tolidine), 3,3'-Dimethylbenzidine	204-358-0	119-93-7	721		yes		
Biphenyl-4-ylamine, Biphenyl-4-ylamine (4-Aminobiphenyl) and its salts	202-177-1	92-67-1	726		yes		
o-aminoazotoluene , 4-o-Tolylazo-o-toluidine, Solvent Yellow 3 , o-Aminoazobenzene	202-591-2	97-56-3	989	yes	yes		

4-aminoazobenzene, 4-Aminoazobenzene, Solvent Yellow 1	200-453-6	60-09-3	990		yes		
Nickel (Ni)8 , Nickel, Nickel (Ni)	231-111-4	7440-02-0	1093	yes	yes		
2,4,5-trimethylaniline, 2,4,5-Trimethylaniline [1], 2,4,5-Trimethylaniline	205-282-0	137-17-7	1158		yes		
4,4'-thiodianiline, 4,4’-Thiodianiline and its salts, 4,4'-Thiodianiline	205-370-9	139-65-1	1159		yes		
4,4'-oxydianiline , 4,4’-Oxydianiline (p-Aminophenyl ether) and its salts, 4,4'-Oxydianiline	202-977-0	101-80-4	1160		yes	yes	
6-methoxy-m-toluidine, 6-Methoxy-m-toluidine; (p-Cresidine), 4-Methoxy-m-toluidine	204-419-1	120-71-8	1162		yes		
Naphthalene	202-049-5	91-20-3	1167		yes		
Phenol	203-632-7	108-95-2	1175		yes		yes
5-nitro-o-toluidine, 5-Nitro-o-toluidine [1], 5-Nitro-o-toluidine	202-765-8	99-55-8	1195		yes		
Solvent Red 1 (CI 12150), when used as a substance in hair dye products, Solvent red 1	214-968-9	1229-55-6	1231				
2,2’-[(3,3’-Dichloro[1,1’-biphenyl]-4,4’-diyl)bis(azo)]bis[3-oxo-N-phenylbutanamide] (Pigment Yellow 12) and its salts, when used as a substance in hair dye products, Pigment yellow 12	228-787-8	6358-85-6, 15541-56-7	1263				
1-[(2-Chloro-4-nitrophenyl)azo]-2-naphthol (Pigment Red 4; CI 12085) and its salts when used as a substance in hair dye products, 1-[(2-Chloro-4-nitrophenyl)azo]-2-naphthol and its insoluble barium, strontium and zirconium lakes, salts and pigments, Pigment red 4	220-562-2	2814-77-9	1345				

3-Hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide (Pigment Red 112; CI 12370) and its salts when used as a substance in hair dye products, 3-Hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide, Pigment red 112	229-440-3	6535-46-2	1346				
N-(5-Chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulphonyl]-2-methoxyphenyl]azo]-3-hydroxynaphthalene-2-carboxamide (Pigment Red 5; CI 12490) and its salts when used as a substance in hair dye products, N-(5-Chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulphonyl]-2-methoxyphenyl]azo]-3-hydroxynaphthalene-2-carboxamide, Pigment red 5	229-107-2	6410-41-9	1347				
Calcium 3-hydroxy-4-[(1-sulphonato-2-naphthyl)azo]-2-naphthoate (Pigment Red 63:1; CI 15880) when used as a substance in hair dye products, Calcium 3-hydroxy-4-[(1-sulphonato-2-naphthyl)azo]-2-naphthoate, Pigment red 63:1	229-142-3	6417-83-0	1349				
Tetrasodium 6-amino-4-hydroxy-3-[[7-sulphonato-4-[(4-sulphonatophenyl)azo]-1-naphthyl]azo]naphthalene-2,7-disulphonate (Food Black 2; CI 27755) when used as a substance in hair dye products, Tetrasodium	218-326-9	2118-39-0,	1354				

6-amino-4-hydroxy-3-[[7-sulphonato-4-[(4-sulphonatophenyl)azo]-1-naphthyl]azo]naphthalene-2,7-disulphonate, Pigment black 2							
8,18-Dichloro-5,15-diethyl-5,15-dihydrodiindolo[3,2-b:3′,2′-m]triphenodioxazine (Pigment Violet 23; CI 51319) when used as a substance in hair dye products, 8,18-Dichloro-5,15-diethyl-5,15-dihydrodiindolo[3,2-b:3',2'-m]triphenodioxazine, Pigment violet 23	228-767-9	6358-30-1	1360				
8,18-Dichloro-5,15-diethyl-5,15-dihydrodiindolo[3,2-b:3′,2′-m]triphenodioxazine (Pigment Violet 23; CI 51319) when used as a substance in hair dye products, 8,18-Dichloro-5,15-diethyl-5,15-dihydrodiindolo[3,2-b:3',2'-m]triphenodioxazine, Pigment violet 23	228-767-9	6358-30-1	1360				
8,18-Dichloro-5,15-diethyl-5,15-dihydrodiindolo[3,2-b:3′,2′-m]triphenodioxazine (Pigment Violet 23; CI 51319) when used as a substance in hair dye products, 8,18-Dichloro-5,15-diethyl-5,15-dihydrodiindolo[3,2-b:3',2'-m]triphenodioxazine, Pigment violet 23	228-767-9	6358-30-1	1360				
6-Chloro-2-(6-chloro-4-methyl-3-oxobenzo[b]thien-2(3H)-ylidene)-4-methylbenzo[b]thiophene-3(2H)-one (VAT Red 1; CI 73360) when	219-163-6	2379-74-0	1365				

used as a substance in hair dye products, 6-Chloro-2-(6-chloro-4-methyl-3-oxobenzo[b]thien-2(3H)-ylidene)-4-methylbenzo[b]thiophene-3(2H)-one, Pigment red 181							
5,12-Dihydroquino[2,3-b]acridine-7,14-dione (Pigment Violet 19; CI 73900) when used as a substance in hair dye products, 5,12-Dihydroquino[2,3-b]acridine-7,14-dione, Pigment violet 19	213-879-2	1047-16-1	1366				
(29H,31H-Phthalocyaninato(2-)-N29,N30,N31,N32)copper (Pigment Blue 15; CI 74160) when used as a substance in hair dye products, (29H,31H-Phthalocyaninato(2-)-N29,N30,N31,N32)copper, Pigment blue 15	205-685-1	147-14-8	1367				
Disodium [29H,31H-phthalocyaninedisulphonato(4-)-N29,N30,N31,N32]cuprate(2-) (Direct Blue 86; CI 74180) when used as a substance in hair dye products, Disodium [29H,31H-phthalocyaninedisulphonato(4-)-N29,N30,N31,N32]cuprate(2-), Direct blue 86	215-537-8	1330-38-7	1368				
Polychloro copper phthalocyanine (Pigment Green 7; CI 74260) when used as a substance in hair dye products, Polychloro copper phthalocyanine, Pigment green 7	215-524-7	1328-53-6	1369				
Polychloro copper phthalocyanine (Pigment Green 7; CI 74260) when used as a substance in hair dye products, Polychloro copper phthalocyanine, Pigment green 7	215-524-7	1328-53-6	1369				

Diethylene glycol (DEG); 2,2'-oxydiethanol for traces level, see Annex III, 2,2'-oxydiethanol Diethylene glycol (DEG), Diethyleneglycol	203-872-2	111-46-6	1370				
Isopropyl 4-hydroxybenzoate (INCI: Isopropylparaben) Sodium salt or Salts of Isopropylparaben, Isopropylparaben	224-069-3	4191-73-5	1374				
Isobutyl 4-hydroxybenzoate (INCI: Isobutylparaben) Sodium salt or Salts of Isobutylparaben, Isobuthylparaben	224-208-8	4247-02-3	1375				

Based on the above discussion, it is clear that there are various hazardous substances included in CPR Annex II. Some of these have EU harmonised classification to reflect their hazards; some have classifications recognised and notified by their suppliers, while others do not have classifications under CLP but are known to be hazardous for humans. Preventing the use of these substances in tattoo inks could therefore avoid potentially significant health impacts in the event that they are used in the future. This is further elaborated upon in the justification below.

1.2.4. Reasoning for inclusion of Annex II CPR substances without individual risk assessment

Annex I of REACH, para 0.5 states that "Where available and appropriate, an assessment carried out under Community legislation (e.g., risk assessments completed under Regulation (EEC) No 793/93) shall be taken into account in the development of, and reflected in, the chemical safety report. Deviations from such assessments shall be justified." Therefore the Dossier Submitter recommends that substances included in Annex II of the CPR based on an assessment of the SCCS and supported by the Member States when agreeing to an amendment of the CPR, should be restricted in tattoo inks and PMU taking into account section 1.4. However, note that not all inclusions in annex II is based on SCCS opinions. E.g., if industry does not want to defend a substance or the substance is a drug or classified CMR, it can be included as well. The Dossier Submitter recommends that substances on Annex II of the CPR without an SCCS opinion should also be restricted in tattoo inks and PMU.

1.3. Technical background to the justification

1.3.1. The dermal absorption route of chemicals into the body

Skin forms part of the so called physiological "first line of defence", acting as a physical barrier preventing external agents from entering the body (DEPA, 2012a). It is composed of three layers: the epidermis, dermis and subcutaneous layer.

In order to reach the viable *epidermis*, the *dermis* and the *vascular network* (blood and lymph

vessels), substances - including for example those contained in cosmetics applied to the skin - must first penetrate into the *stratum corneum,* a complex lipid membrane, composed of the outer 3-5 cell layers of the epidermis (DEPA, 2012a) (ECHA, 2014a).

The passage of compounds across the skin, can be divided into three main stages: *penetration* is the entry of a substance into a particular skin layer or structure such as the *stratum corneum*; *permeation* is the penetration through one layer into another, which is both functionally and structurally different from the first one; *resorption* is the uptake of a substance into the vascular system (lymph and/or blood vessel) (ECHA, 2014a).

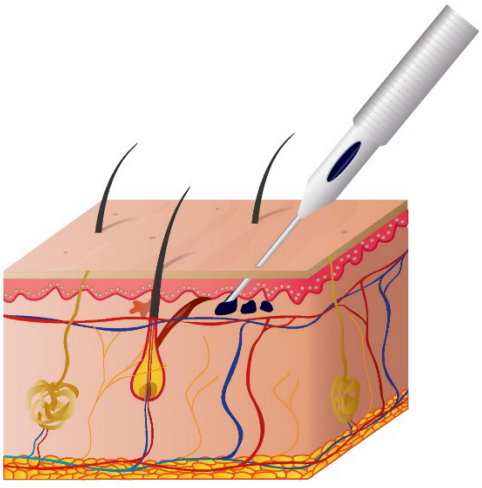
Dermal absorption of a substance is influenced by many factors. These include the physicochemical properties of the substance, such as: its physical state; physical and chemical structure; molecular weight; charge; solubility; and lipophilicity. Dermal absorption also depends on the substance's vehicle, concentration, and exposure pattern; on the thickness and composition of the *stratum corneum* (which depends on the body site); and on the amount of topically applied product. As an example, liquids and substances in solution are taken up more readily than dry particulates; similarly, molecular weight less than 100 favours dermal uptake, while molecules above 500 may be too large to be absorbed by the skin (ECHA, 2014a).

The *stratum corneum* acts as the skin's greatest barrier against penetration of chemical substances and in particular of hydrophilic compounds. Penetration of substances across the *stratum corneum* is difficult; when skin is intact, only small fat-soluble molecules can pass and in small amounts (DEPA, 2012a).

1.3.2. Tattoo inks and the intra-dermal exposure route

Tattoo inks are injected or pricked with needles around 0.1-0.5 mm into the skin in the outer third of the dermis, where pigments become distributed just under the epidermis and below the basement membrane (DEPA, 2012a). PMU is applied similarly, although some state that PMU is deposited shallower into the skin than inks for decorative tattoos (De Cuyper, 2010). Hence, tattoo inks and PMU are applied in such a way as to bypass the natural protection barrier given by the epidermis and enter the human body. This exposure pathway is defined as *intradermal exposure*, which occurs when the integrity of the skin is disrupted by the use of consumer products (e.g., by earrings, piercings or tattoo inks) (ECHA, 2016c).

Figure 4 Injection of tattoo ink under the epidermis.



Source: Photolia.

Evidence from the literature suggests that the intradermal route may result in greater bio-availability of a substance following exposure. For example, a 2016 study by Jungmann et al. reported that while formaldehyde[16] is unable to penetrate through an intact epidermal barrier, direct injection into living and vascularised dermal tissue may result in immediate systemic bioavailability (Jungmann, et al., 2016).

Whilst the knowledge about the fate of tattoo pigments once injected into the body is still limited, the initial quantity of pigment injected in human skin during the tattooing process has been proven to decrease over time (JRC, 2016b), and some of the pigments deposited in the skin are known to migrate from the skin and pass into the body. The 2016 JRC report (JRC, 2016b) indicates several mechanisms which can explain these findings, such as dispersion in the skin, metabolism, and transportation through the lymphatic or blood vessel systems. The report also indicates that pigment particles have been found in macrophages, in the cytoplasm of cells in secondary lysosomes and in lymph nodes. Studies have also shown the migration of the pigments via the lymph and blood system to other organs such as the liver. (Sepehri, et al., 2017)

Moreover, tattoos often fade if they have been applied in parts of the body normally exposed to light. This suggests the occurrence of photochemical decomposition by visible and/or UV light, where particles may be decomposed to form other chemical substances or to be metabolised locally in the skin (DEPA, 2012a). For instance, irradiation of the widely used red-violet 2,9 dichloroquinacridone has been shown to produce, among other things, 4-chloroaniline which is itself a suspected carcinogen (Petersen, 2015), cited by (JRC, 2016b).

Finally, impurities, byproducts and additives contained in tattoo inks such as polycyclic aromatic hydrocarbons, primary aromatic amines and heavy metals could be transported away from the skin and into the body. PAHs (originally present in black inks) were reported to remain partially in skin but were also found in the regional lymph nodes (JRC, 2016b).

1.4. Justification for inclusion of substances within the restriction

The substances included in CPR Annex II are prohibited for use in cosmetics, regardless of the concentration expected to be applied/received. The information presented in Sections 1.3.1 and 1.3.2 of this appendix indicates that the intradermal injection of a substance into the body through tattooing is expected to be at least as high, and in most cases higher, than an equivalent amount of the same substance administered to the skin in a cosmetic product. The CoE resolutions reflect this by requiring provisions for tattoo inks and PMU that are at least as strict as those for cosmetic products under the CPR. This is therefore also reflected by Member States that base their national legislation on CoE resolutions.

Therefore, taking into account the decisions of the Member States and recommendations of the expert committees for inclusion of substances in CPR Annex II, it may be concluded that:

- As the natural protection barrier of the epidermis is broken, the risks of a dose applied beneath the skin (in tattoo inks) is likely to pose at least as high (if not higher) risk to human health than an equivalent dose applied on the skin.

[16] Reaction products of formaldehyde are included in the Annex II of CPR, entry 1128. Formaldehyde is classified as carcinogenic category 1B, mutagenic category 2, skin sensitising category 1, skin corrosive category 1B, and acute toxic category 3.

- The CPR Annex II prohibits the use of a number of substances for use in cosmetic products. It does not establish a safe dose in cosmetic products for the application of these Annex II substances on the skin.
- There is therefore a basis for recommending that these substances should be restricted in tattoo inks and PMU relying on the decisions made for inclusion of the substances under CPR Annex II without a detailed risk assessment of each substance.

There are, however, a number of uncertainties associated with the application of such a rationale, as outlined in the following section.

1.5. Uncertainties and possible adverse impacts

Having reviewed the rationale for restricting the substances in CPR Annex II in tattoo inks and PMU, providing some examples on some individual substances listed, a number of observations are made:

- The rationale for inclusion of some of the CPR Annex II substances is clear, particularly in relation to recent amendments to the CPR/CPD where there is an associated opinion of the SCCS. However, for many of the substances there are no such associated opinions. For example, some of the inclusions relate specifically to certain uses in cosmetic products (e.g., hair dyes or substances used as a fragrance ingredient) and not others. It is uncertain to what extent other uses have been examined in the decision to place the substance on Annex II and what the implications are for risks associated with potential use in tattoo inks.

- Based on the earlier discussion, the risks associated with exposure to a substance at an equivalent dose are expected to be at least as high, if not higher, for exposure via tattooing compared to exposure via cosmetics. However, in some cases this conclusion may not hold true considering that a tattoo may only be applied once, or a limited numbers of times, and while it leads to long-term exposure, this exposure may be different than the exposure associated with a cosmetic product applied and removed multiple times (up to daily application over most of a lifetime).

- The number of substances included in CPR Annex II that have actually been used in tattoo inks is unknown, but the above suggests that the number is at least 69, or around 5% of the total substances included on Annex II. A restriction would therefore likely cover various substances that would never find use in tattoo inks.

- While Annex II of the CPR does not include any concentration threshold for substances prohibited from use, adapting this for a restriction on tattoo inks might require consideration of such a low concentration limit. In particular, some substances might be present in detectable but toxicologically negligible concentrations, with their removal being impractical or would require substantial resources, exceeding any benefits of their elimination. Examples of such situations have not been collected on the basis of the experience of the Member States with national legislation based on the two resolutions. However, enforcement of Annex II under the CPR allows for the non-intended presence of traces of some substances, stemming from impurities of natural or synthetic

ingredients, the manufacturing process, storage, migration from packaging, which is technically unavoidable in good manufacturing practice, and if the presence of the substance can be evaluated as safe for the consumer.

- It would be important to ensure that any restriction on substances in tattoo inks based on CPR Annex II remains relevant over time.[17] In particular, since CPR Annex II is frequently adapted to reflect the latest scientific information (e.g. through addition of new substances), a mechanism would presumably be required to ensure that the restriction on tattoo inks is also kept up-to-date.

1.6. Concentration limit

As stated above, substances on Annex II are prohibited in cosmetic products; therefore, they are currently enforced at a limit of detection (LoD) by Member States with national legislation. As the justification for risk is based on conclusions that intradermal exposure is at least as risky as dermal exposure, the appropriate measure would be to restrict these substances in the same way as under the CPR, i.e. tattoo inks shall not contain substances on annex II to the CPR (RO1).

The one disadvantage to this approach is that it would be difficult to differentiate between intentional and non-intentional use, which the CPR does effectively by allowing traces of prohibited substances if not intentionally added but found in cosmetic products, due to e.g., impurities or as a result of the manufacturing process. Therefore, the Dossier Submitter proposes a second restriction option (RO2), which allows small amounts of these substances, i.e., less than 0.1% w/w, in tattoo inks and PMU. The 0.1% w/w concentration limit is proposed as a practical limit aiming to discourage intentional use.

[17] It should be further investigated whether from a legal point of view a technical amendment of the current restriction to reflect future amendments of Annex II of the CPR is sufficient without the preparation of a new Annex XV dossier.

Appendix B.5. Inclusion of Cosmetic Product Regulation Annex IV substances in the proposed restriction

1.1. Scope of the possible restriction on CPR Annex IV substances

Annex IV of Directive 76/768/EEC (the Cosmetic Products Directive, CPD), which later became Annex IV of the CPR (Cosmetic Products Regulation (EC) NO 1223/2009), is part of the Council of Europe Resolution ResAP(2008)1 and its predecessor ResAP(2003)2.[18] The ResAP recommends that tattoo and permanent make up (PMU) products only be used if they do not contain substances listed in column 2 to 4 of Annex IV of the CPD), now reflected in Annex IV of the CPR, column 'g'. The ResAP (2008)1 and (2003)2 are the benchmark for those Member States having national legislation and for those taking restrictive measures against hazardous tattoo inks on the market based on general safety requirements.

Article 14 of the CPR establishes that cosmetic products shall not contain any colourants other than those listed in Annex IV (List of colourants allowed in cosmetic products). For a number of these substances, Annex IV also establishes specific conditions outside of which their use in cosmetics is prohibited. Such conditions are specified, in terms of product type (rinse-off or leave-on) and of body parts for which the use of substances is allowed or prohibited (e.g., lips, eyes, etc.), the maximum concentration allowed in ready for use preparation, as well as other conditions (e.g. purity requirements).

The conditions are specified in columns "g" to "i" in Annex IV of the CPR:

- Column "g" in the CPR: "Product type/Body part" contains information formerly summarised in columns 1 to 4 of the CPD: "Field of application" as follows:
    - Column 1 of the CPD – Colouring agents allowed in all cosmetic products.
    - Column 2 of the CPD – Colouring agents allowed in all cosmetic products except those intended to be applied in the vicinity of the eyes, in particular eye make-up and eye make-up remover. CPR labels these colourants in column g as colourants "not to be used in eye products"
    - Column 3 – Colouring agents allowed exclusively in cosmetic products intended not to come into contact with the mucous membranes. CPR labels these colourants in column g as colourants "not to be used in products applied on mucous membranes".
    - Column 4 – Colouring agents allowed exclusively in cosmetic products intended to come into contact only briefly with the skin. CPR labels these colourants in column g as colourants allowed in "rinse-off products".
- Columns "h" and "i" in CPR,[19] respectively "Maximum concentration in ready for use preparation" and "other" correspond to the former column "Other limitations and requirements".

[18] Council of Europe Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up (superseding Resolution ResAP(2003)2 on tattoos and permanent make-up), 20 February 2008.

[19] One additional column has been added in the CPR: "j": "Wording of conditions of use and warnings". To date, no conditions have been specified in this column for any of the colourants on Annex IV.

According to these specific conditions for use it is possible to identify, the following groups of colourants are proposed to be included in the scope of the restriction, as follows:

- The use of the following colourants in tattoo inks to be restricted (i.e., not to be allowed):
    - Colourants allowed in rinse-off products only;
    - Colourants not to be used in products applied on mucous membranes;
    - Colourants not to be used in eye products;
- The use of the following colourants to be allowed in tattoo inks under the conditions specified for use in cosmetic products:
    - Colourants allowed in all cosmetic products in concentrations not exceeding the limits specified in Annex IV or subject to other conditions specified in columns "g" to "i" of the Annex (e.g., purity requirements)

Substances allowed only in rinse-off products are considered to pose risks to human health when their use leads to a prolonged exposure. Substances that must not be used in products applied on mucous membranes or in the vicinity of the eyes are considered to pose risks to human health when used via bypassing of the epidermal barrier (or rather providing conditions for an easier penetration of the epidermal layer, in comparison to skin). As use of inks in tattoo applications leads both to prolonged exposure and to circumvention of the skin barrier, the use of these substances in tattoo applications is considered to pose at least equal risks as the above uses (for an equivalent dose). Given this, there is merit in adopting comparable measures in Annex XVII to the conditions in Annex IV of the CPR on colourants in use in tattoo inks and PMU.

In addition, some colourants used in cosmetic products have been shown to pose a risk to human health when applied to the skin in concentrations exceeding the limits specified in Annex IV or other conditions specified in columns "g" to "i" of the Annex (e.g., purity requirements). Therefore, given the similarities in exposure potential (i.e., prohibited or allowed to be used under specific conditionsin cosmetic products which by definition (art. 2 of CPR) are applied, among other, on the external parts of the human body, which include the epidermis), there is merit in considering a comparable restriction for use of these colourants in tattoo inks and PMU. This is also the basis of a similar argumentation for including substances on Annex II of the CPR in the scope of the proposed restriction.

The following justification provides a more detailed explanation for inclusion of the list of substances on Annex IV column 'g' to 'i' and which are included in the categories described above within the scope of the proposed restriction.

1.2. Analysis of CPR Annex IV substances

1.2.1 Coverage of Annex IV

The CPR (and its predecessor, the Cosmetic Product Directive, CPD) requires that a cosmetic product made available on the market shall be safe for human health[20] when used under

[20] The environmental concerns that substances used in cosmetic products may raise are considered through the application of 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) and establishing a

normal or reasonably foreseeable conditions of use. It also requires that, prior to placing a cosmetic product on the market, the cosmetic product has undergone a safety assessment. Where the Commission has concerns about the safety of a substance, an opinion can be requested of the Scientific Committee on Consumer Safety (SCCS)[21]. Following the scientific opinion from the SCCS, the Commission puts the draft amendment of the CPR annexes (including Annex IV - allowing, prohibiting or restricting the use of the substance assessed in cosmetics products), to the vote of the Member States represented in the Standing Committee on Cosmetic Products. Further to a positive opinion of the Standing Committee, the Commission adopts the amendment to the annexes.

When the Cosmetics Products Directive entered into force in 1976, a total of 311 colourants were allowed for use[22]. A list of colourants was provisionally included in Annex IV for a period of three years during which further assessment took place (SMPA, 2017). Following further investigation, substances in the Annex were:

- either definitively permitted,
- or definitively prohibited (Annex II),
- or retained for a further period of three years in Annex IV,
- or deleted from all Annexes to the Directive (Art 5 CP Directive).

In 1986, the SCCS (or rather its predecessor committee, the SCC) evaluated a large number of substances and produced more than 60 opinions which resulted in the current list of 153 entries in Annex IV of the CPR (SMPA, 2017). These opinions were reported in a 1988 published document (SCC, 1988). Thereafter, only occasional assessments were made which amended Annex IV (in 2000[23], 2005[24] and 2007[25]) (SMPA, 2017).

1.2.2. CPR Annex IV substances included within scope of the proposed restriction

This section presents an analysis of the substances in Annex IV of the CPR falling in the scope of the proposed restriction (also summarised in the table below). These include substances that, based on the specifications in Annex IV, can only be used in rinse-off cosmetic products, or that must not be used in eye products or in products applied on mucous membranes.

Note that according to definitions provided in the CPR:

- "*Rinse-off product* means a cosmetic product which is intended to be removed after application on the skin, the hair or the mucous membranes";
- "*Product applied on mucous membranes* means a cosmetic product which is intended to be applied on the mucous membranes

European Chemicals Agency(4) OJ L 396, 30.12.2006, p. 1. (4), which enables the assessment of environmental safety in a cross-sectoral manner (recital 5 of CPR).

[21] The SCCS replaced the previous Scientific Committee on Consumer Products (SCCP, 2004-2009) which in turn replaced the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP; 1997-2004) which was superseded by the Scientific Committee on Cosmetology (SCC, 1977-1997).

[22] These included substances listed in Annex III and Annex IV of the Directive.

[23] The use of dye CI 42640 was prohibited due to carcinogenic properties.

[24] Three azo dyes were removed from Annex IV part 1 due to their potential degradation into aromatic amines.

[25] A dye containing iodine was deleted from Annex IV.

— of the oral cavity,

— on the rim of the eyes,

— or of the external genital organs";

- "*Eye product*" means a cosmetic product which is intended to be applied in the vicinity of the eyes;"

It should be noted that the different entries in the legislative text of CPR Annex IV are mainly identified by a Colour index number (CI number). Since several of the relevant CI numbers can be associated with more than one substance, the European Commission's database for information on cosmetic substances (Cosmetic ingredient database, CosIng) has been used as a source file to identify the correct CAS and EC numbers for the entries in Annex IV. Historical information entries have also been included. For more information see Annex E.1.

There are currently a total of 74 substances included in the 'restricted field of application' categories in Annex IV. Of these, 45 can only be used in rinse-off products, 9 are not allowed in eye products, 20 are not allowed in products to be applied on mucous membranes. In addition, 119 substances are allowed with specific conditions on purity, concentration limit or physical form. Sixty-nine colourants on Annex IV are allowed with no conditions; therefore, no action is proposed for these substances.

The table below provides details on these substances by category, highlighting the following:

- 48 of Annex IV colourants proposed to be included in the scope of the restriction are registered under REACH.
- 91 of these substances have notified classifications from companies under the CLP Regulation, with notifications relating to various hazard classes. Two have harmonised classification under the CLP.
- For 27 of these substances there is information that they have been used in tattoos according to a study by the JRC.
- 25 of these substances are also included in Annex II; the inclusion of these substances relates in most cases to the prohibition of use in hair dyes, which are regulated by a specific provision[26]. Therefore, the inclusion of these colourants in Annex II did not lead to the automatic exclusion from Annex IV.

[26] https://ec.europa.eu/growth/sectors/cosmetics/products/hair-dye_en

Table 105 Summary of analysis of substances in Annex IV CPR included in the scope of the proposed restriction

	Rinse-off products only	**Not allowed on eyes**	**Not allowed on mucous membranes**	**Allowed with conditions**	**Total**
Number of substances in Annex IV	45	9	20	119	193
Registered under REACH	15	2	8	41	66
With harmonised classification	0	0	0	2	2
With notified classification	25	3	14	54	96**
Used in tattoos (JRC, 2015b)	10	2	3	28	43
Included in Annex II*	8	2	12	12	34

Notes: *these colourants are prohibited for use in hair dyes, ** excludes those with not classified notifications only

Source: Amec Foster Wheeler, based on data extraction on Annex IV and Annex II substances provided by ECHA on 27 April 2017.

On Annex IV, 119 colourants are allowed with specific conditions as shown in Table 106. Of those:

- Five colourants are not allowed if the maximum concentration of the colourant in ready to use mixtures exceeds the specified limit (but allowed in cosmetic products otherwise), e.g.:
    - Entry #9 (1-[(2-Chloro-4-nitrophenyl)azo]-2-naphthol and its insoluble barium, strontium and zirconium lakes, salts and pigments, CI 12085, Red) is not allowed if it exceeds 3%
    - Entry #25 (Sodium 2-[(2-hydroxynaphthyl)azo]naphthalenesulphonate and its insoluble barium, strontium and zirconium lakes, salts and pigments, CI15630, Red) is not allowed if it exceeds 3%
    - Entry #74 (Disodium 2-(3-oxo-6-oxidoxanthen-9-yl)benzoate, CI45350, Yellow) is not allowed if it exceeds 6%
    - Entry #77 (3',6'-Dihydroxy-4',5'-dinitrospiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, CI45396, Orange) can be used in all products without restriction on the concentration limit, except when used in applications on the lips. For lip products (which leads to exposure of similar or greater risk than cutaneous application), the colourant can be in free acid form only and in concentration not exceeding 1%
    - Entry #126a Carbon Black nano is not allowed if it exceeds 10%
- Six are not allowed if they contain specific pigment constituents (in excess of the specified concentrations), e.g.:
    - Entry #75 (4',5'-Dibromo-3',6'-dihydroxyspiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one and its insoluble barium, strontium and zirconium lakes, salts and pigments, CI 45370, Orange), #76 (Disodium 2-(2,4,5,7-tetrabromo-6-oxido-3-oxoxanthen-9-yl)benzoate and its insoluble barium, strontium and

zirconium lakes, salts and pigments, CI 45380, Red), and #79 (3,4,5,6-Tetrachloro-2-(1,4,5,8-tetrabromo-6-hydroxy-3-oxoxanthen-9-yl)benzoic acid and its insoluble barium, strontium and zirconium lakes, salts and pigments, CI 45410, Red) are not allowed in cosmetic products if they contain more than 1% 2-(6-hydroxy-3-oxo-3H-xanthen-9-y1) benzoic acid and 2% 2-(bromo-6-hydroxy-3-oxo-3H-xanthen-9-yl) benzoic acid

- Entries #129 (Chromium (III) oxide, CI77288, Green) and #130 (Chromium (III) hydroxide, CI77288, Green) are allowed if they are free from the chromate ion
- Entry #138 (Ferric Ammonium Ferrocyanide, CI77510, Blue) is allowed if it is free from the cyanide ion

- 98 colourants are allowed subject to purity requirements as set out in Commission Directives 95/45/EC or specific purity requirements for individual pigments or particle size requirements for nano forms, e.g.,
  - Carbon black (entry 126): purity > 97%, with the following impurity profile: Ash content ≤ 0.15%, total sulphur ≤ 0.65%, total PAH ≤ 500 ppb and benzo(a)pyrene ≤ 5 ppb, dibenz(a,h)anthracene ≤ 5 ppb, total arsenic ≤ 3 ppm, total lead ≤ 10 ppm, total mercury ≤ 1 ppm
  - Carbon black - nano (entry 126a): has similar purity requirements as for non-nano form and specifies that the pigment cannot be used in applications that may lead to exposure of the end user's lungs by inhalation. The nano form of carbon black can be used if the maximum concentration in ready to use preparations exceeds 10% and if the primary particle size is less than 20 nm.

These purity requirements are of particular importance for tattoo inks as the pigments are not exclusively produced for tattoo applications but are often developed for automotive, textile or printing applications among others. Therefore, they are often of lower purity (on average about 25% of the pigment is impurities as per (JRC, 2015b). For these reasons, the CoE ResAP recommends that colourants in general (not only those allowed in cosmetic products but also those not listed on Annex IV) meet the minimum requirements for organic impurities in foodstuffs and cosmetic products as set out in Directive 95/45/EEC (as well as the concentrations of specific impurities listed in Table 3 of ResAP(2008)1).

1.2.3 Extent of use of CPR Annex IV substances in tattoo inks

There is limited information about the extent to which the CPR Annex IV substances subject to the proposed restrictions have historically been used in tattoo inks or continue to be used today. However, from the list of 193 colourants in scope, 43 have been identified as having been used in tattoo inks in the past, based on a study by the JRC (JRC, 2015b).

Table 106 reports additional information on these 43 Annex IV substances which belong to the categories in the scope of the proposed restriction, and which have been reported to be used in tattooing. Of these, 21 are registered under REACH for multiple compositions and a variety of registered uses which include inks, dyes, cosmetics and personal care products, and finger paints. Nine of these substances are also included in Annex II due to the prohibition of use in hair dyes.

Based on our review of the 1986 assessment (SCC, 1988), 1 substance listed does not appear to have any SCCS opinion assessing its risks; the remainder were assessed in 1986. In addition, no opinion could be expressed for three of the substances because of a lack of data;

for seven substances the SCCS established that use in cosmetic products could be maintained for the time being, but that additional data was required[27]. However for none of these substances further data was compiled and no SCCS opinion was produced after 1986, as confirmed in the findings of the Swedish MPA (SMPA, 2017) as well as review undertaken in preparation of this restriction proposal.

Due to the lack of a specific risk assessment and in some cases the lack of the scientific evaluation of the Committee as highlighted above, there is a high level of uncertainty around the risks of 11 (detailed in the previous paragraph) out of 153 entries in Annex IV. Moreover, some of the substances in Annex IV have been identified as hazardous or regulated in hair dyes and food (SMPA, 2017) as mentioned in Section 1.2.1. This is partly reflected by the inclusion of certain Annex IV substances in Annex II (i.e., List of substances prohibited in cosmetic products). Preventing the use of these substances in tattoo inks could therefore avoid potentially significant health impacts in the event that they are used in the future.

[27] The colouring agent CI 73900 does not appear in the 1986 assessment; however the abovementioned study from the Swedish MPA indicates that an SCCS opinion was produced establishing that 'use in cosmetic products could be maintained for the time being, but that additional data was required'.

Table 106 Summary of analysis of selected substances in Annex IV CPR historically used in tattoo inks/PMU

Chemical Name (Annex IV reference)	**CAS number**	**CI number**	**Registered under REACH**	**Registered uses under REACH**	**SCCS opinion available**	**Annex II**
Substances only allowed in rinse off products						
5,12-dihydro-2,9-dimethylquino[2,3-b]acridine-7,14-dione (ref 103)	980-26-7	73915	9 registered compositions (2 with impurities); 19 active registrations; 1000 - 10000 tonnes manufactured and/or imported in EEA per year.	Product categories: **inks** and toners, coating products, fillers, putties, plasters, modelling clay, finger paints, polymers, paper chemicals and dyes, leather treatment products and **cosmetics and personal care**.	1986: no opinion could be expressed because of a lack of data.	N
5,12-dihydroquino[2,3-b]acridine-7,14-dione (ref 102)	1047-16-1	73900	7 registered compositions; 9 active registrations; 1000 - 10000 tonnes manufactured and/or imported in EEA per year.	Product categories: **inks** and toners, coating products, fillers, putties, plasters, modelling clay, **finger paints**, leather treatment products, paper chemicals and **dyes**, polymers and **cosmetics and personal care products**.	1986: use in cosmetic products could be maintained for the time being, but for which additional data was required.	Y
2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[N-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutyramide] (ref 48)	5567-15-7	21108	9 registered compositions (1 with impurities & 1 with additives); 18 active registrations; 1000 - 10000 tonnes manufactured and/or imported in EEA per year.	Product categories: coating products, **inks** and toners, polymers, fillers, putties, plasters, modelling clay and **finger paints**.	1986: use in cosmetic products could be maintained for the time being, but for which additional data was required.	N
8,18-dichloro-5,15-diethyl-5,15-dihydrodiindolo[3,2-b:3',2'-m]triphenodioxazine (ref 85)	6358-30-1	51319	Pre-registered only	n.a.	1986: no opinion could be expressed because of a lack of data.	Y
3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide (ref 11)	6535-46-2	12370	6 registered compositions of which 2 with impurities; 6 active registrations; 1000 - 10000 tonnes manufactured and/or imported in EEA per year.	Product categories: coating products, **inks** and toners and polymers.	1986: use in cosmetic products could be maintained for the time being, but for which additional data was required.	Y
Disodium [29H,31H-	1330-38-7	74180	Pre-registered only	n.a.	1986: use in cosmetic	Y

phthalocyaninedisulphonato(4-)-N29,N30,N31,N32]cuprate(2-) (ref 106)					products could be maintained for the time being, but for which additional data was required.	
N-(4-chloro-2-methylphenyl)-4-[(4-chloro-2-methylphenyl)azo]-3-hydroxynaphthalene-2-carboxamide (ref 12)	6471-51-8	12420	Pre-registered only	n.a.	1986: no opinion could be expressed because of a lack of data.	N
Substances not allowed in eye products						
Polychloro copper phthalocyanine (ref 107)	1328-53-6	74260	18 registered compositions of which 1 with additives; 29 active registrations; 1000 - 10000 tonnes manufactured and/or imported in EEA per year.	Product categories: **inks** and toners, coating products, polymers, **finger paints** and fillers, putties, plasters, modelling clay.	No SCCS opinion is available.	Y
Substances not allowed in products applied to mucous membranes						
2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-N-phenylbutyramide (ref 4)	2512-29-0	11680	4 registered compositions; 4 active registrations; 100 - 1000 tonnes manufactured and/or imported in EEA per year.	Product categories: coating products, inks and toners and polymers.	1986: use in cosmetic products could be maintained for the time being, but for which additional data was required.	N
2-[(4-chloro-2-nitrophenyl)azo]-N-(2-chlorophenyl)-3-oxobutyramide (ref 5)	6486-23-3	11710	6 registered compositions; 6 active registrations; 100 - 1000 tonnes manufactured and/or imported in EEA per year.	Product categories: coating products, inks and toners and polymers.	1986: use in cosmetic products could be maintained for the time being, but for which additional data was required.	N
Bisbenzimidazo[2,1-b:2',1'-i]benzo[lmn][3,8]phenanthroline-8,17-dione	4424-06-0	71105	2 registered compositions of which 4 with additives; 1 active registration; 10 - 100 tonnes manufactured	Product categories: ECHA has no public registered data indicating whether or in which chemical products the substance might be used.	1986: use in cosmetic products could be maintained for the time being, but for which	N

(ref 97)			and/or imported in EEA per year.		additional data was required.	

1.2.4. Basis for restricting Annex IV CPR substances in tattoo inks and PMU without individual risk assessment

Annex I of REACH, paragraph 0.5 states that "Where available and appropriate, an assessment carried out under Community legislation (e.g., risk assessments completed under Regulation (EEC) No 793/93) shall be taken into account in the development of, and reflected in, the chemical safety report. Deviations from such assessments shall be justified." Therefore, similar measures should be considered under REACH for the use in tattoo inks and PMU of substances included in Annex IV of the CPR with specific conditions on field of application, concentration limit, purity requirements, etc. based on an assessment of the SCCS and supported by the Member States when agreeing to an amendment of the CPR. However, it should be noted that not all inclusions in Annex IV of CPR is based on SCCS opinions.

1.3. Basis for restricting Annex IV substances allowed only in rinse-off products

As explained above, the original cosmetics directive (76/768/EEC) included, in Annex IV, a number of 'fields of application' for which use of certain colouring agents was allowed or prohibited. "Field of application 4" included "Colouring agents allowed exclusively in cosmetic products intended to come into contact only briefly with the skin", i.e., allowed in rinse-off products but prohibited for all other cosmetic product uses.

In the CPR, Annex IV takes these conditions and translates them into conditions in columns g, h and i. In particular, for the substances previously included in "field of application" under the CPD, these are now allowed for use under the condition that they are only used in "rinse off products".

The CPR requires that cosmetic products be safe for human health when used under normal or reasonably foreseeable conditions of use, taking into account, amongst others, instructions for use and disposal. The provisions in Annex IV aim at concretising this obligation for colouring agents. They ensure that certain colouring agents are not used in certain cosmetic products, as these cosmetic products are generally assumed to lead to a relatively high exposure to certain cosmetic ingredients (EC, 2006).

To some extent, the provisions related to rinse-off products take into account the possibility that cosmetic products may be applied wrongly (i.e., not according to the use instructions) or that a product may accidentally come into contact with certain parts of the human body, as long as this is "reasonably foreseeable" (EC, 2006). This is similar for all substances with specified fields of application.

Nonetheless, as per the provisions of the original CPD, the requirements of Annex IV for colourants only allowed in rinse-off products are set out on the basis that the ingredients are only expected to have brief contact with the skin.

The fact that these colourants are allowed in cosmetic products but only in those that are expected to have brief contact with the skin implies that the risks to human health are considered acceptable when the substances have only brief contact with the skin, but not acceptable if such contact is longer.

It is assumed that substances used in tattoo inks are expected to have prolonged contact with certain skin layers, therefore they are equivalent to leave-on cosmetic products. In fact the exposure and therefore the risks to these substances may be greater still in tattoo inks. The Dossier Submitter therefore proposes to restrict these substances in tattoo inks and PMU as for cosmetic products the legislator and scientific committees for the CPR have

concluded that the use of such substances in Annex IV should not be allowed in leave-on cosmetic products.

1.4. Basis for restricting Annex IV substances prohibited in eye products and mucous membranes

According to Annex IV of the CPR, a number of the colourants listed may be used, but not in products applied on mucous membranes or in eye products in the vicinity of the eyes and on mucous membranes, the skin barrier (thickness) is more penetrable than normal skin, and the danger of irritation for example is higher. Products applied on mucous membranes are those intended to be applied on the mucous membranes of the oral cavity, on the rim of the eyes, or of the external genital organs. Eye products are those intended to be applied in the vicinity of the eyes. There are specific test results required in relation to irritation of mucous membranes and of the eyes in the assessment of products, when these are being assessed by the SCCS under the CPR.

According to the (SCCS, 2016), "skin and mucous membranes are protected from microbial attack by a natural mechanical barrier and various defence mechanisms. However, these may be damaged and slight trauma may be caused by the action of some cosmetics that may enhance microbial infection. This may become of particular concern when cosmetics are used around the eyes, on mucous membranes in general [etc.]".

Likewise, skin and mucous membrane irritation are frequently observed reactions after the application of cosmetic products (SCCS, 2016).

As with rinse-off products, these two product types were among the restricted fields of application in the original cosmetics directive, and the limitations on the use of substances in these product types have been carried over to the CPR.

When people are exposed to substances via the eyes or mucous membranes, there is potential in many cases for more severe effects than when applied to the epidermis (other factors being equal).

The fact that tattoo inks bypass the natural protective barrier of the skin has been explored in other parts of the restriction proposal. Such exposure can be likened to exposure via the eyes and mucous membranes, which also bypasses this protective layer more easily. It can therefore be argued that substances which are not safe to be used in cosmetic products applied to the eyes or mucous membranes, should also not be safe when present in tattoo inks.

1.5. Basis for regulating Annex IV substances allowed for use under specific conditions under REACH

A number of colourants on Annex IV (119 in total) are allowed for use in all cosmetic products under specific conditions. These conditions include maximum concentration limits for the substance and its constituents or purity requirements for the colourant or its constituents. The information presented in the preceding pages of this paper and in the justification for the inclusion of the substances indicates that the penetration of a substance into the body via the skin through tattooing is expected to be at least as high, and in most cases higher, than an equivalent amount of the same substance administered to the skin in a cosmetic product. Therefore, if under the CPR it has been demonstrated that the higher concentration limits or lower purity would lead to human health risks due to the presence of the colourants in products applied on the skin, the provisions for tattoo inks and PMU need to be at least as strict as those for cosmetic products under the CPR/CPD. There is therefore

a basis for recommending comparable measures to the use of these substances in tattoo inks in Annex XVII of REACH, relying on the decisions made for inclusion of the substances under CPR Annex II without a detailed risk assessment of each substance.

1.6. Justification for regulating Annex IV substances under REACH

The evidence presented in Section 1.4 indicates that the substances in Annex IV proposed to be included in the scope of this restriction may present equal or greater risk when used in tattoo applications. CoE ResAP(2008)1 seems to take this into account by requiring provisions for tattoo inks and PMU that are at least as strict as those for cosmetic products under the CPR/CPD. This is therefore also reflected by member states that base their national legislation on CoE ResAP(2008)1 or its predecessor.

Taking into account the decisions of the relevant authorities and recommendations of the expert committees for inclusion of substances in CPR Annex IV, it may be concluded that:

- For one group of substances, relevant authorities has concluded that the use of these should only be allowed in rinse-off cosmetic products. These should only be in contact with the skin for short periods of time, and substances present are therefore less bioavailable than in leave-on cosmetics products. It is therefore considered appropriate that such substances should not be allowed in tattoo inks which remain in prolonged (almost indefinite) contact with the dermis.
- For another group of substances, the relevant authorities have concluded that there is a higher risk when these are applied in the vicinity of the eyes or on mucous membranes as compared to applications on the skin. It can therefore be argued that these substances should also not be used in tattoo inks which equally bypass the protective skin layer.
- For another group of substances, the relevant authorities have concluded that there is higher risk when these are applied on the skin in concentrations exceeding those specified in column "h" or not meeting content or purity requirements specified in column "i". It can therefore be considered appropriate that such substances should not be allowed also in tattoo inks or PMU if they do not meet these conditions specified in columns "h" and "i" of Annex IV of the CPR.
- There is therefore a basis for regulating under REACH the above substances in tattoo inks relying on the conditions for the substances under CPR Annex IV.

There are, however, a number of uncertainties associated with the application of such a rationale which need to be taken into account, as outlined in the sections above.

1.7. Uncertainties and possible adverse impacts

Having reviewed the rationale for regulating under REACH the subgroup of substances in CPR Annex IV, as well as examining some of the individual substances within the list, a number of observations are made:

- Based on the earlier discussion, the risks associated with exposure to a substance at an equivalent dose are expected to be at least as high, if not higher, for exposure via tattooing compared to exposure via cosmetics. However, in some cases this conclusion may not hold true considering that a tattoo may only be applied once, or a limited numbers of times, and while it leads to long-term exposure, this exposure may be different than the exposure associated with a cosmetic product applied and removed multiple times (which could be up to daily application over most of a

lifetime).

- The number of substances included in CPR Annex IV that have actually been used in tattoo inks is unknown, but historical information is available for 43 of the 193 restricted field of application substances. A restriction would therefore likely cover various substances that would never find use in tattoo inks.
- While Annex IV of the CPR includes a concentration threshold for only a few of the substances in the 'restricted field of application' product types, adapting this for a restriction on tattoo inks might require consideration of such a low concentration limit for all of these substances. In particular, some substances might be present in detectable but toxicologically negligible concentrations, with their removal being impractical or would require substantial resources, exceeding any benefits of their elimination. Examples of such situations have not been collected on the basis of the experience of the Member States with national legislation based on the two resolutions. However, enforcement of Annex IV under the CPR allows for the non-intended presence of technically unavoidable traces of some substances, stemming from impurities of natural or synthetic ingredients, the manufacturing process, storage, migration from packaging, unless a purity requirement is stated. The concentration thresholds for cosmetic products would also presumably require updating to make them relevant for tattoo inks.
- It would be important to ensure that any regulation under REACH on substances in tattoo inks based on CPR Annex IV remains relevant over time. In particular, since CPR Annex IV may be adapted to reflect the latest scientific information (e.g. through addition or removal of substances), a mechanism would presumably be required to ensure that the restriction on tattoo inks is also kept up-to-date.

Finally, this justification only concerns the conditions related to colourants used in cosmetic products and regulated under the CPR. Historical information shows that pigments other than those on Annex IV have also been used in tattoo inks. There are currently no conditions on their use, other than those related to the groups of substances included in the scope of this restriction proposal. Further work is necessary to identify these other pigments that are currently in use and to specify conditions for their use, if necessary, in particular related to their purity.

1.8. Concentration limit for Annex IV substances with restrictions

Following the same rationale for substances on Annex II, under RO1 it is proposed that those substances on Annex IV with specific use restriction (i.e., allowed in cosmetic products with restrictions on their use on mucous membranes or eye products, and allowed in rinse-off products only) are not allowed in tattoo inks and PMU.

Again, in order to give more flexibility regarding the enforcement of the unintentional presence of small traces of these substances, a second restriction option is proposed – RO2 – with a practical limit of 0.1% w/w. It is worth noting that Annex IV substances are coluorants and therefore, more likely to be found in tattoo inks and PMU only if intentionally added, although some exceptions are possible.

For the remaining 119 substances with conditions on their use in columns h and i of annex IV, it is proposed, under both RO1 and RO2, that those substances are also allowed in tattoo inks and PMU if the specified requirements for their use in columns h to i are met (e.g., for purity, constituents, concentration limits, particle size, etc.).

Appendix B.6. Risk assessment of arsenic (As)

Quantitative risk assessment for arsenic compounds

1.1 Introduction

Arsenic and its compounds have been produced and used commercially for centuries with a wide range of possible applications. Arsenic has chemical and physical properties that are between those of a metal and a non-metal; it is often referred to as a metalloid or semi-metal. The three major groups of arsenic compounds that are important toxicologically include inorganic arsenic compounds, organic arsenic compounds and arsine gas. The common inorganic compounds include arsenic trioxide, sodium arsenite, arsenic trichloride (all trivalent compounds), arsenic pentoxide, arsenic acid and lead and calcium arsenates (pentavalent compounds) (IARC, 2012).

Arsenic compounds have historically been used in pigments, such as those with red and yellow colour. They may have been deliberately used in some tattoo inks in the past (Poon, 2008). Arsenic may also be present as an impurity in other pigments in tattoo inks.

Arsenic and its compounds are included in the list of substances prohibited in cosmetic products under Annex II of the cosmetic products regulation (CPR, EC No 1223/2009). A restriction on CPR Annex II substances is considered elsewhere within the restriction dossier.

Arsenic has been detected in a wide range of tattoo inks, as set out in a number of recent reports ((JRC, 2015b), (DEPA, 2012a), (New Zealand MoH, 2013)). However, it was not clear where the arsenic comes from. However, the concentrations are very low and thus it is assumed that it is an impurity (non-intended). The highest concentrations around 0.9 ppm we found in the red inks. The lowest we found in white ink from below DL to 0.04 ppm. The literature reviewed does not mention the specific arsenic compounds present in the tattoo inks, and the methods used to detect arsenic generally involve analytical techniques that do not allow the original arsenic species to be determined (or indeed whether it is present intentionally in inks or as impurities). For example, the analytical techniques often involve digestion in acid and detection using ICP-MS, which often allow identification of arsenic, but not the specific arsenic species.

Under Council of Europe Resolution ResAP(2008)1 (described elsewhere within the restriction proposal), the maximum allowed concentration of arsenic as an impurity in products for tattoos and permanent make-up is 2 ppm. While this limit is reflected in the legislation of those member states that have national legislation based on ResAP(2008)1, it is not necessarily reflected in the legislation of member states that have instead based their legislation on ResAP (2003)2, which does not include this limit[28]. Furthermore, other member states do not have legislation based on either of these resolutions.

Arsenic has been detected in tattoo inks at levels up to 60 ppm, exceeding the concentration limit in ResAP(2008)1 in several cases. Furthermore, there is also a need to

[28] There is a variety of approaches adopted across the EU, with some member states having legislation (or draft legislation) based on ResAP(2008)1, some having legislation based on ResAP(2003)2 and others having separate national provisions or simply reference to REACH, CLP and the GPSD (JRC, 2015a). ResAP(2003)2 does not include the maximum allowed concentrations of impurities in products for tattoos and PMU (table 3) which is included in ResAP(2008)1. Therefore those member states basing their legislation on ResAP(2008)1 will generally have concentration limits for arsenic in tattoo inks while others generally will not.

revisit the concentration limit in ResAP(2008)1, in the context of current knowledge about the hazards of arsenic compounds, and of potential exposure via tattoo inks.

1.2 Classification

The table below summarises the classification of a number of relevant arsenic compounds in terms of health effects. Several of the compounds are also classified for effects on aquatic organisms, or physical hazards; these classifications are not included here.

Table 107: Harmonised classification of selected arsenic compounds (health effects only, simplified)

Substance Name	CAS No	Carcinogen 1A	Carcinogen 1B	Mutagen	Reprotox 1A	Reprotox 1B	Acute toxic	Skin sensitiser / corrosive	STOT RE
Arsenic	7440-38-2						3*		
Arsenic acid and its salts *	-	1A					3*		
Arsenic compounds *							3*		
Arsine	7784-42-1						2*		2*
Diarsenic pentaoxide; arsenic pentoxide; arsenic oxide	1303-28-2	1A					3*		
Diarsenic trioxide; arsenic trioxide	1327-53-3	1A					2*	Corr 1B	
Gallium arsenide	1303-00-0		1B			1B			
Lead hydrogen arsenate	7784-40-9	1A			1A		3*		2*
Nickel diarsenide [1]; nickel arsenide [2]	12068-61-0 [1]; 27016-75-7 [2]	1A						Sens 1	1
Tert-butylarsine	4262-43-5						2*		
Triethyl arsenate	15606-95-8	1A					3*		
Trinickel bis(arsenate); nickel(II) arsenate	13477-70-8	1A							1
Trinickel bis(arsenite)	74646-29-0	1A							1

The two arsenic species registered under REACH in the largest quantities (arsenic acid and diarsenic trioxide, both at 100-1000 tpa) both have carcinogenicity identified as the most sensitive endpoint used in derivation of a DNEL/DMEL[29]. The following section reviews the health effects of arsenic compounds, with a focus on carcinogenicity.

1.3 Health effects

1.3.1 Overview of health effects

The health effects of various arsenic compounds have recently been reviewed by ECHA's risk assessment committee (RAC) (ECHA, 2017c) in the context of the proposed amendment of Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (CMD). This opinion includes a review of:

- Toxicokinetics – ADME (absorption, distribution, metabolism and excretion)

[29] ECHA brief profiles, accessed 9 June 2017.

- Acute toxicity
- Specific target organ toxicity / repeated dose toxicity
- Irritation and corrosion
- Sensitisation
- Genotoxicity
- Reproductive toxicity
- Carcinogenicity including mode of action of carcinogenicity of arsenic acids and its salts

The primary health endpoint of concern is carcinogenicity, and the outcome of the RAC's review on this is set out in the following sections.

1.3.2 Derivation of health effects data for cancer risk assessment

Dose response function

A dose response function is set out in the RAC's reference dose response relationship for inorganic arsenic compounds (ECHA, 2013). The following is based on that document, which is also incorporated in the recent opinion of the RAC (ECHA, 2017c).

As set out by the RAC (ECHA, 2013), a review was performed of the carcinogenic dose responses of three inorganic arsenic compounds (diarsenic pentoxide, diarsenic trioxide and arsenic acid). While other arsenic species are potentially also relevant in tattoo inks, the 2013 RAC review only considered these three. However, as set out more recently by the RAC (ECHA, 2017c), the evaluation of carcinogenicity applies to arsenic and its inorganic compounds in general.

Diarsenic trioxide is a trivalent arsenic substance, diarsenic pentoxide and arsenic acid are pentavalent arsenic substances. Arsenic compounds produce lung tumours in both animals and humans, following inhalation, oral or parenteral exposures. Exposure to high levels of arsenic compounds in drinking water has been associated with skin and urinary tract / bladder cancer in humans. Tumours at sites including the adrenal glands, bladder and liver have also been reported in some studies in animals.

The cancer mode of action of arsenic and its inorganic compounds has not been established, but it appears not to be related to direct DNA reactive genotoxicity and therefore it is possible that the arsenic carcinogenicity has a threshold exposure level. However, the available data do not allow the identification of threshold exposure levels for key events in the modes of action proposed in the scientific literature.

For **oral exposure**, based on human epidemiology data, the World Health Organization (WHO/FAO, 2011) derived a $BMDL_{0.5}$, by applying a number of models to lung and bladder cancer mortality data from the Taiwanese drinking water cohorts, using data from the most recent publications of Chen et al (2010a, 2010b)[30]. The four models with a good fit to the data were gamma, log-logistic, multistage and quantal linear. The $BMDL_{0.5}$ does not describe the shape of the dose response curve, but because a quantal linear model has a good fit to the data, a linear dose response relationship can be assumed.

[30] $BMDL_{0.5}$ is the benchmark dose lower confidence limit, as set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), that resulted in a 0.5% increase in lung cancer.

The WHO/FAO risk estimates for the oral route are recommended over the other published cancer risk estimates for several reasons. The RAC considered that the assessment was well described and used a variety of models to find the best fit to the data from a number of studies, in order to find the most conservative cancer risk estimates using the defined approach. This assessment used the most up-to-date data from the Taiwanese drinking water cohort. Although this does not produce the greatest excess risk per unit exposure, it was considered by the RAC to be the most robust assessment for oral arsenic exposure available at the present time.

The following relationship for the oral route (systemic exposure), which assumes linearity, was derived:

> Excess lifetime risk of lung tumours = 1.7×10^{-3} per µg As/kg bw/day (as a systemic exposure)

Because there are inadequate data to support a threshold value for cancers associated with oral exposure, RAC concluded that the dose response relationship can be regarded as linear.

For **dermal exposure**, although there is no evidence that dermal exposure to inorganic arsenic compounds has caused skin or other tumours in humans and dermal penetration of arsenic is likely to be low (based on physicochemical properties), RAC has also established risk values for systemic exposure via the skin. Epidemiological studies of workers from smelter plants included investigations of general health and tumours at a wide range of sites. Hence, it would be anticipated that, had there been any significant increases in skin tumours, these would have been noticed and recorded. No adequate studies investigating the carcinogenicity of inorganic arsenic compounds in experimental animals exposed via the dermal route were available.

For a dermal assessment of systemic cancer risk RAC however considered it appropriate to extrapolate from the oral risk estimates. Dermal absorption of 1% was assumed.

The following dose-relationship for the dermal route was derived:

> Excess lifetime risk of lung tumours = 1.7×10^{-5} per µg As/kg bw/day (as a dermal exposure)

In the case of tattoo inks, it does not seem appropriate to use the relationship for dermal exposure given that the skin barrier is effectively bypassed. Therefore, the oral dose-response relationship above is used in the following assessment.

Uncertainties based on substances covered

As highlighted by the RAC (ECHA, 2013), dose response relationships were derived by linear extrapolation. Extrapolating outside the range of observation inevitably introduces uncertainties. As set out by the RAC, the mechanistic evidence is suggestive of non-linearity; it is therefore acknowledged that the excess risks in the low exposure range might be an overestimate.

For the three substances covered in the reference dose-response relationship, carcinogenic potency following oral exposures to their solid form is expected to be similar because solubility will not be a limiting factor at human exposure levels. The solubility of arsenic compounds varies somewhat, so the conclusions on the risks will depend upon which arsenic species are present in any given tattoo ink. The extent to which the risks will vary depending on solubility is unknown. Most arsenic compounds are soluble in water, as illustrated below (ECHA, 2017c):

- Potassium arsenate: soluble in cold water (190 g/l at 6°C), very soluble in hot water
- Lead arsenate: soluble in water (850 g/l at 25°C)
- Magnesium arsenate: soluble in water (270 g/l at 17°C)
- Arsenic: insoluble
- Arsenic trioxide: soluble in water (37 g/l at 20°C and 115 g/l at 100°C)
- Arsenic pentoxide: soluble in water (1500 g/l at 16°C and 767 g/l at 100°C)
- Arsenic trichloride: decomposed by water
- Arsenic trisulphide: insoluble in cold water, slightly soluble in hot water
- Sodium arsenite: very soluble in water (1000 g/l at 25°C)

Therefore, the conclusions are likely to be applicable to most arsenic compounds found in tattoo inks. In the remainder of this assessment, no specific consideration is given to insoluble, or less soluble, arsenic compounds, i.e. no separate risk assessment or concentration limit is derived.

1.4 Exposure assessment

1.4.1 Generic exposure scenario

Based on the generic exposure assessment for tattoo inks (see Exposure assessment), the following is assumed in terms of estimating a realistic worst case exposure to substances in tattoo inks:

Table 108: Parameters to be applied in the exposure assessment for tattoo inks

Parameter	**Value**
Size of tattoo per session (cm^2)	300
Pigmentation covering (%)	100
Weight of tattooed person (kg)	60
Amount of ink used per cm^2 (mg)	14.36
Amount of ink used per session (mg)	4 308
Bioavailability of pigments - Percentage of pigment removed from tattoo area by body fluids	100%
Bioavailability of impurities - Percentage of ink-fluids and soluble substances including impurities removed from the tattoo area	100%
Excretion of pigments	100%
Excretion for soluble substances incl. impurities	100%

It is conservatively assumed that uptake of pigments is 100%, that impurities released from pigments are excreted and that the continuous release of impurities does not exceed the concentration in the ink supplied to the body in the initial ink.

The exposure scenario assumes isolated single tattoo sessions on 300 cm^2 skin repeated until most of the body is covered. This exposure scenario is considered protective for both people with full body tattoos and for others with single or a few tattoos. It is assumed that the person will (on average) go to the tattoo artist once a month. A full body tattoo would

be completed in 61.5 months or 5.2 years. The repeated exposure over a period of ca. 5 years supports the approach that, in the risk characterisation, the exposure with 4,308 mg ink (14.36 mg ink/cm^2 * 300 cm^2) should be compared with a DNEL/DMEL related to lifetime exposure.

The exposure assessment assumes that, as a conservative approach, the risk for a consumer exposure scenario can be characterised by comparing the event exposure over a day to the relevant DNEL/DMEL value. It is further concluded that, in the risk characterisation, the DNEL/DMEL related to lifetime exposure is still relevant even if the exposure event results from an "only one use" event.

In the exposure assessment for arsenic in tattoo inks, exposure is calculated for two reference concentrations of arsenic:

- The maximum allowed concentration according to Resolution ResAP(2008)1 = 2ppm
- The maximum concentration reported in the literature = 60ppm

These two scenarios are considered in the following sections.

1.4.2 Exposure at maximum concentration according to ResAP(2008)1

Based on the maximum allowed concentration of arsenic of 2 ppm, the estimated daily internal dose is calculated as shown in the table below.

Table 109: Estimated arsenic dose based on maximum concentration according to ResAP(2008)1

Parameter	**Value**
Maximum permitted level of As according to ResAP(2008)1 (mg / kg) (ppm)	2
Correction factor (mg / kg to fraction)	1×10^{-6}
Maximum permitted As concentration in ink (mg / mg)	2.0×10^{-6}
As present in ink used per session at this max. conc. (mg) (based on 4308 mg ink per session)	0.00862
Estimated daily As dose for a 60kg person (mg As / kg bw / d)	0.0001436
Correction factor (mg to µg)	1 000
Estimated daily As dose (µg As / kg bw / d)	0.1436

1.4.3 Exposure at maximum concentration identified in tattoo inks

Based on a report by the JRC (JRC, 2015b) (page 62), arsenic has been detected in tattoo inks in the range 0.2-60 mg/kg (ppm). As a realistic worst case, therefore, the estimated dose through use of exposure to tattoo inks with arsenic at this concentration is calculated below.

Table 110: Estimated arsenic dose based on maximum concentration reported in the literature

Parameter	**Value**
Concentration of As present in tattoo inks and PMU (mg / kg)	0.2 - 60
Maximum As concentration in ink (mg / kg) (ppm)	60
Correction factor (mg / kg to fraction)	1×10^{-6}
Maximum As concentration in ink (mg / mg)	6×10^{-5}
As present in ink used per session at this max. conc. (mg) (based on 4308 mg ink per session)	0.2585
Estimated daily As dose for a 60kg person (mg As / kg bw / d)	0.004308
Correction factor (mg to µg)	1 000
Estimated daily As dose (µg As / kg bw / d)	4.308

1.5 Risk characterisation

As set out above, RAC has developed a reference dose-response relationship for arsenic compounds. They concluded that, based on current knowledge, it is prudent to assume a linear dose-response relationship at low doses. There is therefore not assumed to be a threshold for adverse effects in the carcinogenicity assessment.

In this assessment, therefore, risk characterisation is undertaken based on comparison of predicted exposure to a DMEL. According to ECHA (ECHA, 2016d), although there is no EU legislation setting the 'tolerable' risk level for carcinogens, a cancer risk level of 10^{-6} could be seen as an indicative tolerable risk level when setting DMELs for the general population.

Since the leading health effect is a non-threshold effect for which a DMEL has been derived (in this case non-threshold carcinogenicity), a semi-quantitative risk characterisation can be conducted, as follows:

If exposure < DMEL → Exposure is controlled to a risk level of low concern

If exposure > DMEL → Risk is NOT controlled

As set out above, ECHA (ECHA, 2013) (ECHA, 2017c) have established a reference dose-response relationship for both oral and dermal exposure. Whilst that for oral exposure is considered most relevant in the case of tattoo inks, both are used in the following analysis, to illustrate the sensitivity of the results to the choice of dose-response relationship.

A DMEL value is derived by combining the dose-response relationship (for the oral route) with an indicative tolerable risk level[31]. This DMEL is then compared to the predicted exposure (external dose), as calculated above. The following tables illustrate the results of the risk characterisation, firstly for the exposure predicted based on arsenic concentration at the maximum permitted concentration according to ResAP(2008)1 and secondly at the maximum concentration reported in the literature. Results based on the dermal dose-response relationship are not included as this is not considered appropriate for tattoo inks.

[31] According to ECHA (ECHA, 2016), although there is no EU legislation setting the 'tolerable' risk level for carcinogens, a cancer risk level of 10-6 could be seen as an indicative tolerable risk level when setting DMELs for the general population.

Instead the systemic dose-response relationship is used based on the oral dose-response relationship, considered to be more representative for tattoo inks.

Table 111: Risk characterisation based on maximum concentration according to ResAP(2008)1 and maximum concentration reported in the literature

Parameter	**ResAP(2008)1 limit**	**Max reported in literature**
Assumed concentration in tattoo ink (mg/kg)	2	60
Lifetime excess cancer risk per µg As/kg bw/d (RAC dose-response, systemic)	1.7×10^{-3}	1.7×10^{-3}
Indicative tolerable risk level	1×10^{-6}	1×10^{-6}
Tolerable external dose (DMEL) (µg As/kg bw/d)	0.0005882	0.0005882
Exposure estimate as daily dose from above (µg As/kg bw/d)	0.1436	4.308
Ratio of exposure to DMEL	244	7 324
Lifetime excess cancer risk	2.4×10^{-4}	7.3×10^{-3}

As can be seen from the above, taking into account the realistic worst case assumptions on exposure, it is concluded that the risks are "not controlled" with a concentration of arsenic in tattoo ink at the level of the maximum reported in the literature. There is therefore merit in considering an alternative (lower) concentration limit than that set in ResAP(2008)1. (Note that, even assuming exposure based on the maximum permitted concentration in ResAP(2008)1, and applying the less conservative dermal dose-response relationship, the risks would still be concluded to be not controlled.)

1.6 Derivation of a specific concentration limit

Based on the above approach to exposure assessment and risk characterisation, a possible concentration limit is derived by back-calculating from an indicative tolerable risk level (and hence tolerable arsenic dose) to the concentration of arsenic in tattoo inks that would give that risk level. The results are shown below, again using both the oral dose-response relationships.

Table 112: Derivation of a specific concentration limit

Parameter	Value
Calculation of exposure to ink	
Daily exposure to ink (mg ink / kg bw / d) [1]	71.8
Correction factor (mg to µg)	1000
Daily exposure to ink (µg ink / kg bw / d) [2]	71 800
Tolerable external dose	
Lifetime excess cancer risk per µg As/kg bw/d (RAC dose-response, systemic)	1.7×10^{-3}
Indicative tolerable risk level	1×10^{-6}
Tolerable external dose (DMEL) (µg As / kg bw / d)[3]	0.0005882
Estimation of concentration limit	
Tolerable As concentration in ink (µg As / µg ink) [4]	8.2×10^{-9}
Correction factor (Fraction to mg / kg)	1×10^{6}
Tolerable As concentration in ink (mg / kg) (ppm) [5]	0.008

Notes: [1] Based on the generic exposure assessment, which assumes 4308mg ink applied per session and a body weight of 60kg. [2] Daily exposure multiplied by correction factor. [3] Indicative tolerable risk level divided by lifetime excess cancer risk per µg As/kg bw/d. [4] Calculated by dividing the tolerable external dose by the daily exposure to ink. [5] Tolerable As concentration in ink (as µg As / µg ink) multiplied by correction factor.

If the (preferred) oral reference dose-response relationship is used, the tolerable concentration limit is therefore 0.008 ppm (mg/kg), which is a factor of 244 lower than the maximum allowable concentration according to ResAP(2008)1. Expressed as a percentage, the value is 0.00000082%. (Note that if the dermal relationship had been used, the calculated tolerable concentration limit would be 0.8 ppm (mg/kg), which is a factor of 2.4 lower than the maximum concentration under ResAP(2008)1).

Given that this potential concentration limit is significantly lower than that in ResAP(2008)1, it is relevant to confirm the feasibility of detecting arsenic in tattoo inks at such a level. Examples of some available methods are set out below.

Table 113: Detection limits for arsenic using various methods

Method	LOD (mg/kg)	LOQ (mg/kg)
Value for soluble elements in toy materials (EN 71-3:2013+A1:2014) [1]	0.027	0.055
Value for tattoo inks using ICP-MS in the Netherlands (EN 71-3:2013+A1:2014)	0.004-1.46	0.008 – 2.93
Value for ICP-MS screening of tattoo inks [2]	0.04	-
US EPA method 1669 – detection limit in water [3]	0.000003	-
US EPA method 1669 – 'minimum level' for water	0.00001	-

Sources: [1] (JRC, 2015a) [2] (DEPA, 2012a) [3] (US EPA, 1996). Values in (US EPA, 1996) given in µg/l and converted to mg/kg by applying a factor of 1000.

Based on the above, it is concluded that there are available analytical methods that could be used to detect arsenic in tattoo inks at and well below the possible concentration limit. For

example, the US EPA method 1669 minimum level of 10 ng/l (0.00001 mg/kg) is able to detect arsenic at 0.1% of the lower of the calculated arsenic concentration limit of 0.008 mg/kg. However, it is of note that the possible concentration limit is below the detection limit of some of the commonly-applied techniques for detection of elements such as arsenic in tattoo inks as illustrated in the table above.

It is further noted that no consideration has been given herein to the technical feasibility of achieving a concentration limit of 0.008 ppm in tattoo inks. By way of comparison, the level of arsenic in natural waters generally ranges between 1 and 2 µg/l (0.001 to 0.002 ppm), but concentrations may be elevated in areas with volcanic rock and sulphide mineral deposits, in areas containing natural sources, where levels as high as 12 mg/l (12 ppm) have been reported (WHO, 2011a). This aspect of technical feasibility would warrant further investigation.

Appendix B.7. Risk assessment of barium (Ba)

Risk evaluation of barium compounds in tattoo and PMU inks

Introduction

Barium compounds have historically been used in pigments, such as those with red, yellow, orange and green colour (DEPA, 2012) (Laux, et al., 2016). The insoluble barium sulfate is used in the flocculation of organic pigments to optimise their dispersability. It is used not only as a white colour, but also because of its lightening effect and adjustment of colour strength. In addition, it is also employed in the production of lakes and as filler (JRC, 2015b).

Barium sulfate is insoluble and thus, relatively inert in contrast to ionic barium and soluble barium salts such as e.g. the chloride, nitrate and hydroxide (from which the barium ion is released in the body). Ionic barium is toxic to several organs and tissues, including the nervous system, kidney and heart (DEPA, 2012a). Barium sulfate may have free soluble barium compounds as impurities (Laux, et al., 2016).

Barium can react with titanium dioxide thereby forming the insoluble salt barium titanate. This might be of particular importance in tattoo inks in which both soluble barium salts and titanium dioxide occur. The extent of degradation of the insoluble barium sulfate and barium titanate locally in the skin thereby releasing the toxic barium ion is not known (DEPA, 2012a).

Under Council of Europe Resolution ResAP(2008)1, the maximum allowed concentration of barium as an impurity in products for tattoos and permanent make-up is 50 ppm (mg/kg). While this limit is reflected in the legislation of those member states that have national legislation based on ResAP(2008)1, it is not necessarily reflected in the legislation of member states that have instead based their legislation on ResAP (2003)2, which does not include this limit[32]. It is unclear on what basis this limit is set in ResAP(2008)1.

Barium has been detected in a wide range of tattoo inks at levels exceeding the recommended concentration limit of 50 ppm (mg/kg) set in the CoE ResAP (2008)1 (JRC, 2015b) (DEPA, 2012) (New Zealand MoH, 2013). In the elements' analysis described by JRC (JRC, 2015b), barium was found in 886 samples with Ba contents up to 17737 mg/kg (equivalent to 1.77%) reported. Around 20% of the samples had Ba levels higher than the recommended CoE limit value. The reported concentrations refer to the total content of the element after complete digestion of the samples, as set in the CoE ResAP(2008)1 (JRC, 2015b).

The analyses of barium in tattoo inks performed by authorities have been criticised for only examining the total content of barium, and not the soluble form (Prior, 2014). The lack of a quantitative method for the soluble fraction of barium has been mentioned by German

[32] There is a variety of approaches adopted across the EU, with some member states having legislation (or draft legislation) based on ResAP(2008)1, some having legislation based on ResAP(2003)2 and others having separate national provisions or simply reference to REACH, CLP and the GPSD (JRC, 2015a). ResAP(2003)2 does not include the maximum allowed concentrations of impurities in products for tattoos and PMU (table 3) which is included in ResAP(2008)1. Therefore those member states basing their legislation on ResAP(2008)1 will generally have concentration limits for barium in tattoo inks while others generally will not.

authorities (JRC, 2015a) (JRC, 2015b) and according to the Danish EPA are some of the substances (e.g. barium from barium sulfate) found in their investigation probably released due to the analytical method used for the analyses of the tattoo inks. This means that the analytical method chosen in the Danish investigation did not reveal the pigments, coformulants and chemical impurities that are actually present in the analysed tattoo inks and to which a tattooed person is exposed to (DEPA, 2012a).

According to information by JRC (JRC, 2015b), the following barium containing pigments and compounds have been found in tattoo inks (table below).

Table 114. Barium pigments/compounds found in tattoo and PMU inks (JRC, 2015b).

Substance name	**Colour Index no (Generic name)**	**CAS no**	**Chemical class**	**Use in tattoo**	**Use in PMU**
Barium sulfate	CI 77120	7727-43-7	In-organic	X	
Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]	CI 15585:1 Pigment Red 53:1	5160-02-1	Monoazo	X	X
Barium bis[2-[(2-hydroxynaphthyl)azo]naphthalenesulphonate]	CI 15630:1 Pigment Red 49:1	1103-38-4 (not in CosIng)	Monoazo	X	X
Barium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate	CI 15865:1 Pigment Red 48:1	7585-41-3 (not in CosIng)	Monoazo	X	
Barium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate	CI 15850:2 Pigment Red 57:2	17852-98-1 (not in CosIng)	Monoazo	X	X
Barium(2+) hydrogen 2-[(2-hydroxy-3,6-disulphonato-1-naphthyl)azo]benzoate	CI 16105:1 Pigment Red 60:1	1325-16-2	Monoazo	X	
Barium bis[4-[(2-hydroxy-1-naphthyl)azo]-2-methylbenzenesulphonate]	CI 15580 Pigment Red 51	5850-87-3	Monoazo	X	X

Various other cadmium-barium co-precipitates as well as barium chromate ($BaCrO_4$, barium tetraoxochromate(VI), CAS no 10294-40-3; water solubility 2.6 mg/L water at 20°C, practically insoluble in dilute acids (PubChem)) has been used as a yellow/orange/red tattoo pigments (Laux, et al., 2016).

Barium salts are included in the list of substances prohibited in cosmetic products under Annex II of the cosmetic products regulation (CPR, EC No 1223/2009) with the following wording: "Barium salts, with the exception of barium sulphide under the conditions laid down in Annex III, and of barium sulfate, lakes, salts and pigments prepared from colouring agents when listed in Annex IV". A restriction on CPR Annex II substances is considered elsewhere within the restriction dossier.

According to the list of colourants allowed in cosmetic products (CPR Annex IV), a total of 17 entries (including entries #23 barium bis[4-[(2-hydroxy-1-naphthyl)azo]-2-methylbenzenesulphonate] and #122 barium sulfate) could potentially contain barium. Entries #3, 9, 21, 25, 27, 28, 31, 35, 37, 44, 60, 75, 76, 79 and 80 are all included in Annex IV with the following wording "chemical name of parent compound" followed by "…

and its insoluble barium, strontium and zirconium lakes, salts and pigments". It should be noted that many of these entries will be captured by proposed restrictions on CPR Annex II and Annex IV substances described elsewhere in this restriction.

Of the barium containing pigments found in the JRC report (Table 115), barium sulfate/CI 77120 (CAS no 7727-43-7) and Pigment Red 51/CI 15580 (CAS no 5850-87-3) are listed in CPR Annex IV (lists of colourants allowed in cosmetic products) without any restrictions. Pigment Red 53:1/CI 15585:1 (CAS no 5160-02-1) is listed in Annex II and is not allowed to use in cosmetic products. The CAS numbers for Pigment Red 49:1/CI 15630:1 (1103-38-4), Pigment Red 57:2/CI 15850:2 (17852-98-1) and Pigment Red 48:1/CI 15865:1 (7585-41-3) could not be found in the European Commission's Cosmetic Ingredient database (CosIng database). However it is not clear if these pigments are allowed according to Annex IV as their "parent" CI numbers (CI 15630 = entry # 25; CI 15850 = entry # 27 and CI 15865 = entry # 28) indicate they should be allowed. Pigment Red 60:1 (CAS no 1325-16-1) is not included in Annex IV and can thus not be used as a colourant in cosmetic products.

There is a need to revisit the concentration limit of 50 ppm (mg/kg) for barium impurities set in the CoE ResAP(2008)1, in the context of current knowledge about the hazards of barium compounds, and of potential exposure via tattoo inks. The approach taken in this risk evaluation is to look into the toxicological data for the barium pigments/compounds found in tattoo and PMU inks with reference to the JRC report (Table 115). Relevant information for these barium chemicals was collected from ECHA registration dossiers and database searches (search terms: substance name and CAS no) in TOXNET, Web of Science and Google.

To assess the toxicity of soluble barium, studies on barium carbonate and barium dichloride have been included in the evaluation below.

Toxicity of soluble barium (Ba^{2+}) based on available data on barium (Ba; CAS no 7440-39-3), barium carbonate ($BaCO_3$; CAS no 513-77-9) and barium chloride dihydrate ($BaCl_2.2H_2O$; CAS no 10326-27-9)

The toxicity of barium compounds depends on their solubility and soluble Ba^{2+} salts are deadly poisons at high concentrations, e.g. they have been used as rodenticides. Solid Ba is a soft, silvery alkaline earth metal (the heaviest non-radioactive earth metal) that vigorously reacts with water forming Ba^{2+} ions. $BaCO_3$, also called witherite, consists of white crystals that are soluble in most acids (not in sulfuric acid, as insoluble barium sulfate forms) but has low solubility in water (14 mg/L at 20°C) and is insoluble in ethanol. $BaCl_2$ dissolves readily in water. Ba^{2+} mediates toxicity in the kidneys, muscles (Ba^{2+} is a muscle poison) and the cardiovascular system, and causes hypertension. Ba^{2+} is a physiological antagonist of potassium (Ba^{2+} partly blocks the K^+-channels in the Na-K pump in cellular membranes). Poisoning is accompanied by severe hypokalemia (low blood serum K^+). There are presently no well-established biomarkers of Ba^{2+} salt exposure or effects. The general population is exposed to barium mainly via food (e.g. nuts (particularly Brazil nuts), bread and other cereal products, and vegetables and fruit), then drinking water (WHO guideline value from 2011 is 0.7 mg/L) (WHO, 2011, 2004). In most cases, drinking water only makes a small contribution to the total intake (6-10 µg/kg/day in adults, 22-25 µg/kg/day in 1-4 year-old children) of barium (Oskarsson, 2015).

ADME

After inhalation, Ba^{2+} is readily absorbed in the lung. After oral intake, $BaCO_3$ can dissolve and form Ba^{2+} in the acidic stomach which is readily absorbed in the gastrointestinal tract. After absorption, Ba^{2+} mainly accumulates in bone and teeth containing up to 90% of the body burden (some accumulation also in the eye, heart and submaxillary gland). Systemically available solubilized Ba^{2+} is excreted primarily in the feces following inhalation and parenteral (e.g. intravenous injection) administration, a smaller part is excreted into urine. The blood plasma elimination half-life in humans is 3.6 days (Baselt, 1982), in bone about 50 days (Ba^{2+} does not accumulate in bone with age). The biological half-life for soluble barium in rat is 90-120 days. Ba^{2+} can react with sulfate (SO_4^{-2}) forming $BaSO_4$.

Carcinogenicity

No evidence of carcinogenic activity was found in rats (male and female Fischer 344) or mice (male and female B6C3F1) after barium chloride dihydrate ($BaCl_2.2H_2O$, 99%) administration at 500, 1250 or 2500 ppm through drinking water for up to 2 years (NTP, 1994). GLP, use of a guideline is not stated but procedures appear similar to those in the OECD 451 guideline for carcinogenicity studies.

Genotoxicity

The $BaCO_3$ REACH registrants have included study summaries for three genotoxicity studies for $BaCl_2.2H_2O$ in the registration dossier(s) for which read across is performed for $BaCO_3$ ($BaCl_2$ forms Ba^{2+} and is more water soluble than $BaCO_3$), all three found no evidence of genotoxicity (negative outcome):

-In vitro gene mutation study having tested five strains of *S. typhimurium* bacteria ±S9 mix. Methodology equivalent or similar to OECD 471 (Bacterial Reverse Mutation Assay), GLP (ECHA, 1994). This is presumably a study performed by NTP which was included in their 1994 publication regarding carcinogenicity studies, but there is no reference in the REACH registration in the ECHA dissemination site.

-In vitro cytogenetic /chromosome aberration study in Chinese Hamster Ovary (CHO) cells ±S9 mix. Methodology equivalent or similar to OECD 473 (In Vitro Mammalian Chromosome Aberration Test), GLP (ECHA, 1994). This is presumably a study performed by NTP which was included in their 1994 publication regarding carcinogenicity studies.

-In vitro gene mutation study in mouse lymphoma L5178Y cells ±S9 mix. OECD 476 (In Vitro Mammalian Cell Gene Mutation Test), GLP, 2010.

In addition, the carcinogenicity studies by NTP in 1994 also included an In vitro gene mutation study in mouse lymphoma L5178Y cells ±S9 mix (GLP, methodology equivalent or similar to OECD 476). It also had a negative outcome.

Reproductive toxicity

The $BaCO_3$ REACH registrants have included study summaries of four reproduction toxicity studies as well as one developmental/teratogenicity study for $BaCl_2$ for which read across is performed for $BaCO_3$. All five had a negative outcome:

-In a subchronic/single-generation reproductive toxicity study in rats by (Dietz, et al., 1992), $BaCl_2.2H_2O$ (99.5% pure) was given for 92 days to Fischer 344/N rats in their drinking water at levels up to 4000 ppm. In parallel, a premating study was performed with rats given 0, 1000, 2000 or 4000 ppm. The premating exposure period with $BaCl_2.2H_2O$ was 60 days for males and 30 days for females. There were no indications of a substantial impairment of fertility in rats up to the highest dose (4000 ppm = NOAEL) tested. No

anatomical effects on offspring were seen (examined after birth), but rats receiving 4000 ppm exhibited marginal reductions in pup weight. However, there was no exposure of females during gestation (period from conception until birth).

-In the same subchronic/single-generation reproductive toxicity study by (Dietz, et al., 1992), a premating study was also performed for B6C3F1 mice using similar experimental procedures but $BaCl_2$ doses of 0, 500, 1000 and 2000 ppm. Also for mice, there were no indications of a substantial impairment of fertility up to the highest dose (2000 ppm = NOAEL) tested, and no anatomical effects on offspring were seen (examined after birth). However, there was no exposure of females during gestation.

-Analyses of the reproductive organs from the 2-year rat (Fischer 344) $BaCl_2$ carcinogenicity study (NTP, 1994), revealed no increased incidence of nonneoplastic lesions of the genital system (male and female) that could be related to the test substance. NOAEL = 2500 ppm.

-Similarly, analyses of the reproductive organs from the 2-year mouse (B6C3F1) $BaCl_2$ carcinogenicity study (NTP, 1994), revealed no increased incidence of non-neoplastic lesions of the genital system (male and female) that could be related to the test substance. NOAEL = 2500 ppm.

-An OECD 414 (Prenatal Developmental Toxicity Study) GLP study administered $BaCl_2.2H_2O$ dissolved in water via gavage in mated female rats (RccHan: WIST strain) from gestation day 0 up to and including gestation day 20 (ECHA, 2014). Doses were 0, 10, 30, or 100 mg $BaCl_2.2H_2O$/kg bw. Maternal toxicity occurred at the highest dose (spontaneous death of two animals and conditional decline in one animal at gestation day 21) at the end of the study (the NOAEL for maternal toxicity was 30 mg $BaCl_2.2H_2O$/kg bw/day). Foetal examination did not reveal any treatment-related effects.

Skin irritability

The $BaCO_3$ REACH registrants have included two skin irritability studies for $BaCl_2.2H_2O$ for which read across to $BaCO_3$ is performed, both had a negative outcome:

An in vitro skin irritation: Reconstructed Human Epidermis (RhE) Test Method (OECD draft proposal for a new guideline) GLP study with $BaCl_2.2H_2O$ administration on cultured adult human-derived epidermal keratinocytes (ECHA, 2010a) found no evidence for irritation. At least 10 mg of the solid test substance was applied (spread out) directly on top of the keratinocytes. Skin irritation potential was classified according to remaining cell viability following exposure. Negative and positive controls were included.

An OECD 429 (Skin Sensitisation: Local Lymph Node Assay) GLP study with $BaCl_2.2H_2O$ administration in CBA mice (ECHA, 2010b) found no evidence for sensitization.

Repeated exposure toxicity (STOT-RE)

An overview of the most important findings in the animal and human studies reviewed in this assessment is presented in Table 116 and Table 117.

Table 115. Animal studies reviewed in this assessment:

Author	**Species and Strain**	**Test compound and administration**	**Doses**	**Duration**	**NOAEL**	**LOAEL effect**	**AF**	**DNEL consumers[33]**
(Dietz, et al., 1992)[34] (NTP, 1994)	Fischer 344/N rats M+F	$BaCl_2.2H_2O$ in drinking water	0, 125, 500, 1000, 2000, 4000 ppm	13w	2000 ppm (61.1 mg/kg bw/d for M, 80.9 for F)	Reduced bw gain, lesions in the kidney (tubular dilatation) and lymphoid tissue	100 (inter- and intraspecies variation)	0.61 mg Ba/kg bw/day
(Dietz, et al., 1992) (NTP, 1994)	$B_6C_3F_1$ mice M+F	$BaCl_2.2H_2O$ in drinking water	0, 125, 500, 1000, 2000, 4000 ppm	13w	2000 ppm (164.7 mg/kg bw/d for M, 165.8 for F)	Reduced bw gain, lesions in the kidney (toxic nephrosis) and lymphoid tissue	100 (inter- and intraspecies variation)	1.65 mg Ba/kg bw/d
(NTP, 1994)	Rats M+F	Barium chloride dihydrate in drinking water	0, 500, 1250, or 2500 ppm	2 y	60 mg/kg/d in M (NOEL)	bw loss and reduced drinking water intake F: Increased kidney weight interim	100	0.60 mg Ba/kg bw/d
(NTP, 1994)	Mice M+F	Barium chloride dihydrate in drinking water	0, 500, 1250, or 2500 ppm	2 y	75 mg Ba/kg bw/d in M	Nephropathy, kidney lesions	100	0.75 mg Ba/kg bw/d
(Perry, et al., 1989)[35]	female Long-Evans rats	Ba (as $BaCl_2$)	0, 1, 10, or 100 ppm barium (as $BaCl_2$)	1-16 months		Increased systolic blood pressure after 8 months of exposure to 10 ppm (0.82 mg Ba/kg bw/day) and by 12 mm Hg after 1 month of exposure to 100		

[33] based on the lowest NOAEL in the study.

[34] single-generation reproductive toxicity studies, later included in the NTP report as 13 w studies. No reproductive toxicity observed.

[35] Applied by the Norwegian Scientific Committee for Food Safety (VKM, 2004) to derive a TDI.

						ppm (7.4 mg/kg bw/day).		

Table 116. Available human studies

Author and study type	**Exposure route**	**Dose Ba**	**Effect studied**	**Study applied in setting recommended limits**	**Resulting recommendation max value in drinking water**
(Brenniman, et al., 1981)	drinking water	Low dose community 0.1 mg/L High dose community 7.3 mg/L	Differences in mean blood pressure levels	WHO Danish EPA	0.7 mg/L 0.021 mg Ba/kg bw (calculated from 7.3 mg Ba/L)
(Brenniman & Levy, 1984)	Drinking water	Low dose community <0.1 mg/L High dose community 2-10 mg/L	Cardiovascular disease and mortality	US EPA (cited in (Dallas & Williams, 2001)	0.07 mg Ba/kg/d
(Wones, et al., 1990)	Drinking water	5 or 10 mg/L/d (0.11 or 0.21 mg/kg bw/d) for 4 weeks	Cardiovascular risk factors		

In a single-generation reproductive toxic study by Dietz et al. (Dietz, et al., 1992)- which was later included as a 13w study in the NTP report (NTP, 1994) - $BaCl_2$. $2H_2O$ (99.5% pure, 244.26 g/mole) was given for 92 days (subchronic) to Fischer 344/N rats in their drinking water at levels of 0, 125, 500, 1000, 2000, 4000 ppm. In rats, the NOAEL was 2000 ppm (corresponding to 61.1 (M) and 80.9 (F) mg Ba/kg bw/day) and the LOAEL 4000 ppm based on depressed bw gains, elevated phosphorous levels, neurobehavioral effects and chemically related lesions in the kidney and lymphoid tissue at the highest dose. Extrapolating the NOAEL in male rats to humans (performed here), the DNEL becomes 0.61 mg Ba/kg bw/day if using an assessment factor of 100 for inter- (10) and intraspecies (10) variation. The NOAEL in mice was higher than in rats resulting in a corresponding higher DNEL which was not taken forward in the risk characterisation.

In extensive toxicology and carcinogenesis studies by (NTP, 1994), F344/N rats and B6C3F1 mice were administrated $BaCl_2.2H_2O$ through the drinking water for 15 days, 13 weeks (the Dietz study (Dietz, et al., 1992)) or 2-years, respectively. The report does not state a NOAEL for the 2-year studies. In the 15 day studies, rats (5M/5F) were administrated 0, 125, 250, 500, 1000, or 2000 ppm (corresponds to 0, 10, 15, 35, 60, 110 mg Ba/kg bw/day for M and F), and mice (5M/5F) 0, 40, 80, 173, 346 or 692 ppm (M: 0, 5, 10, 20, 40, 70 mg Ba/kg bw/day; F: 0, 5, 10, 15, 40, 85 mg Ba/kg bw/day). Increased relative liver weights were observed at the highest dose for mice (both sexes), but not in rats. There were no histopathological evidence of toxicity or clinical toxicity for either mice or rats. The results from the 13 w studies are given above.

In the 2-year studied, both rats (60M/60F) and mice (60M/60F) were administrated 0, 500, 1250 or 2500 ppm (M rats: 0, 15, 30, 60 mg Ba/kg bw/day; F rats: 0, 15, 45, 75 mg Ba/kg bw/day; M mice: 0, 30, 75, 160 mg Ba/kg bw/day; F mice: 0, 40, 90, 200 mg Ba/kg bw/day).

In rats, survival was similar to that of the controls. At the highest dose, final mean bw and water consumption was lower than controls. There were no chemical-related clinical or hematology findings. In mice, survival was significantly lower at the highest dose due to renal toxicity. At the end of the study, pathology examinations showed increased incidences of nephropathy at the highest dose in male and female mice (M: 1/50, 0/50, 2/48, 19/50; F: 0/50, 2/53, 1/50, 37/54). Final mean bw was also reduced at the highest dose, whereas the water consumption was not. There were no chemical-related clinical or hematology findings. There was no evidence of carcinogenic activity in rats or mice. Renal toxicity was only seen in mice and not in rats in the 2y study, probably due to the much larger Ba doses per unit body weight in mice compared to rats (two- to fourfold). A DNEL is proposed to be set at 0.60 mg/kg/d (based on findings in rats), and 0.75 mg/kg/d (based on findings in mice).

Dallas and Williams (Dallas & Williams, 2001) suggested a reference dose of 0.6 mg/kg/day using a NOAEL for renal effects of 60 mg/kg/day in rats (uncertainty factor 100) in the 2-year NTP study. The authors stated that the available long-term animal studies were found to be more appropriate for the RfD derivation than the available human studies as they have some utility but suffer from either a small population size, a short exposure regimen, or difficulties in identifying definitive Ba exposure in the study population. Later, based on the 13 week NTP female rat data (NTP, 1994), ATSDR (ATSDR, 2007) determined an intermediate-duration oral minimal risk level (MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure) of 0.2 mg barium/kg/day

based on a NOAEL of 65[36] Ba/kg/bw/d (LOAEL = 115) for increased kidney weights in female rats and an uncertainty factor of 300 (10 to account for animal to human extrapolation and 10 for human variability and a modifying factor of 3). The modifying factor of 3 was included to account for the lack of an adequate developmental toxicity study. Also, based on the 2 year NTP male mice data (NTP, 1994), ATSDR (ATSDR, 2007) determined a chronic-duration oral MRL of 0.2 mg barium/kg/day based on a calculated BMDL05 of 61 mg barium/kg/day for a 5% increase in the incidence of nephropathy (the 95% lower confidence limit on the BMD (BMDL) was 61.13 mg barium/kg/day; the BMD 80.06 mg barium/kg/day) and an uncertainty factor of 300 (10 to account for animal to human extrapolation and 10 for human variability and a modifying factor of 3). The modifying factor of 3 was included to account for the lack of an adequate developmental toxicity study. The dose corresponding to a predicted 5% incidence was selected over the typically 10% incidence as a precaution due to the severity of the observed effects (moderate to marked severity nephropathy), which resulted in marked weight loss and increased mortality. Since the additional OECD 414 developmental study was negative, there appears to no longer be any support for the inclusion of a modifying factor of 3 (which particularly was included as an additional developmental study was lacking in 2007), implying that both the derived oral MRLs (intermediate and chronic-duration) can be set to 0.6 mg barium/kg/day. In an opinion related to barium exposure from toys, RIVM (RIVM, 2008) supported the BMDL05 approach, but cites an ATSDR draft from September 2005 (final report (ATSDR, 2007)) in which a TDI of 0.6 mg barium/kg/day (assessment factors of 100) was suggested. In another opinion related to barium exposure from toys, SCHER (SCHER , 2012) supported the ATSDR (ATSDR, 2007) BMDL05 approach (TDI of 0.2 mg barium/kg/day) (notably, also this was before the negative OECD 414 (2014) study had been performed) and, in addition, suggested a TDI of 0.02 mg barium/kg/day from toy exposure (arguing that only 10% of the TDI should come from toys).

In an epidemiological study, Brenniman et al (Brenniman, et al., 1981) examined differences in mean blood pressure levels between a high (7.3 mg barium/L drinking water) and a low (0.1 mg/L) barium community. The data suggests that elevated levels of barium (dissolved Ba^{2+} form assumed) in drinking water does not significantly elevate blood pressure levels in adult males or females. No threshold values were reported. The present WHO guideline value (0.7 mg/L; using an uncertainty factor of 10 to account for intraspecies variation) is based on the Brenniman data (WHO, 2011, 2004). In a report prepared for the Danish EPA, Nielsen and Ladefoged (Nielsen & Ladefoged, 2006; published 2013), considered a NOAEL of 0.21 mg/kg bw/day based on the Brenniman data (a mean of 7.3 mg Ba/l corresponds to 0.21 mg Ba/kg bw/day assuming water ingestion of 2 L/day and 70 kg bw) (Brenniman, et al., 1981). However, it is stated that the NOAEL may be higher and data corresponding to a LOAEL for blood pressure was not reported in the Brenniman 1981 study. There is also no information regarding actual intakes of barium from food and drinking water, concentrations of other elements in the water, etc. Assuming a NOAEL of 0.21 mg/kg bw/day and a total uncertainty factor of 10 (for interindividual differences), a tolerable daily intake (TDI) = 0.021 mg Ba/kg bw was calculated. In a 2012 report by the Danish EPA (DEPA, 2012) it is stated that the calculated TDI in the Nielsen/Ladefoged 2006 report corresponds, in principle, to a DNEL. Thus the DNEL was set to 0.02 mg Ba/kg bw/day.

[36] Value not identical to other references, probably given here as the mean of initial and final Ba dose.

In a retrospective study, Brenniman and Levi (Brenniman & Levy, 1984) compared mortality in a population living in Illinois with elevated barium levels in municipal drinking water (2-10 mg/L) with a control population with low barium levels (<0.1 mg/L). Significantly elevated mortality rates for cardiovascular diseases (combined) and heart diseases (atherosclerosis) were found in high-barium communities, particularly for those over 65 years of age. However, also this study did not control for several important confounders, and it is not possible to conclude on a positive relationship between cardiovascular disease and barium exposure.

Wones et al (Wones, et al., 1990) administered barium chloride in the drinking water (daily consumption 1.5 L) to 11 healthy male volunteers and studied the effects on cardiovascular risk factors. The experiment lasted for 10 weeks, starting with 2 weeks with no added barium, followed by two periods of 4 weeks with 5 and 10 mg/L barium, respectively, in the drinking water. The corresponding doses were 0.11 and 0.21 mg/kg/day. There were no changes in blood pressure, plasma cholesterol, or in lipoprotein, serum potassium, or urine catecholamine levels, and no arrhythmias related to barium exposure. A small, but statistically significant (when normalized to albumin) increase in serum calcium was found, but considered not to be clinically important.

Perry et al (Perry, et al., 1989) chronically exposed female Long-Evans rats for 1-16 months (from weaning) to 0, 1, 10, or 100 ppm barium (as $BaCl_2$), and reported increased systolic blood pressure by 6 mm Hg in rats after 8 months of exposure to 10 ppm (0.82 mg Ba/kg bw/day) and by 12 mm Hg after 1 month of exposure to 100 ppm (7.4 mg/kg bw/day). However, the animals received a diet with a low content of trace elements, including calcium and potassium, which may have caused a higher sensitivity to the cardiovascular effects of barium ((ATSDR, 2007), (Oskarsson, 2015)). From the results of this study, The Norwegian Scientific Committee for Food Safety (VKM, 2004) derived a TDI of 51 µg/kg bw, employing an uncertainty factor of 10 (10 for variations among humans and 1 for extrapolation from rats to humans, since it has been shown that humans are not more sensitive than rats).

The $BaCO_3$ REACH registrants states that the DNEL is 3.5 mg $BaCO_3$ (equivalent to 2.4 mg Ba)/kg bw/day for the general population using an overall assessment factor of 25 and repeated dose toxicity as the most sensitive endpoint (chosen study not specified, but likely the (Dietz, et al., 1992) rat study is used for which the NOAEL of 2000 ppm corresponds to 87.8 mg $BaCO_3$/kg bw/day for males).

There are also several older repeated studies not mentioned here (judged to be less relevant).

Other toxicities

An OECD 405 (Acute Eye Irritation /Corrosion) GLP study with $BaCO_3$ administration in Himalayan rabbits (ECHA, 2010c) found no evidence for eye irritation. Included in the ECHA dossier for $BaCO_3$.

Risk evaluation

Ba^{2+} can be distributed in the body. $BaCO_3$ particles are expected (from its insolubility in ethanol and low solubility at the slightly basic pH in skin) to dissolve slowly into Ba^{2+} over time, but experimental data is lacking. However, as $BaCO_3$ could dissolve during preparation of tattoo ink, $BaCO_3$ should in terms of risk characterization be considered a soluble Ba^{2+} salt along with other more soluble barium salts: chloride ($BaCl_2$), hydroxide ($Ba(OH)_2$),

acetate ($Ba(C_2H_3O_2)_2$), nitrate ($Ba(NO_3)_2$), and sulfide (BaS), to which read across can be performed although threshold values need to be adjusted for molecular weight differences (Ba: 137.33 g/mole; $BaCO_3$: 197.34 g/mole).

From the repeated dose toxicity studies mentioned above, reported threshold levels vary depending on the study and interpreter, and there are large discrepancies. The epidemiological studies do not seem applicable. Perry (Perry, et al., 1989) reported increased blood pressure in rats already at repeated low barium exposures, but the study does not seem fully reliable, and NTP (NTP, 1994) found no increased blood pressure or serum K^+ changes in their 13 week studies at considerable higher barium exposures. Short repeated studies appear more relevant than long (e.g. 2 year) ones. A transient slight increase in blood pressure is also likely to be tolerable for most individuals (should this occur after the tattoo event) and circulatory system available Ba^{2+} is relatively rapidly excreted in humans.

A DNEL of 0.60 mg Ba/kg bw/day is supported by the derived DNEL based on results from the Dietz male rat study (Dietz, et al., 1992), the derived reference dose by Dallas and Williams (Dallas & Williams, 2001) for the NTP studies, and the derived updated intermediate- and chronic duration MRLs by ATSDR (ATSDR, 2007) for the NTP 13 week female rat and 2-year male mouse studies, and implies that a 60 kg person should maximally be injected with 36 mg Ba/day (60 kg x0.60 mg Ba/kg/day) or 52 mg $BaCO_3$/day (36 mg x197.34 g/mole/137.33 g/mole) for the risk characterization ratio (RCR) not to exceed 1.

For a single 300 cm^2 tattoo, 4308 mg (14.36 mg ink/cm^2 x 300 cm^2) ink is injected. The concentration limit (CL) becomes (36 mg /4308 mg =0.0084=0.84% (w/w)) 8400 ppm soluble/dissolved Ba. High concentrations of Ba (min to max: 50-17737 mg/kg (=up to 1.7737% (w/w)) have been reported in tattoo ink ((JRC, 2015b), but it is unclear if Ba existed in soluble or insoluble (i.e. $BaSO_4$) form.

Conclusion

For soluble Ba^{2+} the concentration limit (CL) of 0.84% equals RCR = 1. A CL of 8400 ppm (0.84% (w/w)) water soluble/dissolved barium is suggested.

As a content of barium (Ba) has been reported up to 1.77% in tattoo inks, there is a need to set a CL for Ba. However, it should be noted that most/all analytical methods cannot differentiate between soluble and insoluble barium (see further details in the introduction).

Barium sulfate ($BaSO_4$; CAS no 7727-43-7)

Commercial barium sulfate ($BaSO_4$ (s), Mw 233.38 g/mole) is traded with varying purity (79-99.999%) and exists as naturally occurring barite or synthetically manufactured blanc fixe that is free of unwanted accompanying substances, matching the strict regulation regarding the solubility of barium (Dirks, 2015). At high purity it is a white crystalline powder which is nearly insoluble in water (2.5-3.1 mg/L at 25 °C), dilute acids, alcohol and organic solvents, very slightly soluble in alkalis, but soluble in hot concentrated sulfuric acid. $BaSO_4$ is generally regarded nontoxic due to its insolubility. Toxicity is commonly due to impurities by soluble barium salts.

$BaSO_4$ is a common ingredient in tattoo and permanent makeup (PMU) ink, either on its own (giving a white colour as an inorganic pigment) or together with dyes and other pigments. $BaSO_4$ is included in the List of colourants allowed in cosmetic products (Commission Regulation (EC) No 1223/2009, Annex IV). It is of utmost importance that

$BaSO_4$ is analyzed for presence of soluble barium salts as described for medical or cosmetic products; failure to do so could have fatal consequences (Prior, 2015). In tattoo ink, $BaSO_4$ is used as a filler (spatial framework material to influence storage properties) and flocculation partner for organic pigments (controlled flocculation of organic pigments optimizes manufacturing properties (e.g. dispersibility), facilitates redispersion of the sediment that develops during long storage, and no demixing of pigments with different densities occurs) (Dirks, 2015). Dyes are precipitated on $BaSO_4$, becoming insoluble and more stable to light and other chemicals, and organic pigments show higher colour strength when mixed with $BaSO_4$. In human medicine, $BaSO_4$ (in suspension) has been used as diagnostic (X-ray imaging, etc.) agent for over 100 years mainly as a gastrointestinal contrast medium taken by mouth or rectally (formulation and volume depends on tract investigated).

ADME

$BaSO_4$ particles are not soluble in cellular membranes, but cells may take them up by pinocytosis (most cell types) or phagocytosis (macrophages). Formed intracellular vesicles fuse with lysosomes (pH 4.5-5.0) and the particles are subject to breakdown attempts, but it is unclear in which cell types and at what rates this occurs. $BaSO_4$ nanoparticles are rapidly cleared from the circulatory system (which could accidentally occur due to a faulty tattoo technique) by hepatic macrophages (Kupfer cells) that may dissolve them and release Ba^{2+} which accumulates in bone (Konduru, et al., 2014), before Ba^{2+} is slowly excreted by the liver (into bile) or kidneys. $BaSO_4$ particle accumulation in bone is unlikely. To prevent particle disintegration, factors such as particle size and stability may be important. If the $BaSO_4$ particles are not get broken down in dermis, dissolved Ba^{2+} may only leak out from the particles' surfaces, not from their core portions.

After oral administration, dissolution of $BaSO_4$ can potentially occur in the acidic stomach (gastric pH 1-2 in humans, pH 3-4 in rats/mice (McConnell, et al., 2008)), but the bioavailability of $BaSO_4$ is generally assumed negligible unless there is a breach of the gastrointestinal tract. The acute oral LD_{50} in rats is 307 g/kg bw. Dissolved Ba^{2+} is excreted primarily in the faeces following inhalation and parenteral (e.g. intravenous injection) administration, but it is also excreted in urine.

In the lungs, barium (form unclear) absorption after $BaSO_4$ exposure via inhalation has been reported to occur, but conditions in the lungs (having extensive first defense against inhaled particles) could be quite different from those in dermis. Inhalation of $BaSO_4$ dust causes a pulmonary reaction with mobilization of polymorphonuclear leukocytes and macrophages. Long-term exposure can result in baritosis, a benign form of pneumoconiosis. Lung studies suggest that $BaSO_4$ dust is partly removed by ciliary action (following, particles are often swallowed) and partly by 'lung-to-blood transfer mechanisms' (probably macrophage activity), and barium (unclear form) can thus be absorbed. A Syrian hamsters lung study determined a biological half-life of 8-9 days with subsequent urinary clearance, indicating some solubility, possible in colloidal form. In an inhalation study (Einbrodt, et al., 1972), rats were daily exposed for 5 h to a 40 mg $BaSO_4/m^3$ aerosol for 2 months and thereafter observed for 4 weeks (total period of 3 months). The barium (form not specified) content in lungs, lymph nodes, jaw and femur bones was determined over time using atomic absorption spectroscopy (AAS). The lung content was high after 2 weeks, then decreased over the next 4 weeks, but increased again at the end of the study period. The content in bones initially (2 weeks after beginning inhalation) increased, but then gradually decreased. No lymph transport was observed. One study found that lung clearance is

dependent on $BaSO_4$ particle area. A recent nanoparticle study (Konduru, et al., 2014) predominantly observed ^{131}Ba transfer from lung into bone (form could not be determined) versus other organs after 4 weeks of rat lung instillation of defined neutron-activated $^{131}BaSO_4$ nanoparticles (purity 93.8%). Lung clearance occurred with a half-life of 9.6 days.

$BaSO_4$ had a biological half-life of 26 days after intramuscular injection, suggesting migration and/or solubilisation (Thomas, et al., 1973).

Carcinogenicity

The $BaSO_4$ REACH registrants refer (read-across as a worst case scenario) to the rat and mouse 2-year carcinogenicity (NTP, 1994) $BaCl_2$ studies that both had a negative outcome (see section above for Ba and $BaCO_3$).

In one rat study (Cember & Watson, 1958) having administrated radioactive $BaS^{35}O_4$ particles by intratracheal injection, bronchogenic carcinoma (squamous cell type) developed. However, this study is likely not relevant due to the co-exposure from the strong local radioactivity.

Genotoxicity

The $BaSO_4$ REACH registrants have included three genotoxicity studies for $BaCl_2.2H_2O$ for which read across is performed ($BaCl_2$ forms Ba^{2+} and is much more water soluble than $BaSO_4$), all three had a negative outcome, (see section above for Ba and $BaCO_3$).

No mutagenicity studies on $BaSO_4$ itself were found which might relate to the fact that $BaSO_4$ cannot be dissolved in most media. $BaSO_4$ (as undissolved powder) did not induce DNA damage in isolated human peripheral lymphocytes at 10-1000 µg $BaSO_4$/ml (Braz, et al., 2008) or in cultured murine fibroblasts at 10-1000 µg $BaSO_4$/ml (Ribeiro, 2009) using the Comet assay (genotoxicity).

Reproductive toxicity

No studies were found. The ECHA $BaSO_4$ registrants has not included any reproduction or developmental/teratogenicity toxicity studies (data waiving).

Skin irritability

$BaSO_4$ is not a skin sensitizer and is safe for use in the present practices of use and concentrations in rinse-off (up to 0.99% w/v) and leave-on (up to 37% w/v) cosmetic products, when formulated to be non-irritating (in absence of other irritants) (CIR, 2014). The $BaSO_4$ REACH registrants has included one skin irritation and one skin sensitization for $BaCl_2.2H_2O$ to which read across is performed, both had a negative outcome, see section for Ba and $BaCO_3$.

Repeated exposure toxicity (STOT-RE)

The $BaSO_4$ REACH registrants states that the DNEL is 13 000 mg/kg bw/day for the general population via oral route (systemic effects) having used an overall assessment factor of 30 (chosen study not specified).

Other toxicities

The $BaSO_4$ REACH registrants has included one Acute Eye Irritation/Corrosion study for $BaCl_2.2H_2O$ to which read across is performed. It had a negative outcome, see section for Ba and $BaCO_3$.

Risk evaluation

Due to its high insolubility in most media, $BaSO_4$ would not be expected to dissolve during preparation of, or within, the tattoo ink. Risk characterization ratios (RCRs) for different scenarios can be calculated assuming that all tattoo injected $BaSO_4$ would be converted into Ba^{2+} per a chosen time period (e.g. days to years). However, it is difficult to calculate a probabilistic scenario as the rate of $BaSO_4$ dissolution in dermis (expected to be very low or even zero) is presently not known (data is lacking). If e.g. assuming that all the injected $BaSO_4$ (Mw 233.38 g/mole) would be dissolved into Ba^{2+} at a constant rate (equal amount dissolved per day) during 6 months (182.5 days) and using a DNEL of 0.60 mg Ba/kg bw/day from the repeated dose toxicity studies mentioned above (see section for Ba and $BaCO_3$), this implies that a 60 kg person should maximally be injected with 11 g $BaSO_4$ (182.5 days x61.2 mg/day) (60 kg x0.6 mg Ba/kg/day x233.38 g/mole /137.33 g/mole=61.2 mg $BaSO_4$/day) for the RCR not to exceed 1. Since only 4.308 g (14.36 mg ink/cm^2 x 300 cm^2) ink (largely made of liquids) is injected in a single tattoo, there seems to be no need for a CL for $BaSO_4$ even for larger, several, or tattoos in lighter individuals, unless the injected $BaSO_4$ would dissolve very fast inside the dermis (seems unlikely and supporting data is lacking). Over time, any eventually formed Ba^{2+} is also excreted.

Conclusion

There are presently no available relevant studies warranting a CMR classification for $BaSO_4$. The rate of $BaSO_4$ solubilisation in the skin (or $BaSO_4$ migration) is not known but expected to be low although this is uncertain. $BaSO_4$ is not a skin irritant/sensitizer. Sufficient information that would warrant $BaSO_4$ prohibited in tattoo and PMU ink appears presently not available and restricting the amount of $BaSO_4$ appears not relevant.

Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate] (CAS no 5160-02-1)

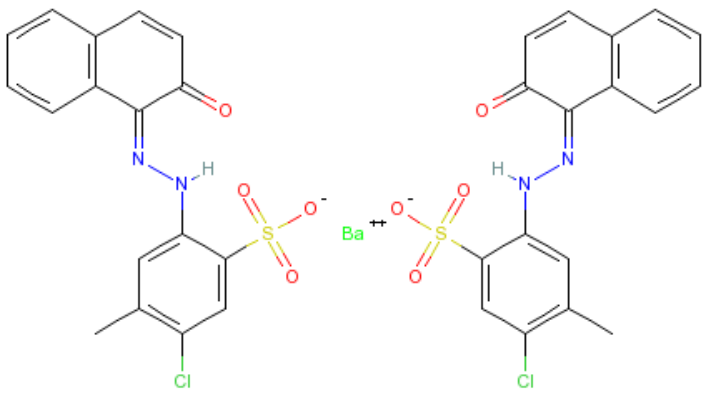
Pigment Red 53:1/CI 15585:1/D&C Red No. 9

CAS no.: 5160-02-1

Molecular formula: $C_{34}H_{24}BaCl_2N_4O_8S_2$

Molecular weight: 888.9 g/mol

Water solubility: 3 mg/L at 23°C (immediately after filtration) and <0.01 mg/L at 23°C (after one week) (ECHA).



Classification

ECHA: No harmonized classification.

Hazard Class and Category and Hazard statements:

Acute Tox. 4 H302

Acute Tox. 4 H332

Acute Tox. 3 H301

Skin Sens. 1 H317

Pigment Red 53 (CAS no 2092-56-0) is listed in CoE ResAP(2008)1, Table 2, which lists colourants, particularly with regard to their carcinogenic, mutagenic, reprotoxic and/or sensitising properties which tattoo and PMU products should not contain (JRC 2015). Pigment Red 53/CI 15585 (CAS no 5160-02-1 and CAS no 2092-56-0) is also listed in CRP Annex II and is not allow to use in cosmetic products. Pigment Red 53:1 (CAS no 5160-02-1) belongs to the β-Naphtol pigment lakes. Lake pigments are manufactured by precipitating water-soluble dyes on inert binders, usually salts of calcium, magnesium, barium or strontium (e.g., $BaSO_4$). The β-naphthol pigment lakes are characterized by a general structure which includes a β-naphthol coupling component with ionisable groups (e.g., $–SO_3^-$, $–COO^-$) on the ring(s) opposite the β-naphthol moiety and with additional substitutions (Herbst and Hunger 2004 cited by (Health Canada, 2016)).

ADME

An in vivo absorption/metabolism study was identified for PR53:1. In this study, groups of four male F344 rats were fed ^{14}C-labelled PR53:1 (called "Red No. 9") for 24 hours in the diet at a concentration of 3 000 parts per million (ppm) (equivalent to a dose of 150 mg/kg-bw) followed by a 24-hour recovery period (Chadwick et al. 1984, cited by (Health Canada, 2016)). The PR53:1 was labelled on either the benzene or the naphthol ring in order to distinguish between two azo bond cleavage products on either side of the azo bond, ^{14}C-benzene for Red Lake C amine and ^{14}C-naphthol for 1-amino-2-naphthol. Urine, feces and blood samples were taken during the 0- to 48-hour study period. Substantial metabolism was noted, as the majority of the applied dose was recovered in urine as Red Lake C amine (35%) and 1-amino-2-naphthol (54%), while only a small fraction of the dose (< 1%) was recovered as unchanged PR53:1. Overall, the results of this study suggest that a large fraction of PR53:1 and/or its azo bond cleavage products is absorbed by the oral route, with substantial azo bond cleavage occurring either by bacteria of the gastrointestinal tract or by tissue enzymes in vivo.

In an in vitro study, substantial (~99%) azo bond cleavage of PR53:1 was observed following an overnight incubation with a rat fecal preparation (Dillon et al. 1994, cited by Health Canada), while another study demonstrated gradual but consistent azo bond cleavage of PR50:1 in a 24-hour anaerobic culture with a human fecal homogenate (BRI 2013, cited by (Health Canada, 2016)). These in vitro studies support the above oral study suggesting that azo bond cleavage of the β-naphthol pigment lakes can occur, and likely occurs, in the mammalian gastrointestinal tract, based on the similar structures and physical-chemical properties among these substances.

The only available dermal absorption study identified was an in vitro skin penetration study using human skin, reporting a maximum of 0.06% of ^{14}C-benzene-labelled PR53:1 detected in the receptor fluid (Franz 1983, cited by (Health Canada, 2016)). Based on the close

structural similarity between β-naphthol pigment lakes and BONA pigment lake analogue, PR57:1* (Ca^{2+} salt) and PR57* (Na^{+} salt), a conservative estimate of 1% dermal absorption for BONA pigment lakes is applied also for the β-naphthol pigment lakes.

Collectively, the β-naphthol pigment lakes are considered to be bioavailable by the oral route and to undergo metabolism, including azo bond cleavage, in the gastrointestinal tract, with some degree of dissociation of the Ba^{2+} ion expected to occur.

Genotoxicity

PR53:1 was generally reported as negative in most in vivo and in vitro studies, with some weak and/or equivocal responses reported in a few in vitro tests (Salmonella mutagenicity following incubation with a faecal preparation, in vitro chromosomal aberrations, in vitro cell transformation assay). The other positive response was observed for in vivo SCEs in bone marrow of mice.

Results from the standard Ames assay in Salmonella in the identified studies were almost entirely negative (Brown et al. 1979; Muzzall and Cook 1979; Miyagoshi et al. 1983; Longstaff et al. 1984; BUA 1993; Zeiger et al. 1988; Dillon et al. 1994, cited by (Health Canada, 2016)), and weakly positive or equivocal responses were reported in strains TA97 and TA98 only at doses resulting in precipitation of the test material. A reverse mutation assay in Escherichia coli was also reported as negative (Hoechst AG 1985, cited by (Health Canada, 2016)). *In vitro* genotoxicity tests in mammalian cells were also primarily negative, including the *tk* locus mutation assay in mouse lymphoma L5178Y cells (Myhr and Caspary 1990), chromosomal aberrations and SCEs in CHO cells (Ivett et al. 1989) and UDS in primary rat hepatocytes (Kornbrust and Barfnecht 1985; Williams et al. 1989, all cited by (Health Canada, 2016)). For *in vivo* genotoxicity studies, following intraperitoneal injection of PR53:1 at doses of 500–2000 mg/kg-bw, no statistically significant increase in UDS was observed in rat hepatocytes or in micronuclei of the bone marrow (Westmoreland and Gatehouse 1992). This result confirms an earlier negative result for UDS in rat hepatocytes at a lower dose of 500 mg/kg-bw. A negative result was reported for chromosomal aberrations in bone marrow following intraperitoneal injection (1250–5000 mg/kg-bw) in mice, whereas positive results for SCEs in bone marrow were reported from the same study (NTP 2013). PR53:1 was also reported as negative for mutagenicity in Drosophila (Foureman et al. 1994, cited by (Health Canada, 2016)).

Carcinogenicity

There is inadequate evidence in humans for the carcinogenicity of D&C Red No. 9. There is limited evidence in experimental animals for the carcinogenicity of D&C Red No. 9. Overall evaluation: D&C Red No. 9 is not classifiable as to its carcinogenicity to humans (Group 3). (IARC, 1993a)

The carcinogenicity of PR53:1 was tested in several chronic studies in rats and mice (NTP 1982; CTFA 1982; Davis and Fitzhugh 1962; OECD 1999a, cited by (Health Canada, 2016)). The chronic assay by the NTP (1982) included 2 year exposure of PR53:1 in the diet to F344 rats (50/sex/dose, control, 1000 ppm or 50 mg/kg-bw per day, 3000 ppm or 150 mg/kg-bw per day) and B6C3F1 mice (50/sex/dose, 1000 ppm or 130 mg/kg-bw per day, 2000 ppm or 260 mg/kg-bw per day).

An increased incidence of spleen sarcomas was observed only in high-dose male F344 rats. The NTP considered the association of PR53:1 exposure with the spleen sarcomas to be unequivocal and "positive" evidence for the carcinogenicity of PR53:1 (NTP 1982). Similar

types of spleen tumours were observed in Sprague-Dawley rats in the high-dose group (10 000 ppm = 500 mg/kg-bw per day) of another chronic dietary study with PR53:1. The tumours were considered as likely exposure related, although their incidence was not statistically significant (OECD 1999a). No spleen tumours were observed in the exposed female F344 rats from the NTP study (NTP 1982). Other chronic oral studies in mice (CTFA 1982; NTP 1982) and Osborne-Mendel rats (Davis and Fitzhugh 1962) did not demonstrate an observable tumorigenic response to PR53:1 exposure. The reason for the apparent sex and species sensitivity of this tumour type is not known.

In the chronic oral NTP studies in mice and rats, the incidence of hepatic neoplastic nodules observed in the liver of male F344 rats was statistically significant at both test doses and considered as "positive" evidence for carcinogenicity. These effects were also associated with non-neoplastic liver effects in male rats, while a statistically significant trend ($p = 0.039$) for neoplastic nodules of the liver was also observed for female rats and considered "equivocal" evidence for carcinogenicity (NTP 1982).

Charles River rats (CD strain) with in utero and lifetime exposure to D & C Red No. 9 (pigment red 53:1) in the diet reveals a small number of highly unusual mesenchymal neoplasms of the spleen. The increased incidence of these tumours was not statistically significant in the dosed animals in this study; however, due to their highly unusual nature and the possibility of tumour origin in nonneoplastic fibrosis it is highly likely that these tumours were compound induced (OECD SIDS, 1999).

Reproductive toxicity

In a 30-months chronic toxicity and potential carcinogenicity study with in utero and lifetime exposure, rats of the Charles River CD strain was given D & C Red No. 9 (pigment red 53:1) via its incorporation into the basal diets at doses of 0 and 10,000 ppm (OECD SIDS, 1999). The reproductive performance of the F0 generation was also evaluated. The effect of test material for the in-utero phase was evaluated via mortality, clinical observations, body weight, food consumption, sex ratio, pup viability data and gross necropsy observations on selected animals. There was no evidence for an impairment of reproductive functions in animals.

The PR53:1 REACH registrants included one one-generation reproductive study (1977) basically performed according to OECD Guideline 415 (One-Generation Reproduction Toxicity Study). The test material, D&C Red No. 9, was administered to rats in the diet at concentrations of 100, 200 and 500 ppm for 8 weeks prior to mating. The treatment was continued during gestation and lactation. The test material was judged not to have an effect on body weight, food consumption, or fertility of the F0 generation rats. Similarly, no effect was evident on the viability or growth of F1 pups from birth to weaning.

Skin irritability

The PR53:1 REACH registrants included one study on rabbit skin performed according to "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959) of the US Association of Food and Drug Officials (AFDO). Erythema score and primary dermal irritation was observed 24 and 72 hours after treatment for 24 hours with 0.5 g PR53:1 on a 2.5 x 2.5 cm gauze patch applied to the prepared skin. The primary irritation index as the measure of the acute irritation to the skin of rabbits was found to be 0. Therefore the test article was considered as non-irritant to the skin of rabbits.

No other data was found.

Eye irritation

The PR53:1 REACH registrants included one study on rabbit skin performed according to "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959) of the US Association of Food and Drug Officials (AFDO). Cornea opacity score, iris score and conjunctivae score was observed 24 hours and 2, 3, 4 and 7 days after treatment with 0.1 g for 30 seconds. The primary-irritation index, which serves as a measure of the acute irritation provoked by the substance, was found to be 0 for all three endpoints. Therefore, the test item was considered as non-irritant to the eye of rabbits. No other data was found.

Skin sensitisation

The PR53:1 REACH registrants included one study in mice performed according to OECD Guideline 429 ((Skin Sensitisation: Local Lymph Node Assay). No symptoms of local toxicity at the ears of the animals and no systemic findings were observed during the study period. Eventual erythema of the ear skin could not be evaluated due to the colour of the test item. The submitter concluded that PR53:1 is not sensitising.

No other data was found.

Repeated exposure toxicity

The repeated-dose oral toxicity studies available for PR53:1 demonstrate some common non-cancer effects, with the spleen, blood, liver and kidney as the primary target organs or tissues in the tested species in the following general order of sensitivity: dog greater than rat, greater than mouse (Health Canada, 2016). The most consistent reported observations across the studies involved spleen toxicity and were observed in both sexes of several strains of rat from short-term, sub-chronic and chronic studies (Davis and Fitzhugh 1962; CTFA 1982; NTP 1982, cited by (Health Canada, 2016)). Splenic toxicity was also observed in studies in dogs (CTFA 1983a, cited by (Health Canada, 2016)) as well as in short-term and sub-chronic studies in mice (NTP 1982). Overall, spleen toxicity was observed at LOAELs of 30 mg/kg-bw per day in the 2-year dog study (CFTA 1983, cited by (Health Canada, 2016)), 50 mg/kg-bw per day in the chronic rat study (NTP 1982) and 163 mg/kg-bw per day in the 13-week mouse study (NTP 1982). The spleen toxicity is likely associated with, or secondary to, splenic clearance following primary toxicity on the red blood cells.

Other common non-cancer observations involved effects in the liver and kidney. Liver toxicity in rats (Davis and Fitzhugh 1962; CTFA 1982; NTP 1982, in (Health Canada, 2016)) and dogs (CTFA 1983a, cited by (Health Canada, 2016)) involved increased liver size/weight and hemosiderosis as well as additional effects (basophilic cytoplasm changes and centrilobular necrosis) in male rats (NTP 1982). Observations in the kidneys included increased pigmentation in both sexes of rats following short-term, sub-chronic and chronic exposure and kidney tubule regeneration in female rats after chronic exposure (NTP 1982). Other less common effects were reported in studies in rats by the NTP (testes tubule degeneration, dilatation of mammary acini, hyperplasia of bronchiolar lymph nodes; NTP 1982) and the US FDA (bone marrow hyperplasia; Davis and Fitzhugh 1962) and a study in mice (chronic inflammation of the stomach; CTFA 1982).

Repeated oral administration of pigment red 53:1 in high dosages (at 3000 ppm and above) for 90 days in rats resulted in haematological findings and effects on spleen liver and kidneys (OECD SIDS, 1999). Daily administration of pigment red 53:1 for 90 days in mice led to comparable findings. The NOEL for mice was determined as 90 mg/kg bw/day.

A 20-week subacute feeding study using 5 male and 5 female weanling Osborne Mendel rats per level and levels of 2%, 1%, 0.5%, 0.25% and 0% of D & C Red No. 9 (pigment red 53:1) in the diet produced no mortality but resulted in lowered average haemoglobin and haematocrit values (OECD SIDS, 1999). At autopsy, splenomegaly was noted in rats on all substance test levels, and liver enlargement was noted at the 1% and 0.5% colour-feeding levels. 5 groups of 50 3-week old Osborne-Mendle rats were started on a two-year feeding experiment on D & C Red No. 9 at dose levels of 1%, 0.25%, 0.05%, 0.01% and 0% (controls). The test substance had no apparent effect on the growth rate, mortality or occurrence of tumours in the test rats. Haemoglobin levels were slightly lowered and abnormal shape of red blood cells were observed in rats on the 1% and 0.25% feeding levels (no further information given). At autopsy, survivors on the 1 % feeding level showed moderate splenomegaly and rats on the 0.25% level showed slight splenomegaly. Histopathologic findings attributable to the colour feeding consisted of moderate splenomegaly at 1%, slight splenomegaly at 0.25%, and slight bone marrow hyperplasia at both levels. The 1% feeding level rats also showed slightly increased splenic haemosiderosis and some had splenic infarcts. At 0.05% and 0.01% there were no gross or microscopic pathologic changes attributable to D & C Red No. 9 (pigment red 53:1). The No Observed Effect Level (NOEL) was determined as 25 mg/kg bw/day (0.05% colour in the diet).

In the PR 53:1 studies, clear effects in the spleen of both rats and mice were observed in the 13wk study of PR53:1 at doses of 150 and 163 mg/kg/d respectively. This corresponds to equivalent doses of Ba^{2+} ion of 23 and 25 mg/kg bw per day for rat and mice (assuming 100% of PR53:1 dissociates to release free Ba^{2+} ion). No similar effects were observed in the sub-chronic component of a barium chloride drinking water study (NTP, 1994) at Ba^{2+} ion doses 10-20x higher in rat and mouse respectively (200mg Ba^{2+} ion /kg bw per day in rat, 495mg Ba^{2+} ion /kg bw per day mouse).

Therefore any PR53:1 related effects in the blood or spleen seem unrelated to the contribution of Ba^{2+} ion. Similar analysis supports the conclusion that Ba^{2+} ion is unlikely to be contributing to the PR53:1 related liver lesions. Therefore, these observed effects in PR53:1 are likely due to the organic moiety of this substance as either the parent azo dye and/or the azo cleavage products (Health Canada, 2016).

Derivation of DNELs

Derivation of a DNEL based on the barium moiety of the pigment and a subsequent risk evaluation was not performed based on the assumption that the observed effects are due to the parent azo dye and/or the azo cleavage products.

Barium bis[2-[(2-hydroxynaphthyl)azo]naphthalenesulphonate] (CAS no 1103-38-4)

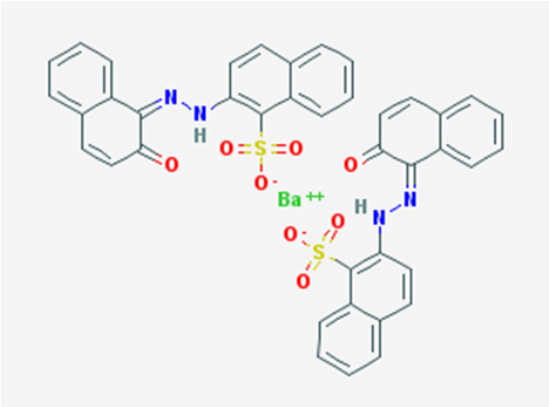
Pigment Red 49:1/CI 15630:1/D&C Red No. 12

CAS no.: 1103-38-4

Molecular formula: $C_{40}H_{26}BaN_4O_8S_2$

Molecular weight: 892.1 g/mol

Water solubility: 1672.9 µg/l at 23°C (immediately after filtration) and 32.5 µ/L at 23°C (after one week) (ECHA).



Classification

ECHA: No harmonized classification

Hazard Class and Category Codes and Hazard statements:

Acute Tox. 4 H302

Pigment Red 49:1 belongs to the same group as Pigment Red 53:1, the β-Naphtol pigment lakes. For more chemical information, see under the evaluation of this pigment (Pigment Red 53:1; Barium bis[2-chloro-5-[(2-hydroxy-1-naphthyl)azo]toluene-4-sulphonate]).

Sodium 2-[(2-hydroxynaphthyl)azo]naphthalenesulphonate and its insoluble barium, strontium and zirconium lakes, salts and pigments (CI 15630) is listed as entry #25 in CPR Annex IV with a maximum concentration in ready for use preparations of 3%. According to the CosIng database, CI 15630 is associated with CAS no 1248-18-6.

The barium containing Pigment Red 49:1/CI 15630:1 (CAS no. 1103-38-4) could not be found in the CosIng database. However it is not clear if this pigment is allowed according to Annex IV or not, as its "parent" CI number (CI 15630 = entry #25) indicate it should be allowed.

ADME

No absorption/metabolism studies were identified for Pigment Red 49:1. However, the absorption, distribution, metabolism and excretion (ADME) are assumed largely similar for pigments belonging to the β-naphthol pigment lakes. Thus, the ADME described for Pigment Red 53:1 is supposed to apply to Pigment 49:1 (Health Canada, 2016). A degree of bioavailability is considered after oral exposure to PR49:1 since a dissociation of the Ba^{2+} -ion and the organic azo anion is likely to occur. Based on this, studies on PR49*, the sodium salt, were also considered informative for the toxicity of PR49:1 and therefore included as a basis for the evaluation (Health Canada, 2016).

Genotoxicity

Pigment Red 49:1 has been reported as negative in the standard Ames assay (Muzzall and Cook, 1979, cited by (Health Canada, 2016)). Furthermore, Pigment Red 49:1 was reported inconclusive and negative for mutagenicity without and with S9 activation, respectively, in the mouse lymphoma assay (Seifried et al., 2006, cited by (Health Canada, 2016)).

Based on several read-across and supporting experimental results, the PR49:1 REACH registrant concluded that PR49:1 is not genotoxic.

Carcinogenicity

The PR49:1 REACH registrant refers no carcinogenicity studies performed on PR49:1, but based on read-across one key carcinogenicity study (probably the NTP-study referred for PR53:1) and several supporting studies are presented. No conclusion is drawn.

No studies on carcinogenicity of PR49:1 or PR49* were identified. However, some read-across for the β-naphthol pigment lakes has been performed (Health Canada, 2016). The increased incidence of spleen tumours in male rats from the studies on PR53:1 is considered to be secondary to the non-neoplastic splenic toxicity observed. This consideration is based on the similarity of the observed tumours to that observed for aniline (Environment Canada, Health Canada 2011, cited in (Health Canada, 2016), and the absence of strong genotoxicity of PR53:1. Thus, since hemolysis and spleen toxicity was also observed for PR49*, it is concluded that it is reasonable to assume a similar potential for PR49:1 and the other β-naphthol pigment lakes to induce spleen tumours if tested under the same conditions as PR53:1 (Health Canada, 2016).

Reproductive toxicity

No studies on PR49:1 or PR49* were identified.

The PR49:1 REACH registrant refers to Read-across to the one-generation reproductive study referred for Pigment53:1 (D&C Red No. 9). In this study, the test material was judged not to have an effect on body weight, food consumption, or fertility of the F0 generation rats. Similarly, no effect was evident on the viability or growth of F1 pups from birth to weaning.

Skin irritability

The PR49:1 REACH registrant included one study on rabbit skin performed according to OECD Guideline 404 (Acute Dermal Irritation / Corrosion). The test substance (1.5 ml or 0.5 g) was applied on test sites for 24 hours. Observations were recorded after 24 and 72 hours. The applicant concludes that the test substance is not irritating.

Eye irritation

The PR49:1 REACH registrant included one study on rabbit performed according to OECD Guideline 405 (Acute Eye Irritation/Corrosion). Conjunctivae score was observed 1, 6, 24, 48 and 72 hours after treatment with 0.1 g for 30 seconds. A slight to mild conjunctival reaction was seen in 5/6 eyes one hour after application the compound. After 5 hours the reaction subsided, washed eyes returning to normal slightly more quickly. All were normal by day 3. The ocular reactions were scored by the method described in "Appraisal of the Safety of Chemicals in Food Drugs and Cosmetics" page 51, published by the Association of Food and Drug Officials of the U.S.A. The test item was considered not irritating.

Skin sensitisation

No studies on PR49:1 or PR49* were identified.

The PR49:1 REACH registrant states this substance as not skin sensitizing. This is based on a key study performed according to OECD Guideline 429 (mouse local lymphnode assay) (2012).

Repeated exposure toxicity

The results of a 20-week sub-chronic feeding study conducted by the US FDA indicated no changes in mortality, growth or other gross signs of toxicity (Davis and Fitzhugh 1963 cited

by (Health Canada, 2016)). In this study, five Osborne-Mendel rats of each sex were exposed to a dietary concentration of 0%, 0.25%, 0.5%, 1% or 2% (equivalent to doses of 0, 125, 250, 500 and 1 000 mg/kg-bw per day) of PR49*indicated no changes in mortality, growth or other gross signs of toxicity. However, a slight effect on hematological parameters as well as moderate splenomegaly were observed in the test groups compared with the control group, suggesting a sub-chronic LOAEL of 125 mg/kg-bw per day, the lowest dose tested.

In a chronic 2-year feeding study, also conducted by the US FDA, 25 rats of each sex were exposed to a dietary concentration of 0%, 0.01%, 0.05%, 0.25% or 1% (equivalent to doses of 0, 5, 25, 125 and 500 mg/kg-bw per day) of PR49:1 for 103 weeks. The only exposure-related effects reported were a statistically significant enlargement of the spleen in the 0.25% and 1% groups (125 and 500 mg/kg-bw per day) with associated splenic hemosiderosis and erythropoiesis confirmed by histology, while the spleens of the 0.05% group (25 mg/kg-bw per day) were not histologically different from those of the control group. Moderate bone marrow hyperplasia was also observed at 0.05% and above (≥ 25 mg/kg-bw per day). Blood samples showed no apparent effects on hemoglobin, hematocrit or white cell counts; however, test groups did show "polychromasia, target cells, and occasional normoblasts in peripheral blood." No other obvious differences in effects such as organ weights or tumour incidences were reported between the exposure and control groups (Davis and Fitzhugh, 1963, cited by (Health Canada, 2016)). The chronic LOAELs were considered to be 125 mg/kg-bw per day for the spleen effects (NOAEL = 25 mg/kg-bw per day) and 25 mg/kg-bw per day for bone marrow hyperplasia (NOAEL = 5 mg/kg-bw per day).

In a dietary study in Beagle dogs (six of each sex per group) fed PR49* at a concentration of 0%, 0.015%, 0.1% or 5% (equivalent to doses of 0, 4.5, 30 and 150 mg/kg-bw per day) for 2 years, no exposure-related changes in body weight, feed consumption or behaviour were reported. Hematological parameters were reportedly affected in dogs fed at and above 0.1% (30 mg/kg-bw per day), these effects increased in severity at the 5% dose. Urine bilirubin was also increased at and above 0.1%. A dose-dependent increased incidence of splenomegaly was shown at and above 0.1%, while liver weights increased in the 5% group. Evidence of red blood cell destruction was reported at and above 0.1%, based on histological findings of increased hemosiderosis and erythropoiesis of the spleen, as well as pigmentation of the liver, bone marrow and renal tubules. Severe acute hyperemia was found in tissue sections of all dogs in the 5% group. A more limited 90-day sub-chronic study (one Beagle of each sex per group) in the same dose range as above also demonstrated some evidence of effects on the blood (bilirubinuria, splenomegaly, hemosiderosis of spleen/liver/kidney, increased nucleated red blood cells) and supports the findings from the chronic study (US FDA 1972 cited by (Health Canada, 2016)). Based on these data, the chronic LOAEL was considered to be 30 mg/kg-bw per day, with a NOAEL of 4.5 mg/kg-bw per day.

The PR49:1 REACH registrant supported Read-across from two key studies and one supporting study performed with Pigment Red 53:1. One key study was the range-finder for the NTP 2-year cancer study in rats and mice, performed according to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents, 1982; referred under PR53:1). The other key study was a study in mice performed according to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies, 1978 - 1980). The applicant concluded that the gross and histopathologic evaluation and tumour incidence analyses did not reveal any compound related effects.

Since animal studies of PR49* (Na+ salt) did not include exposure to Ba^{2+} ion, the effects observed in these studies (e.g. hemolysis, splenomegaly, hemosiderosis, bone marrow hyperplasia, urine bilirubin) support that the common organic moieties of both PR53:1 and PR49* are responsible for the effects observed in erythrocytes, spleen and liver for these substances.

Derivation of DNELs

Derivation of a DNEL and a subsequent risk evaluation was not performed based on the assumption that the observed effects are due to the parent azo dye and/or the azo cleavage products as described for PR53:1.

Barium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate (CAS no 7585-41-3)

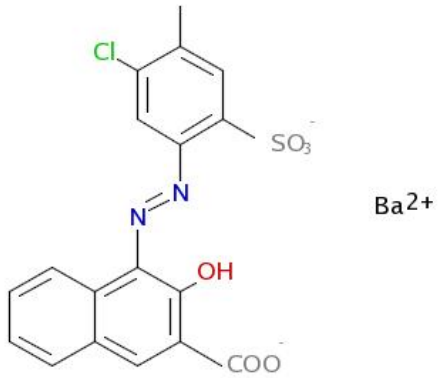
Pigment Red 48:1/CI 15865:1

CAS no.: 7585-41-3

Molecular formula: $C_{18}H_{11}BaClN_2O_6S$

Molecular weight: 556.135 g/mol

Water solubility: < 25 µg/L at 23°C (ECHA)



Classification

ECHA: No harmonized classification

Hazard Class and Category Codes and Hazard statements:

Acute toxicity – oral:	Acute Tox. 4	H302
Acute Toxicity – inhalation:	Acute Tox. 4	H332

Disodium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate and its insoluble barium, strontium and zirconium lakes, salts and pigments (CI 15865), is listed as entry #28 in CPR Annex IV without any restrictions. According to the CosIng database, CI 15865 is associated with CAS no 3561-21-4 and 5280-66-0. CAS no 3561-21-4 8 (CI 15865, Pigment Red 48) is also listed in CPR Annex II when used as a substance in hair dye products.

The barium containing Pigment Red 48:1/CI 15865:1 (CAS no. 7585-41-3) could not be found in the CosIng database. However it is not clear if this pigment is allowed according to Annex IV or not, as its "parent" CI number (CI 15865 = entry #28) indicate it should be allowed.

ADME

No absorption/metabolism studies were identified for Pigment Red 48:1.

However, the PR49:1 REACH registrant refers to a study in rats where Ba was given at a dose of 0.5 ml/100 g bodyweight by gavage. Ba was given either as sulfate, chloride or carbonate and recovery of Ba in blood and organs was reported. The ADME of barium ions is described previously.

Genotoxicity

Based on the result from one key experimental study on Pigment Red 48:1, and Read-across from several key and supporting studies on structural analogues/surrogates, the ECHA PR48:1 submitter concluded that PR48:1 is not genotoxic.

No other data was identified.

Carcinogenicity

The PR48:1 REACH registrant refers no carcinogenicity studies performed on PR48:1, but the read-across of several studies for the substance PR57 (CAS 5858-81-1, the sodium salt). Included are one combined chronic toxicity/carcinogenicity study (performed according to OECD Guideline 453), one carcinogenic study in mice (performed according to OECD Guideline 451 and US FDA requirements for feeding studies of D&C colours), two supporting drinking water NTP studies (NTP, 1994) on barium chloride dihydrate (CAS No. 10326-27-9) and one dermal toxicity study on PR57:1 (1984). The registrant concludes that the structural analogue/surrogate to PR 48:1, PR57:1, is not carcinogenic.

No other data was identified.

Reproductive toxicity

No studies on PR48:1 was identified.

The PR48:1 REACH registrant refers to Read-across of three key studies in rats. The referred studies were a One-generation reproduction toxicity study (OECD Guideline 415) testing the pigment D & C 6 (CAS 5858-81-1, the sodium salt named Pigment Red 57), an OECD combined repeated dose and reproductive/developmental toxicity screening test (Precursor Protocol og GL 422) testing Pigment Red 57:1, and a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD Guideline 422) testing Pigment Red 48:2 (CAS 7023-61-2, the calcium salt of PR 48). No treatment related effects on reproductive parameters were reported.

Developmental toxicity

No studies on PR48:1 was identified.

The PR48:1 REACH registrant refers to Read-across of one key Prenatal developmental toxicity study (OECD Guideline 414) in rats exposed to D & C no 7 (PR 57:1, CAS 5281-04-9). No developmental toxicity was observed in rats at doses of 5, 16 and 50 mg/kg bw.

Skin irritability

The PR48:1 REACH registrant refers two key experimental studies performed in rabbits. In one study (1974), the test substance PR48:1 (50% aqueous solution) was tested using an internal standard method (BASF test). Animals were treated for 1, 5 and 15 minutes and for 20 hours using occlusive conditions. Observations were recorded after 24 hours and 8 days. The registrant concluded that the test substance was not irritating. In another study (1973, according to OECD Guideline 404 (Acute Dermal Irritation / Corrosion) and The Food and Drug Administration of the U.S.A. in The Federal Register (17 September, 1964 §191. 11), 0.5 g of the test substance was applied to test patches of 1 inch x 1 inch on abraded and intact skin for 24 and 72 hours. Erythema and edema was scored. The registrant concluded that the test substance was not irritating.

Eye irritation

The PR48:1 REACH registrant refers one key experimental study performed in rabbits (OECD Guideline 405, Acute Eye Irritation/Corrosion). The registrant concluded that the test substance was not irritating.

Skin sensitisation

No studies on PR48:1 was identified.

The PR48:1 REACH registrant refers to Read-across for three studies performed in mice. Two of the referred studies were performed according to OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay) and one according to OECD Guideline 406 (Skin Sensitisation). One of the OECD Guideline 429 studies and the OECD Guideline 406 study tested the structural analogue/surrogate to PR 48:1, PR57:1. In the second OECD Guideline 429 study, the test substance was not identified.

Repeated exposure toxicity

No studies on PR48:1 was identified. Read-across from the following studies were referred by the REACH registrant:

Two studies according to OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) (1993 in rats: test substance not identified; 2009 in rats: test substance Pigment Red 48:2), one study according to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies) (1981: test substance D & C 6 (CAS 5858-81-1, the sodium salt named Pigment Red 57) and one study according to OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)/EU Method B.7 (Repeated Dose (28 Days) Toxicity (Oral)) (2006 in rats: test substance not identified).

Read-across from one supported OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) study on Pigment Red 57:1 in rats and two supportive NTP Toxicology and Carcinogenesis Studies of Barium Chloride Dihydrate (CAS No. 10326-27-9) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies) were provided by the registrant (NTP, 1994).

Derivation of DNELs

No substance specific toxicological information was available in order to derive a DNEL for PR 48:1. None of the studies on other pigments included for Read-across contained the barium-salts and are thus not relevant to derive a DNEL for PR 48:1.

Barium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate (CAS no 17852-98-1)

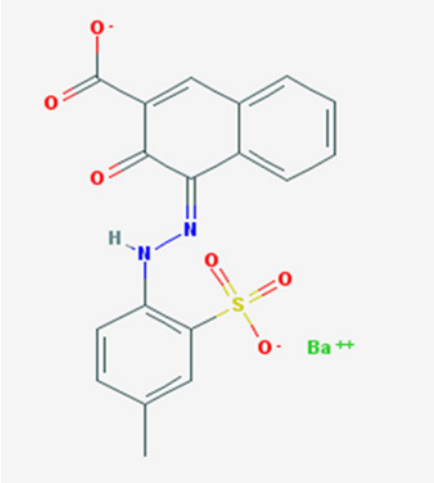
Pigment Red 57:2/CI 15850:2/D&C Red No. 6

CAS no.: 17852-98-1

Molecular formula: $C_{18}H_{12}N_2O_6S.Ba$

Molecular weight: 521.689 g/mol

Water solubility: Not known



Classification

ECHA: No harmonized classification

Hazard Class and Category Codes and Hazard statements:

Acute toxicity:	Acute Tox. 4	H302

Pigment Red 57:2 (Barium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate) is in the REACH Pre-registration process.

Disodium 3-hydroxy-4-[(4-methyl-2-sulphonatophenyl)azo]-2-naphthoate and its insoluble barium, strontium and zirconium lakes, salts and pigments (CI 15850) is listed as entry #27 in CPR Annex IV with purity criteria as set out in Commission Directive 95/45/EC (E 180). According to the CosIng database, CI 15850 is associated with CAS no 5281-04-9 and 5858-81-1.

The barium containing Pigment Red 57:2/CI 15850:2 (CAS no. 17852-98-1) could not be found in the CosIng database. However it is not clear if this pigment is allowed according to Annex IV or not, as its "parent" CI number (CI 15850 = entry #27) indicate it should be allowed.

Toxicological information

No toxicological information was identified for Pigment Red 57:2. However, the PR57:2 REACH registrant referred to Read-across for several studies on Pigment Red 57 (the

sodium salt) and Pigment Red 57:1 (the calcium salt) in their Registration dossier. The following conclusions were drawn by the registrant:

- PR57 and PR57:1 are not carcinogenic.
- No treatment related effects of PR57:1 on reproductive parameters were reported.
- No developmental toxicity of PR57:1 was observed in rats at doses up to 50 mg/kg bw.
- Pigment Red 57:1 does not cause skin sensitization in the local lymph node assay in mice
- NOEL for PR57:1 based on increased incidence of chronic nephritis in aged rats was 0.3% in the diet (189 mg/kg bw/day) for females and 0.05% in the diet (26 mg/kg bw/day) for males.

Derivation of DNELs

No DNEL could be derived due to lack of data.

Barium (2+)hydrogen-2-((2-hydroxy-3,6-disulfonato-1-naphthyl)azo)benzoat (CAS no 1325-16-2)

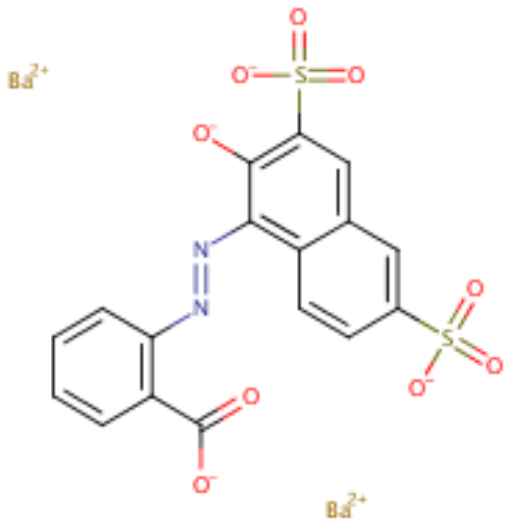
Pigment Red 60:1/CI 16105:1

CAS no.: 1325-16-2

Molecular formula: $C_{17}H_{10}Ba_2N_2O_9S_2$

Molecular weight: 723.0 g/mol

Water solubility: Not found



Classification

ECHA: No harmonized classification

Pigment Red 60:1 (Barium (2+)hydrogen-2-((2-hydroxy-3,6-disulfonato-1-naphthyl)azo)benzoat) is in the REACH Pre-registration process. No registrant dossier was available.

Toxicological information

No toxicological information was identified for Pigment Red 60:1.

Derivation of DNELs

No DNEL could be derived due to lack of data.

Barium bis[4-[(2-hydroxy-1-naphthyl)azo]-2-methylbenzenesulphonate] (CAS no 5850-87-3)

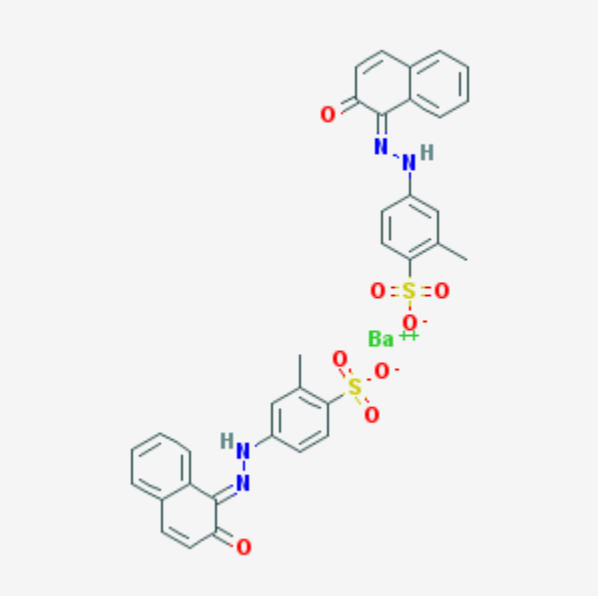
Pigment Red 51/CI 15580

CAS no.: 5850-87-3

Molecular formula: $C_{34}H_{26}BaN_4O_8S_2$

Molecular weight: 820.0 g/mol

Water solubility: 19 µg/l



Classification

ECHA: No harmonized classification

Pigment Red 51 (Barium bis[4-[(2-hydroxy-1-naphthyl)azo]-2-methylbenzenesulphonate), is listed as entry #23 in CPR Annex IV without any restrictions.

Pigment Red 51 (Barium bis[4-[(2-hydroxy-1-naphthyl)azo]-2-methylbenzenesulphonate]) is in the REACH Pre-registration process. No registrant dossier was available.

Toxicological information

No toxicological information was identified for Pigment Red 51.

Derivation of DNELs

No DNEL could be derived due to lack of data.

Overall conclusion for organic Ba-pigments

The assessment demonstrates that the toxicity of two of these pigments (Pigment Red 53:1 and Pigment Red 49:1) is associated with the parent azo dye and/or the azo cleavage products and not with the barium moiety. For the other pigments, there is a lack of data and no DNEL could be derived based on barium. In conclusion, no CL is proposed for these organic barium pigments.

Appendix B.8. Risk assessment of copper (Cu)

Risk evaluation of Cu in tattoo inks

Introduction

In the CoE ResAP(2008)1 the content of soluble copper (Cu) is addressed. In this paper both the soluble form and Cu captured in colourants are addressed initially because most chemical analytical methods are not capable of distinguishing between the two forms.

A wide range of different metals and other elements, including copper (Cu), have been found in tattoo inks (JRC, 2015b) (DEPA, 2012).

In 2012 the total copper content in 61 tattoo inks on the Danish market was investigated (DEPA 2012). The content of copper in the various colours of ink is listed in the table below. The measurements were performed without filtration using ICP/MS, thus assuming that the colourant particles were evenly suspended both dissolved and non-dissolved Cu were measured. In the ICP the samples are transformed into plasma at up to 10 000°C, thus definitely no distinction between soluble and insoluble is possible.

Table 117**.** The content of copper (Cu) in the various colours of inks (DEPA 2012)

Colour of ink	Range of Cu content (ppm = **mg Cu/kg ink** or µg Cu/g ink**)**
Black	0.24 – 3.47
Red	0.17 – 11
Orange	0.64 – 100
Peach	0.68 – 3.4
Violet	0.69 – 1,012
Brown	140
Blue	5,300 – 20,000
Green	1.1 – 17,000
Yellow	0.45 – 13
White	0.52 – 6.3

In 2015 a review on detected metals - including copper - in tattoo inks was performed (JRC, 2015b). In the review result from several analysis performed across Europe was reviewed. The findings are summarised in Table 119. The applied analytical methodology is not mentioned in the reference. However, in the JRC review a distinction was made between soluble copper and total copper in the inks. Considering the percentage (4.5%) it is likely that the method applied, when looking for dissolved copper would also include insoluble copper, like the method applied in DEPA 2012 (DEPA, 2012).

Table 118**.** Copper (Cu) present in tattoo and PMU inks ((JRC, 2015b) p. 62 and p.149).

	CAS no.	**Samples analysed**	**% of samples with contents above 25 ppm**	**Range (min-max) (ppm)**
Soluble Cu	7440-50-8	283	32 (90 in number)	2.5-45,000
Total Cu	7440-50-8	227	31 (71 in number)	0.1-49,500

The CoE ResAP(2008)1, Table 3, recommends a maximum concentration of 25 ppm soluble copper (25 mg Cu/kg ink) in inks for tattoos and PMU. No maximum concentration limit was recommended by CoE ResAP(2003)2. Since background papers for the limit value of the CoE ResAP(2008)1 are not available it is not possible to discuss the reasoning behind the limit value. Moreover, the resolution lacks a definition of 'soluble' Cu in the context of tattoo inks.

The reason for the high contents of copper in green and blue inks is most likely due to the use of green and blue pigments based on the copper ion.

Hazard evaluation

In DEPA 2012, a hazard evaluation of copper was performed. An updated version of the assessment is presented here:

Copper (oxidation state zero) is not classified for health effects according to Annex VI of the CLP Regulation.

Copper flakes (coated with aliphatic acid) has a harmonised classification as Eye Irrit. 2.

Copper chloride is classified with Acute Tox. 4 (H302: Harmful if swallowed). Copper sulphate is classified with Acute Tox. 4 (H302: Harmful if swallowed), Skin Irrit. 2 (H315: Causes skin irritation) and Eye Irrit. 2 (H319: Causes serious eye irritation).

Note that a proposal from France for harmonised classification as Eye Irrit. 2, H319, Aquatic Chronic 2, H411 of granulated copper was out on public consultation until 19 May 2017. Even though a harmonised classification as Eye Irrit. might be relevant for the dossier, until the harmonised classification is adopted it will not be applied in this dossier.

Copper (metallic) or copper compounds have not been evaluated by IARC.

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

In an opinion by the Scientific Committee on Food (SCF, 2003) the Tolerable Upper Intake Level of Copper has been evaluated, the SCF conclude: Liver damage in children appears to be restricted to children with a predisposition for enhanced copper toxicity. The Tolerable Upper Intake Level for Copper is 4 mg/day. Available studies show that the mean copper intakes of adults in EU countries are below the Tolerable Upper Intake Level. The 97.5 percentile of total copper intakes for all age groups are close to the Tolerable Upper Intake Level, which, in the view of the Committee, are not a matter of concern.

The WHO evaluated copper in their "Copper in Drinking-water, Background document for development of WHO Guidelines for Drinking-water Quality (2004)" (WHO, 2004). WHO concludes with a guideline value of 2 mg/l to be protective against the adverse gastrointestinal effects of copper and to provide an adequate margin of safety in populations with normal copper homeostasis. Two mg/l equals a mean total copper intake of 2.2 mg/day (95^{th} percentile would be 5.6 mg), if assuming a bw of 60 kg and a water intake of 1.1 l/d (or with the 95^{th} percentile 2.8 l/d) (US EPA, 2011).

It should be noted that in a REACH registration dossier for copper (ECHA, 2017e) DNELs for the General Population was developed:

Inhalation: 1 mg/m³

Dermal, acute short term: 273 mg/kg bw/day

Oral, RDT: 0.041 mg/kg bw/day

The latter oral DNEL is based on a 90-day oral repeated dose toxicity (rats) National technical program (NTP) study with copper sulphate pentahydrate (NTP, 1993), where a NOAEL of 16.7 mg Cu^{2+}/kg bw/day based on an oral absorption factor of 25% and an assessment factor (AF) of 100 were applied.

Further, the effects described in the CSR for metallic Copper (CAS nr. 7440-50-8) are: "Forestomach lesions consisted of hyperplasia of the squamous mucosa of the limiting ridge at the junction of the forestomach and glandular stomach. Hepatic changes consisted of histopathological changes (chronic active inflammation) plus significant alterations in several clinical chemistry parameters. Hepatic changes appeared most pronounced in males. Renal toxicity consisted of histopathological changes (increase in cytoplasmic droplets), together with significant alterations in some urinary parameters"

However, the NOAEL setting and the derivation of DNELs in the registrations dossier can not be applied without in depth scrutiny. A single 90d study in rats does in the opinion of the Dossier Submitter not add much to the weight of evidence analysis.

Both SCF and WHO are considered very good sources for data to base the limit values on in this case since their assessment is based on studies in humans.

However, the DNEL from the registered NTP study is almost the same as the SCF Tolerable Upper Intake Level (0.041 mg/kg/d vs. 4 mg, i.e. 0.067 mg/kg/d if assuming a bw of 60 mg). Also the forestomach effects etc. in the 90 d study could be assumed to not be relevant to humans, see ECHA guidance R.8, p. 381 (ECHA, 2012).

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

A justification for a classification of copper sulphate as skin irritant has not been provided. The irritant effects might be caused by the solid copper salts upon contact with moisture or by stock solutions with low pH, thus having limited relevance for copper as impurity in tattoo inks. However, further scrutiny and probably research is needed to draw firm conclusions on the irritant effect.

Overall concerning irritation, the data seems to be extremely limited for all other Cu substances than copper sulphate ($CuSO_4$). In order to quantitatively derive DNELs for irritation dose-reponse information is needed, as described in ECHA Guidance R.8 Appendix

R.8-9 (p 109 in the guidance) (ECHA, 2012). According to the Dossier Submitter this information is not available.

Based on the data on skin irritation as reflected in the risk assessment report (V-RAR 2007), and the considerations described here it is considered that even if irritation were considered a critical effect a DNEL for this effect a limit value on this effect could not be derived.

As a classified irritant, copper sulphate ($CuSO_4$) would according to the proposal for restriction in this dossier specifically be limited via the restriction for irritants. For the non-classified Cu compounds it seems relevant to base the restriction on the recommendation given by SCF and WHO.

Based on the above, in order to provide an adequate margin of safety in populations with normal copper homeostasis a tolerable daily intake (TDI) of 2.2 mg/day (for 60 kg bw) is applied, i.e. 0.037 mg Cu/kg bw/d.

Risk evaluation

A safe concentration level for copper is calculated based on the exposure scenario and an adequate margin of safety in populations with normal copper homeostasis corresponding to a TDI of 2.2 mg Cu/day (for 60 kg bw) or 0.037 mg Cu/kg bw/day.

The mean total copper intake is 2.2 mg/day. This can be compared directly to the exposure from tattoo inks assuming 100% uptake for the exposure via subdermal injections.

Based on the exposure scenario an amount of 4308 mg ink/day is applied.

The maximum allowed content in the ink would thus be:

2.2 mg Cu/day / 4,308 mg ink/day x 100% = 0.05% of soluble Cu in the ink. This corresponds to 500 ppm.

Further, in the data collected by JRC (JRC, 2015b) the concentration of copper was found to be up to 5%. In DEPA (2012) concentrations up to 2% has been found.

This would lead to RCRs of 100 and 40, respectively.

Conclusion

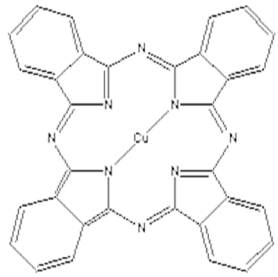
For dissolved copper the RCR seam to be between 100 and 40 assuming that the analytical methods applied have measured the dissolved fraction.

The maximum allowed content in the ink is calculated to be 0.05% soluble Cu. This corresponds to 500 ppm soluble copper.

Appendix B.9. Risk assessment of phthalocyanine

Phthalocyanine used in tattoo inks

According to the JRC (JRC, 2015b), phthalocyanines are macrocyclic compounds having four pyrrole-like subunits linked to form a 16-membered ring in their structure forming coloured complexes with various metals. In the case of copper as the centre metal, several intensely blue or green coloured complexes are formed. These are used in tattoo inks.



Chemical structure of Pigment Blue 15.

In DEPA 2012, the contents of phthalocyanine in tattoo inks were investigated since a high content of copper was assumed to relate to the use of phthalocyanines. The content of phthalocyanine in three green inks, two violet inks and one blue ink was verified – all inks with high contents of copper.

Further, a correlation between the darkness of the ink and the content of copper was found. The highest content of copper was found in the dark blue inks.

On the basis of the information on the labels and from the safety data sheets, 4 colours were found to contain Phthalocyanine Blue 15:3 (Pigment Blue 15), and simultaneously a high content of copper was found by chemical analysis. The content of copper was used to estimate the content of Phthalocyanine Blue 15:3 in these inks. The calculation was carried out by using the relationship between the molecular weights for copper (63.5 g/mol) and Phthalocyanine Blue 15:3 (576.1 g/mol).

Table 119**.** Calculation of content of Phthalocyanine Blue 15:3 in selected inks

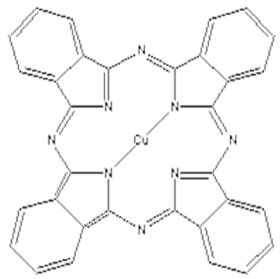
Colour of the ink	Content of Cu µg/g	Calculated content of Phthalocyanine Blue 15:3 µg/g	Percentage by weight % w/w
Dark green	12,300	112,000	11.2
Blue	19,200	174,000	17.4
Blue	20,800	189,000	18.9
Pale blue	5,130	46,500	4.65

This indicates concentrations of 4 to 19%. However, in case the colourant particles have not been evenly suspended, the concentration can both be higher and lower.

Blue and green colourants in tattoo inks

Phthalocyanines

According to the JRC (JRC, 2015b), phthalocyanines are macrocyclic compounds having four pyrrole-like subunits linked to form a 16-membered ring in their structure forming coloured complexes with various metals. In the case of copper as the centre metal, several intensely blue or green coloured complexes are formed. These are used in tattoo inks (see Appendix B.9. Risk assessment of phthalocyanine).



Chemical structure of Pigment Blue 15.

Blue colourants in tattoo inks

In the JRC report (JRC, 2015b), eight blue colourants were identified that seem to be in use, 7 and 5 in tattoo and PMU inks, respectively. See table below.

Table 120. Blue colourants used in tattoo and PMU inks (JRC, 2015b).

Name	**Colour Index (CI) Constitution number**	**CAS number**	**Chemical class**	**Use in tattoo inks**	**Use in PMU inks**
Pigment Blue 15 (PB 15)	74160	147-14-8	Phthalocyanine	X	X
Pigment Blue 17 (PB 17)	74180 (74200)	71799-04-7	Phthalocyanine	X	
Direct Blue 86 (DB 86)	74180	1330-38-7	Phthalocyanine	X	
Pigment Blue 27 (PB 27) (Ferric ammonium ferrocyanide or Prussian blue)	77510	12240-15-2	Inorganic	X	X
Pigment Blue 29 (PB 29) (Lazurite or Ultramarine Blue)	77007	57455-37-5	Inorganic	X	X
Acid Blue 9 (AB 9) (Benzenemethanaminium)	42090	2650-18-2	Triarylmethane	X	X
Pigment Blue 25 (PB 25)	21180	10127-03-4	Diazo	X	
Y Pigment Blue 60 (YPB 60) 6,15-dihydroanthrazine-5,9,14,18-tetrone)	69800	81-77-6	Anthraquinone		X

The regulation of the blue inks in the Cosmetic Product Regulation is described in the following paragraphs and summarized in Table 123.

According to the listing in Annex II of the Cosmetic Product Regulation (entry #1367), Pigment Blue 15 or Pigment Blue 15:1 ((29H,31H-Phthalocyaninato(2-)-N29,N30,N31,N32)copper) is not allowed in cosmetics products when used as a substance in hair dye products. However, it is allowed as a cosmetic colourant in cosmetic products in general according to the listing in Annex IV (entry #105).

According to industry (JRC, 2015b), there is no better alternative to Pigment Blue 15 because the possible substitutes do not result in the same colour brilliance or they have greyish tones when blended with white pigment.

Pigment blue 17 (CAS no. 71799-04-7, CI 74180:1) is not on any lists in the Cosmetic Product Regulation and therefore not included in the CosIng database. Pigment Blue 17 is thus not included on the positive list for colourants on Annex IV and thus not allowed in cosmetics, neither in general nor as a colourant. However, there is no information why it is not allowed in cosmetics. It might thus simply be because nobody prepared a dossier for evaluation by SCCS. Thus the pigment not being allowed in cosmetic cannot alone be used as an argument for restriction in tattoo inks. A risk assessment would be required.

However, also note that for Pigment Blue 17 it is not possible to find a unique Colour Index Constitution Number, as sometimes it was referred to with the same number as the one for Direct Blue 86 (CI 74180) and other times with a different number (CI 74200). According to information in (JRC, 2015b), Pigment Blue 17 is included in Annex IV with a permitted use in rinse off only products. It is unclear why CAS no 71799-04-7 is not included in the CosIng database, as long as it is being associated with CI 74180 (Annex IV entry #106).

Direct Blue 86 (CAS no. 1330-38-7, CI 74180) is listed in Annex II of the Cosmetic Product Regulation (entry # 1368), which prohibits the use in cosmetic products when used as a substance in hair dye products, but it is allowed as a "cosmetic colourant" in rinse-off products (Annex IV entry # 106). Note that according to the CosIng database the CI number 74180 corresponds to both CAS number 1330-38-7 and 1328-51-4.

Pigment Blue 27 (Ferric Ammonium Ferrocyanide) (CAS no. 12240-15-2, CI 77510) is an iron based inorganic salt. It is allowed as a cosmetic colourant according to Annex IV (entry # 138), when free from cyanide ions. According to the CosIng database the chemical composition of Pigment Blue 27 (Prussian Blue) includes the following three substances, CAS no. 12240-15-2, 14038-43-8 and 25869-00-5, all with the same CI number (CI 77510).

Pigment Blue 29 (CAS no. 57455-37-5, CI 77007) is listed as a cosmetic colourant in Annex IV (entry # 120). Note that according to the CosIng database the CI number 77007 also corresponds to CAS numbers 12769-96-9 and 1302-83-6. Annex IV entry # 120 also includes the following CAS no. 1317-97-1, 1345-00-2, 11118-33-5 and 12703-661.

Acid Blue 9 is listed in Annex III (entry # 190) of the Cosmetic Product Regulation with a restricted use in non-oxidative hair dye products (0.5%) The restriction in Annex III refers to CAS no. 3844-45-9, 2650-18-2 and 68921-42-6. Acid Blue 9 (CAS no. 2650-18-2, CI 42090) is also listed as a cosmetic colourant in Annex IV with purity criteria as set out in Commission Directive 95/45/EC (E 133) (entry # 63). According to the CosIng database the CI number 42090 also corresponds to CAS no. 3844-45-9, 68921-42-6, 37307-56-5 and 6371-85-3.

Note that the Annex III does not contain pigments besides the hair dyes and that the pigments will be removed from Annex III and moved to annex IV once the evaluation of all hair dyes for which a dossier has been submitted has been finished by SCCS.

Y Pigment Blue 60 (6,15-Dihydroanthrazine- 5,9,14,18-tetrone) (CAS no. 81-77-6, CI 69800) is allowed as a cosmetic colourant in all cosmetic products according to Annex IV entry # 95.

Pigment Blue 25 (CAS no.10127-03-4, CI 21180) is not included in Annex IV and is thus not allowed to use as a cosmetic colourant.

Green colourants in tattoo inks

JRC et al. (JRC, 2015b) reports that five green colourants are used as ingredients in PMU inks and four in the tattoo inks. Two of them are phthalocyanines.

Table 121. List of green colourants used in tattoo and PMU inks (JRC, 2015b).

Name	Colour index Constitution Number	CAS number	Chemical class	Use in tattoo inks	Use in PMU inks
Pigment Green 7	74260	1328-53-6	phthalocyanine	X	X
Pigment Green 36	74265	14302-13-7	phthalocyanine	X	X
Pigment Green 17	77288	58591-12-1 and 1333-82-0	inorganic pigments	X	X
Pigment Green 18 (Chromium Hydroxide Green, Viridian 3B)	77289	12001-99-9	inorganic pigments		X
Acid Green 25	61570	4403-90-1	anthraquinone	X	X

The regulation of the green inks in the Cosmetic Product Regulation is described in the following paragraphs and summarized in Table 123.

The CoE ResAP(2008)1 recommends not to use Pigment Green 7. Pigment Green 7 (CAS no. 1328-53-6, CI 74260) is listed in Annex II of the Cosmetic Product Regulation (entry # 1369), which prohibits the use in cosmetics, when used as a substance in hair dye products, but it is allowed as a cosmetic colourant according to Annex IV (# 107) in other cosmetic products except eye products.

According to JRC et al. (JRC, 2015b), Pigment Green 36 (CAS no. 14302-13-7, CI 74265) has been used on the market to substitute Pigment Green 7. The chemical structures of those colourants are very similar, the only difference being the substitution of 6 chlorine atoms with 5 bromine ones in Pigment Green 7.

Pigment Green 36 is not included in Annex IV and is thus not allowed as a colourant in general in cosmetic products.

According to information in reported by JRC (JRC 2015b), pigment Green 17 (CAS no. 58591-12-1 and 1333-82-0, CI 77288) is listed as a cosmetic colourant in Annex IV (entry # 129, Chromium (III) oxide). However, according to the CosIng database, the CI no. 77288 only corresponds to CAS no. 1308-38-9. Thus pigment green 17 (CAS no. 58591-12-1 and 1333-82-0) is not included among the cosmetic colourants (CPR Annex IV substances) dealt with in this restriction proposal.

Pigment Green 18 (CAS no. 12001-99-9, CI 77289) is listed on Annex IV (entry # 130, Chromium (III) hydroxide)) as a cosmetic colourant and can thus be used as colourant in cosmetics products. Conditions for use are: "free from chromate ion". According to the CosIng database the CI number 77289 corresponds to both CAS no 1308-14-1 and 12001-99-9.

Acid Green 25 (CAS no. 4403-90-1, CI 61570) is listed on Annex III (nr. 290) in the Cosmetic Product Regulation and a limit value of 0.3% has been established for use in non-oxidative hair dye products. The substance is also listed on Annex IV (entry # 92) and thus it is allowed for use as a colourant.

According to the JRC (JRC, 2015b), the green inorganic chromium oxide pigments, both chromium oxide (Cr_2O_3) and chromium oxide hydrate ($Cr_2O_3 \bullet 3H_2O$), can usually only be found in PMU inks. These pigments might contain chromium VI.

Regulation in cosmetic products for both blue and green colourants

The regulation in cosmetic products for both blue and green pigments has been summarised in the table below.

Table 122. Blue and green pigments used in tattoo and PMU inks and their regulation in the Cosmetic Product Regulation (CPR). Phthalocyanines are marked with light blue

Name of pigment	CAS numbers	Annex II	Annex III, including limit values	Annex IV: allowed as colourants in all cosmetic products
Pigment Blue 15 (PB 15)	147-14-8	X (entry # 1367 - when used as a substance in hair dye products)		X (entry # 105)
Pigment Blue 17 (PB 17)	71799-04-7			Not allowed according to information in the CosIng database
Direct Blue 86 (DB 86)	1330-38-7	X (entry # 1368 - when used as a substance in hair dye products)		X (entry # 106 – rinse-off products only – column g)
Pigment Blue 27 (PB 27)	12240-15-2			X (entry # 138 – free from vyanide ions – column i)
Pigment Blue 29 (PB 29)	57455-37-5			X (entry # 120 – column)
Acid Blue 9 (AB 9)	2650 18-2		X (entry # 190 – hair dye substances in non-oxidative hair dye products - column f and 0,5% max. concentration in ready for use - column g)	X (entry # 63 purity criteria as set out in Commission Directive 95/45/EC (E 133) - column i)
Pigment Blue 25 (PB 25)	10127-03-4			Not allowed
Y Pigment Blue 60 (YPB 60)	81-77-6			X (entry # 95)

Pigment Green 7 (PG 7)	1328-53-6	X (entry 1369 - when used as a substance in hair dye products)		X (entry # 107 – not to be used in eye products – coloumn g)
Pigment Green 36 (PG 36)	14302-13-7			Not allowed
Pigment Green 17 (PG 17)	58591-12-1 and 1333-82-0			Not allowed according to information in the CosIng database
Pigment Green 18 (PG 18)	12001-99-9			X (entry # 130 free from chromate ion – column i)
Acid Green 25 (AG 25)	4403-90-1		X (entry # 290 – hair dye substances in non-oxidative hair dye products (column f) – max. concentration in ready for use preparation 0.3% - column g)	X (entry # 92)

Note that some colourants listed in Annex II, and thus banned in all cosmetic products, are listed only because industry did not submit a dossier for their evaluation as colourant in hair dyes. In these cases no risk has been demonstrated (see Commission Directive 2008/88/EC). If the colourants are listed in Annex IV these colourants are allowed in cosmetic products not intended to colour hair. Substances listed in Annex III are subject to restrictions (with indication of limit values).

Note that for pigments neither listed in Annex IV nor Annex II, they are not allowed in cosmetic products in general unless they have another function than being colourants. In this analysis the pigments are not assumed to have other functions. However, it may also be that there is no information why it is not allowed in cosmetics. It might thus simply be because nobody prepared a dossier for evaluation by SCCS. Thus the pigment not being allowed in cosmetic cannot alone be used as an argument for restriction in tattoo inks. A risk assessment would be required.

For pigments listed on both Annex II and IV the only use allowed is as colourant except for in hair dyes.

For pigments only listed on Annex IV and not on Annex II, which is the case for most colourants, their use is both as a colourant and for other functions if relevant.

For some of the pigments the CI numbers apparently could refer to more than one CAS number.

Hazard evaluation for phthalocyanine

In DEPA 2012, a hazard assessment of phthalocyanine was performed. An updated version is given here.

Phthalocyanines form complexes with most of the elements in the periodic table. In general, all the complexes are of very low solubility in most solvents, including water. The various phthalocyanines are assumed to have similar toxicity profiles.

The phthalocyanine Pigment Blue 15 (CAS No. 147-14-8) has been evaluated in the OECD SIDS program (OECD SIDS, 1997). Further, Pigment Blue 15 is reviewed in a REACH registration dossier (ECHA, 2017d).

The most relevant data in relation to tattooing are summarised here:

The pigment is insoluble in water and stable in most solutions, i.e. it does not dissociate to the phthalocyanine anion and copper ions to a considerable degree in aqueous solution.

In rats, a reduced number of red blood cells was observed after oral administration of the pigment colourant by gavage (1000 mg/kg bw) daily for 28 days. The NOAEL was tentatively established at 200 mg/kg bw per day (ECHA, 2009).

In the 28-day study detailed data is not available (it is a Japanese study where only summary is in English). The hematological changes are described as slight. According to the Guidance on the application of the CLP criteria (ECHA, 2017f) (section 3.9.2.5.2), slight hematological changes which are not accompanied by any other adverse symptoms are considered not to be toxicologically relevant.

In rats and mice, no effects were seen after administration of the pigment in the feed (0.3 to 5%) for 90-days (ECHA, 1979).

Further, no tumours were observed in mice given the pigment for 8 months. No genotoxic effects were observed in a variety of tests.

In rats, no effects on fertility and no effects in offspring were observed after oral administration of the pigment by gavage (0, 40, 200, 1000 mg/kg bw) daily for 42 days (males) and from 14 days before mating to 3 days after giving birth (females).

A NOAEL was tentatively established at 1000 mg/kg bw/day for offspring as well as for the parents.

The critical effect of phthalocyanine after repeated exposure over a prolonged time period is considered to be the decreased number of red blood cells.

However, in the 90-day study, doses up to 4500 mg/kg bw/d were administered. In the 90-day study, no adverse treatment related effects were observed (neither macroscopically nor microscopically). Therefore, clinical chemistry parameters were not tested.

It should also be noted that report, the magnitude of the decrease in the number of red blood cells in exposed animals compared to controls is not presented. Therefore, it cannot be evaluated whether the decrease is statistically and biologically significantly different compared to the control group.

Thus, the available data do not allow any conclusion on repeated dose toxicity. A derivation of a valid DNEL is thus judged by the Dossier Submitter not to be possible.

Laser treatment of phthalocyanine has shown to form 1,2-benzene dicarbonitrile, benzonitrile, benzene, and hydrogen cyanide (Schreiver, et al., 2015a). However, since there are many uncertainties related with laser treatment, which has not been investigated these consideration has not been included in this evaluation.

The main use of copper in tattoo inks are in the phthalocyanine colourants. However, the hazards of the phthalocyanine colourants are not related to the content of copper or the release of copper ions.

Conclusion

Since a DNEL value could not be established based on the current available information it is not possible to assess any risk from the use of phthalocyanine in tattoo inks.

Appendix B.10. Risk assessment of lead (Pb)

1. Introduction

Lead (Pb) has been used by humans for at least 7000 years, because it is easy to extract and work with and widespread. It has found major uses in pipes and plumbing, pigments and paints, gasoline additives, construction materials and lead-acid batteries. Some of its uses have resulted in substantial introductions of lead into environment and human exposure, and are being phased out in many countries (Klaasen, 2013).

As a result of anthropogenic activity, lead can enter the environment at any stage from its mining to its final use, including during recycling, and it contaminates crops, soil, water, food, air and dust. Once lead is introduced, it persists. The important routes of human exposure from these sources are inhalation or ingestion.

Metallic lead (Pb^0) is resistant to corrosion; it is attacked (oxidised) only superficially by air, forming a thin layer of lead oxide that protects it from further oxidation. The metal is not attacked by sulfuric or hydrochloric acids. The two major groups of lead compounds that are important toxicologically include inorganic and organic lead compounds. The common inorganic compounds include lead arsenate, lead carbonate, lead chromate, lead nitrate, lead monoxide, lead dioxide, lead trioxide, lead tetraoxide, lead phosphate, lead sulphate, and lead sulphide. Compounds of lead exist in two main oxidation states: +2 and +4; the former is more common. Inorganic lead(IV) compounds are typically strong oxidants or exist only in highly acidic solutions. PbO is representative of lead's +2 oxidation state. It is soluble in nitric and acetic acids, from which solutions it is possible to precipitate halide, sulfate, chromate, carbonate ($PbCO_3$), and basic carbonate ($Pb_3(OH)_2(CO_3)_2$) salts of lead. The sulfide can also be precipitated from acetate solutions. These salts are all poorly soluble in water. Among the halides, the iodide is less soluble than the bromide, which, in turn, is less soluble than the chloride. Organolead compounds are dominated by Pb^{4+} (IARC, 2006).

Lead compounds have historically been used in various pigments, such as those with red and yellow colour. Lead has been detected in a wide range of tattoo inks, as set out in a number of recent reports (JRC, 2015b), (DEPA, 2012a), mainly present as an impurity in the pigments used.

Under Council of Europe Resolution ResAP(2008) (described elsewhere within the restriction proposal) (CoE, 2008), the maximum allowed concentration of lead as an impurity in products for tattoos and permanent make-up is 2 ppm. While this limit is reflected in the legislation of those member states that have national legislation based on ResAP(2008), it is not necessarily reflected in the legislation of member states that have instead based their legislation on the previous resolution ResAP (2003), which does not include this limit[37]. Furthermore, other member states do not have legislation based on either of these resolutions.

Lead has been detected in tattoo inks at levels exceeding the concentration limit in ResAP(2008)1 in several cases. Furthermore, there is also a need to revisit the concentration

[37] There is a variety of approaches adopted across the EU, with some member states having legislation (or draft legislation) based on ResAP(2008)1, some having legislation based on ResAP(2003)2 and others having separate national provisions or simply reference to REACH, CLP and the GPSD (JRC, 2015a). ResAP(2003)2 does not include the maximum allowed concentrations of impurities in products for tattoos and PMU (table 3) which is included in ResAP(2008)1. Therefore those member states basing their legislation on ResAP(2008)1 will generally have concentration limits for lead in tattoo inks while others generally will not.

limit in ResAP(2008)1, in the context of current knowledge about the hazards of lead compounds, and of potential exposure through tattoo inks.

Lead and its compounds are included in the list of substances prohibited in cosmetic products under Annex II of the cosmetic products regulation (CPR, EC No 1223/2009) (EC, 2009). A restriction on CPR Annex II substances is considered elsewhere within the restriction dossier.

2. Classification

2.1 Classification and Labelling in Annex VI to Regulation (EC) 1272/2008 (CLP Regulation) (ECHA, 2011b)

Several lead compounds are classified in the CLP Regulation (Annex VI in Regulation 1272/2008/EC of the European Parliament and of the Council and on Commission Regulation 790/2009/EC).

The classification of lead compounds depends of the intrinsic properties of the lead cation as well as the intrinsic properties of the anion of the compound. There are several harmonised classifications for lead compounds according to Annex VI to CLP Regulation, under the entry "lead compounds with the exception of those specified elsewhere in this Annex (Index No 082-001-00-6)" (ECHA, n.d.), (ECHA, n.d.).

A proposal for harmonised classification of metallic lead has been submitted to ECHA in 2012 (ECHA, 2012b), on which the scientific opinion of ECHA's Committee for Risk Assessment concluded that (i) all physical forms of metallic lead should be classified as Repr. 1A-H360DF (May damage fertility; May damage the unborn child) similar to the classification that applies for "lead and lead compounds"; (ii) According to the criteria in the CLP Guidance (3.7.2.5) (ECHA, 2017f), the generic concentration limit would underestimate the hazard therefore the metallic lead should be assigned a specific concentration limit of 0.03% for developmental toxicity (H360D, C ≥ 0.03%).

Table 124 summarises the existing classification of lead and inorganic lead salts under the CLP Regulation. Several lead substances are identified as substances of very high concern (SVHCs) and are included in the Candidate List therefore subject to authorisation under the REACH Regulation.

Table 123: Classification of lead and its inorganic salts in Table 3.1 under the EC Regulation 1272/2008 (CLP).

Substance	**EC number**	**CAS Number**	**Classification**	
			Hazard class and category code(s)	Hazard satatement code(s)
Lead hexafluorosilicate	247-278-1	25808-74-6	Repr. 1A Acute Tox. 4 * Acute Tox. 4 * STOT RE 2 * Aquatic Acute 1 Aquatic Chronic 1	H360-Df H332 H302 H373** H400 H410
Lead compounds with the exception of those specified elsewhere in this Annex	-	-	Repr. 1A Acute Tox. 4 * Acute Tox. 4 * STOT RE 2 * Aquatic Acute 1 Aquatic Chronic 1	H360-Df H332 H302 H373** H400 H410
Lead diazide lead azide	236-542-1	13424-46-9	Unst. Expl. Repr. 1A Acute Tox. 4 * Acute Tox. 4 * STOT RE 2 * Aquatic Acute 1 Aquatic Chronic 1	H200 H360-Df H332 H302 H373** H400 H410
Lead diazide; lead azide [≥ 20 % phlegmatiser]	236-542-1	13424-46-9	Expl. 1.1 Repr. 1A Acute Tox. 4 * Acute Tox. 4 * STOT RE 2 * Aquatic Acute 1 Aquatic Chronic 1	H201 H360-Df H332 H302 H373** H400 H410
Lead chromate[#]	231-846-0	7758-97-6	Carc. 1B Repr. 1A STOT RE 2	H350 H360-Df H373**

			Aquatic Acute 1 Aquatic Chronic 1	H400 H410
Trilead bis(orthophosphate)	231-205-5	7446-27-7	Repr. 1A STOT RE 2 * Aquatic Acute 1 Aquatic Chronic 1	H360-Df H373** H400 H410
Lead sulfochromate yellow#; C.I. Pigment Yellow 34; [This substance is identified in the Colour Index by Colour Index Constitution Number, C.I. 77603.]	215-693-7	1344-37-2	Carc. 1B Repr. 1A STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H350 H360-Df H373** H400 H410
Lead chromate molybdate sulfate red#; C.I. Pigment Red 104; [This substance is identified in the Colour Index by Colour Index Constitution Number, C.I. 77605.]	235-759-9	12656-85-8	Carc. 1B Repr. 1A STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H350 H360-Df H373** H400 H410
Lead hydrogen arsenate#	232-064-2	7784-40-9	Carc. 1A Repr. 1A Acute Tox. 3 * Acute Tox. 3 * STOT RE 2 * Aquatic Acute 1 Aquatic Chronic 1	H350 H360-Df H331 H301 H373** H400 H410

Notes:

- '*' indicates that the classification corresponds to the minimum classification for a category;
- for certain hazard classes, e.g. STOT, the route of exposure should be indicated in the hazard statement only if it is conclusively proven that no other route of exposure can cause the hazard in accordance to the criteria in Annex I. Under Directive 67/548/EEC the route of exposure is indicated for classifications with R48 when there was data justifying the classification for this route of exposure.
- The classification under 67/548/EEC indicating the route of exposure has been translated into the corresponding class and category according to this Regulation, but with a general hazard statement not specifying the route of exposure as the necessary information is not available. These hazard statements are indicated by the reference '**'.
- '#' denotes an inclusion in the Candidate List of SVHC for Authorisation.

The most critical harmonised classifications for lead compounds in general are:

- Repr. 1A, H360Df (May damage the unborn child. Suspected of damaging fertility);
- STOT RE 2 * H373 (May cause damage to organs through prolonged or repeated exposure);
- Acute tox. 4 * H302 (Harmful if swallowed);
- Acute tox. 4 * H332 (Harmful if inhaled);
- Aquatic Acute 1, H400 (Very toxic to aquatic life);
- Aquatic Chronic 1, H410 (Very toxic to aquatic life with long lasting effects).

3. Hazard assessment

The health effects of various lead compounds have been reported by ECHA's Committee for Risk Assessment (RAC) in the context of proposed restrictions. The RAC opinion on lead and lead compounds in jewellery from 2011 and the RAC oppinion on lead and its compounds in articles intended for consumer use from 2013 both focus on developmental neurotoxicity as the critical effect. The background documents to the opinions also include reviews of:

- Toxicokinetics – ADME (absorption, distribution, metabolism and excretion)
- Acute toxicity
- Irritation and corrosion
- Sensitisation
- Specific target organ toxicity / repeated dose toxicity manifested in hematological effects, renal effects and effects on the central nervous system
- Mutagenicity
- Carcinogenicity
- Reproductive toxicity

3.1 Toxicokinetics of inorganic lead (IARC, 2006)

The oral and the inhalation routes are the most significant routes of exposure to lead, whereas dermal absorption is considered as minimal.

Lead absorption from the gastrointestinal tract in both humans and experimental animals is strongly influenced by age (neonates and the young absorb a larger fraction than adults), fasting/fed status (fasting humans and experimental animals absorb much larger fractions than their fed counterparts), nutrition (fat and caloric intakes; phosphorus, copper, zinc and especially iron and calcium status, all affect lead absorption), solubility (soluble lead compounds are better absorbed) and particle size (in controlled studies in rats, lead absorption from ingested mining wastes was shown to be inversely proportional to particle size). There are no data indicating that the fraction of lead absorbed from an inhalation exposure is dependent on the amount of lead in the lung. Patterns and rates of particle deposition are highly dependent on particle size and ventilation rate, but all lead deposited deep in the lung is eventually absorbed. Limited studies indicate that dermal absorption of inorganic lead is negligible, although slightly increased by high perspiration rates in humans.

In both humans and experimental animals, absorbed lead is rapidly distributed from blood plasma simultaneously into erythrocytes, soft tissues, and bone. The half-life of lead in blood and soft tissues is 20–30 days in adult humans and 3–5 days in adult rats. In both humans and rats, the soft-tissue concentrations of lead are highest in liver and kidney and much lower in brain. Plasma, rather than whole blood, is generally accepted as the source of lead available for distribution and excretion, although plasma lead comprises only 0.2–0.3% of whole blood lead concentrations when these are < 6 mg/dL. The fraction of whole blood lead in plasma is substantially larger at high blood lead concentrations than at low blood lead concentrations.

The majority of lead is stored in bone (in adults > 90%) and is partitioned mainly into trabecular and cortical bone. The higher rate of remodelling in trabecular bone is reflected in a shorter halflife of lead in trabecular bone (2–8 years) compared with that in cortical bone (> 20 years). Bone can be a significant source of endogenous lead, in particular when the bone resorption rate is increased, such as during pregnancy, lactation, the period just after menopause, and during weightlessness.

After oral ingestion, inorganic lead that has not been absorbed in the gastrointestinal tract is excreted in the faeces. Absorbed lead is excreted in the urine and, via the bile, in the faeces. Excretion of lead through sweat is of minor importance.

3.2 Toxic effects of inorganic lead

Typical clinical manifestations of lead poisoning include weakness, irritability, asthenia, nausea, abdominal pain with constipation, and anaemia.

Lead interferes with numerous physiological processes. In the haeme biosynthetic pathway, it inhibits δ-aminolevulinic acid dehydratase (also known as porphobilinogen synthase), probably through its high affinity for the zinc-binding site in the enzyme. Although lead displaces zinc more readily in one of the alloenzymes of the protein, the relationship between δ-aminolevulinic acid dehydratase genotype and sensitivity to lead at different blood lead concentrations is at present unclear. Lead also causes an increase in zinc protoporphyrin, by a mechanism which is not fully established. Lead inhibits pyrimidine-5’ nucleotidase, resulting in accumulation of nucleotides, and subsequent haemolysis and anaemia.

Renal manifestations of acute lead poisoning include glycosuria, aminoaciduria and phosphaturia. Chronic exposure to low concentrations of lead is associated with increased urinary excretion of low-molecular-weight proteins and lysosomal enzymes. Chronic exposure to high concentrations of lead results in interstitial fibrosis, glomerular sclerosis, tubular dysfunction and, ultimately, in chronic renal failure. Lead has also been implicated in the development of hypertension secondary to nephropathy.

A considerable body of evidence suggests that children are more sensitive than adults to the neurotoxic properties of lead. Although clinical symptoms of toxicity generally become apparent at blood lead concentrations of 70 µg/dL, many important disturbances occur at much lower concentrations. These include electrophysiological anomalies of evoked brain potential in response to auditory stimuli and reduced peripheral nerve conduction. Both cross-sectional and prospective studies of children have found impairments in cognition, attention, and language function at concentrations of lead previously thought to be harmless. In studies with larger samples, better measures of lead burden and neurobehavioural function, and more advanced statistical techniques, effects are detectable at blood lead concentrations below 10 µg/dL. The relative effect is greater below 10 µg/dL than above this level. Recently, attention has shifted from the impact of lead on cognition to its effects on behaviour. Exposure to lead has been found to be associated with attentional dysfunction, aggression and delinquency.

Exposure to lead is associated with cardiovascular effects and with changes in endocrine and immune functions.

Many of the effects of lead exposure in humans have been confirmed in experimental systems. At the cellular level, lead has mitogenic properties; it affects various regulatory proteins, including those that depend on the presence of zinc.

Studies on the reproductive and developmental toxicity of lead did not show consistent effects, morphologically or quantitatively, on markers of male fertility. It is not clear whether the effects are caused by a direct interaction of lead with the reproductive organs, or by modulation of the endocrine control of reproduction, or both.

There is consistent evidence in humans, in the form of case series and epidemiological studies, that the risk for spontaneous abortion (pregnancy loss before the 20th week of gestation, but after the stage of unrecognized, sub-clinical loss) is increased by maternal exposure to high concentrations of lead.

In humans, prenatal lead exposure is associated with an increased risk for minor malformations, low birth weight and reduced postnatal growth rate. The effect on postnatal growth rate is apparent only in those children with continuing postnatal lead exposure.

Differences in reproductive end-points between species make it unlikely that useful conclusions can be extrapolated from animals to humans.

The lead in blood (or PbB) level is considered as the best biomarker for an exposure to lead. Lead in blood does not necessarily correlate with the total body burden of lead, but this value has the advantage that a wealth of information can be linked to the PbB especially the effects of low exposure on the central nervous system functions in children. PbB level increases when exposure rises and stabilizes after a while. However, RAC considered more appropriate to base the assessment in the recent EFSA opinion (EFSA, 2013).

The following table summarises relevant information on the human health endpoints. Further details of these endpoints can be found in various sources.

Table 124: Compilation of the human health effects of lead exposure (ECHA, 2011a), (ECHA, 2013a), (ECHA, 2017g), (ECHA, 2017h)

Endpoint	**Critical lead exposure levels – human data**
Acute toxicity	Very few data exist on acute poisoning. The US National Institute of Occupational Safety and Health (NIOSH) determined that acute lethal dose for an adult is 21 g (equivalent to 450 mg/kg bw) by oral route, and 21,000 mg/m^3 for 30 minutes by inhalation route. However, the latter kind of poisoning is very rare. Obvious signs of acute lead poisoning involve dullness, restlessness, irritation, poor power of concentration, headache, vibrations in muscles, stomach cramps, kidney injuries, hallucinations and loss of memory. These effects can occur at PbB levels of 800-1000 µg/L in children. US EPA has furthermore identified a LOAEL value of 600-1000 µg/L related to colic in children as a

	result of lead poisoning. Then a LOAEL of 800 µg/L and a NOAEL of 400 µg/L could be identified for acute effects in children. However, due to the long elimination half-life of lead in the body, chronic toxicity is a much greater risk.
Irritation	In general, lead and its compounds can be considered non-irritating. Out of nine animal studies investigating dermal and eye irritation, eight were negative. One rabbit study was positive for dermal irritation caused by lead oxide, but this study can only be found in an undocumented IUCLID entry (lead oxide), for which there is no experimental verification. In humans, no studies were found that document eye-, skin- or respiratory irritation resulting from exposure to lead or its compounds. In conclusion, lead and its compounds should be considered non-irritating.
Corrosivity	No studies were found that document corrosivity to the eye, skin or lung in humans or animals following exposure to lead or its compounds (ECHA, 2008). Thus lead and its compounds should be considered as non-corrosive.
Sensitisation	Animal studies indicate an absence of skin sensitizing potential for lead and its compounds (ECHA, 2008). No human studies were found documenting sensitization to lead or its compounds. In view of the large number of workers that historically have been occupationally exposed to lead and its compounds, the lack of reports on sensitization strongly suggests lead is non-sensitizing in humans.
Repeated dose toxicity	As stated previously, some lead compounds are classified as STOT RE 2 (H373 - May cause damage to organs through prolonged or repeated exposure).

	Lead is a poison by chronic accumulation. Signs of chronic lead poisoning include among others: sleepiness, irritation, headache, pains in the joints and problems related to the stomach- and intestinal system. Chronic exposure to lead can also induce neurological effects such as: uneasiness, forgetfulness, irritation, dullness, headache, tiredness, impotence, decreased libido, dizziness and weakness.
	Haematological effects Effects of lead on blood can be detected at low levels of exposure but are not deemed to be adverse. As exposure intensity increases, the constellation of observed effects becomes increasingly diverse until impacts upon haeme synthesis are observed and which would be considered as adverse. At quite low levels of lead (< 100 µg/L) an inhibition of enzymes such as ALAD implicated in the haeme synthesis is observed. These enzymatic effects are not considered as adverse but are sometimes used as biomarkers of lead exposure. At higher levels of lead exposure, the cumulative impacts of lead upon multiple enzymes in the haeme biosynthetic pathway begin to impact the rate of haeme and haemoglobin production. Decreased haemoglobin production can be observed at blood lead levels above 400 µg/L in children. Impacts on haemoglobin production sufficient to cause anaemia are associated with blood lead levels of 700 µg/L or more .
	Renal effects Kidneys are the target organ of lead: some effects can be observed from a PbB level of 100 µg/L. It seems to be the biological function which is affected at the lowest dose. Colic is a recognized symptom of a lead poisoning, which could occurred at PbB from 1000 µg/L. Effects which are generated by lead on kidneys are the same in animals and in humans, the cells brush border in proximal tubules are affected. These effects could lead to a nephropathy with a

	tubular atrophy. In children, a study has demonstrated the effects of lead poisoning on proximal tubules via an environmental exposure from 30-350 µg/L. Blood lead concentration of 15 µg Pb/L associated with a 10% increase of chronic kidney disease in the population. There is no evidence for a threshold in adults (EFSA, 2013). NOAEL of 60 µg/dL, combined with >5 years of lead exposure (ECHA, n.d.). (EFSA, 2013) considered that there is no threshold for renal effects in adults.
	Effect on blood pressure and cardiovascular effects Blood lead concentration of 36 µg Pb/L associated with a 1% increase in systolic blood pressure. This corresponds to a daily lead exposure of 1.50 µg Pb/kg bw per day (EFSA, 2013). There is no evidence of a threshold in adults (EFSA, 2013). Weak positive association between blood lead concentration and blood pressure in general population with average blood lead concentration below 45 µg/dL (ECHA, n.d.). Potential for a 'societal risk'' as opposed to an 'individual risk'. However, lack of dose-response relationship prevents use of this endpoints within a quantitative risk assessment.
Effects on the central nervous system (CNS) - developmental toxicity	In young children, brain is the primary target organ. At higher blood lead levels, lead can cause other neurotoxic effects, and children are especially vulnerable. When PbB level is above 800 µg/L, an encephalopathy can be observed (characterised by ataxia, coma or convulsions). This condition can be fatal. Lead has an effect on the development and the maturation process of the cognitive functions of children. The central nervous system is still under development well over a decade after birth; therefore the IQ effects in children should be considered a developmental. Lead

	causes IQ deficits in children at very low blood lead levels; under 10 µg/dL and since no safe blood lead level has yet been established, lead should be regarded as a non-threshold toxic substance. If prenatal lead exposure occurs, in most studies no effect is reported if the maternal exposure is below 250 µg/L. Nevertheless it was demonstrated that a PbB level of 100 µg/L could induce effects on endpoints of uncertain significance (e.g. neurological soft signs).
Mutagenicity	Occupational exposure to lead has been shown to be associated with increased mitotic activity in peripheral lymphocytes, increased rate of abnormal mitosis and increased incidence of chromosomal aberrations and sister chromatid exchange. These effects occur at PbB levels ranging from 220 – 890 µg/L. However, these results reporting chromosomal aberrations are contradictory since other studies performed with similar PbB ranges did not demonstrate such effects. Moreover, it has been demonstrated that lead exposure can lower the ability of DNA to repair itself, and is therefore responsible for an increase in DNA damage.
Carcinogenicity	According to (IARC, 2006), most inorganic lead compounds are classified as "potentially cancer-causing in humans" (Group 2A), based on epidemiologic studies in which cancers of the stomach and the lungs were noted (human evidence: limited; evidence in experimental animals: sufficient). Organic lead compounds are not classified as to their cancer-causing ability in humans (group 3 'not classifiable as to their carcinogenicity to humans' - human evidence: inadequate; evidence in experimental animals: inadequate). For the organic lead compounds, it has to be noted that these are metabolised, at least in part, to ionic lead both in humans and animals and may consequently exert the toxicities associated with inorganic lead. According to the CLP-legislation, lead acetate is classified and listed in annex VI as Carc. 2 (H351), since carcinogenic effects have been observed in animal studies (ECHA, 2008).

	In Europe, lead acetate is classified as Carc. 2 (H351), since a carcinogenic effect has been observed in animals only. The Lead Development Association International proposes in its risk assessment to extend this classification to all inorganic lead compounds, since they have a greater bioavailability compared to other lead compounds.
Genotoxicity	Humans occupationally exposed to lead show evidence of genotoxicity as measured in a variety of assays. In some studies, these effects were correlated with blood lead concentrations. However, all the human genotoxicity studies involved co-exposure to other compounds, making it difficult to attribute genetic and other effects to lead alone. In a limited number of studies on non-occupationally exposed individuals, no genotoxic effects were found that were correlated with blood lead concentrations.
Reproductive toxicity - fertility	In humans, there are clear indications that high levels of lead cause adverse effects on both male and female reproductive functions. Less is known concerning reproductive effects following a chronic exposure to low levels. However, if the PbB level is above 200 µg/L, an abortion or still-born baby risk exists and several studies reported that the length of gestation is affected at PbB level of 150 µg/L and above. It was reported in 1999 that the risk of spontaneous abortion nearly doubles for every 5 µg/dL increase in blood lead levels. *Male fertility* Effects on sperm may start to appear at blood lead levels of 400 µg/L. Moreover, a Finnish study has observed a significant increase of the risk of spontaneous abortion among the wives of men whose PbB level was 300 µg/L or higher during spermatogenesis. Cross sectional study of 503 men (UK, Italy and Belgium) indicated a threshold for an effect on semen quality at 45 µg/dL of concurrent blood lead. As blood lead concentrations exceed 50 µg/dL, a progressively greater impact on fertility can be expected. *Female fertility* Effects on female reproduction in animal studies are usually not apparent at the blood lead concentrations that impair male fertility.

	Blood lead concentrations much higher than 50 µg/dL are generally needed to see an adverse effect on female fertility. Human data is inconsistent and effects thresholds cannot be estimated with precision. *Lead poisoning in pregnant women* Since lead can easily cross the placental barrier, the exposure of children starts *in utero* and lasts during the lactation period. PbB level is correlated to the serum calcium: the demineralization of the skeleton observed during pregnancy and lactation induces a migration of the lead accumulated in the mother's bone to the fetus and the infant. This transferred amount of lead is directly linked to lead accumulated by the mother (resulting from a cumulated exposure) rather than to the maternal exposure during pregnancy. The maternal and the fetal PbB levels are quite identical. The teratogenic effects observed in animals were not noted for humans, but it seems that the risk of spontaneous abortions, growth retardation and premature delivery appear when PbB level is above 250 µg/L.

Conclusion

The critical effects were considered to be the effects on the nervous, haematopoietic and reproductive systems, and the carcinogenic effect. It should be noted that the mode of action for the carcinogenic effect is not completely understood and that tumours have only been seen at relatively high doses. The carcinogenic effect of lead is therefore, not considered as a critical effect in relation to tattooing.

The most critical effect of lead at low concentrations was considered to be the effects on the developing nervous system. It is still discussed whether there is a threshold for the effects on the developing nervous system. Therefore, a NOAEL or LOAEL for the most critical effect could not be established.

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

In an opinion on lead and lead compounds in jewellery, adopted by the ECHA Committee for Risk Assessment (RAC) in the spring of 2011, it was concluded that no threshold for the

adverse effect has been identified in humans (ECHA, 2011b). In their risk assessment, RAC used 1/10 of the EFSA $BMDL_{01}$ of 0.50 µg Pb/kg bw per day, i.e. 0.05 µg Pb/kg bw per day as a maximum exposure value.

4. Exposure and risk characterisation

4.1 Exposure characterisation

In the exposure assessment for lead in tattoo inks, exposure is calculated for the reference concentrations of lead:

i. defined as the maximum allowed concentration according to Resolution ResAP(2008)1 – 2 ppm (Table 126), and

ii. based on the maximum concentration reported in the literature – 401.5 ppm (Table 127).

Table 125: Estimated lead dose based on maximum concentration according to ResAP(2008)1

Parameter	**Value**
Maximum permitted level of Pb according to ResAP(2008)1 (mg/kg) (ppm)	2
Correction factor (mg/kg to fraction)	1×10^{-6}
Maximum permitted Pb concentration in ink (mg/mg)	2.0×10^{-6}
Pb present in ink used per session at this max. conc. (mg) (based on 4 308 mg ink per session)	0.00862
Estimated daily Pb dose for a 60 kg person (mg Pb/kg bw/d)	0.0001436
Correction factor (mg to µg)	1 000
Estimated daily Pb dose (µg Pb/kg bw/d)	0.1436

Based on a report by the JRC (JRC, 2015b), lead has been detected in tattoo inks in the range 0.015-401.5 mg/kg (ppm). As a realistic worst case, therefore, the estimated dose through use of exposure to tattoo inks with lead at this concentration is calculated below.

Table 126: Estimated lead dose based on maximum concentration reported in the literature

Parameter	**Value**
Concentration of Pb present in tattoo inks and PMU (mg/kg)	0.015 – 401.5
Maximum Pb concentration in ink (mg/kg) (ppm)	401.5
Correction factor (mg/kg to fraction)	1×10^{-6}
Maximum Pb concentration in ink (mg/mg)	40.15×10^{-5}
Pb present in ink used per session at this max. conc. (mg) (based on 4 308 mg ink per session)	1.73
Estimated daily Pb dose for a 60 kg person (mg Pb/kg bw/d)	0.029
Correction factor (mg to µg)	1 000
Estimated daily Pb dose (µg Pb/kg bw/d)	29

5. Risk characterisation

In the scope of the restriction proposal for substances in tattoo inks the Dossier Submitter addressed those of them without reliable dose descriptor for a given endpoint in a purely qualitative approach, e.g. irritation/corrosion, sensitisation, acute toxicity, carcinogenicity and mutagenicity. For most of these substances a threshold cannot be identified. The remaining substances have been addressed in a (semi-)quantitative manner.

The maximum exposure value of 0.05 µg Pb/kg bw/d is supported by the previous RAC opinions based on EFSA's opinion, and implies that a 60 kg person should maximally be injected with less than 3 µg Pb/day (60 kg x 0.05 µg Pb/kg/day) for the risk characterisation ratio (RCR) not to exceed 1.

For a single session tattoo with 300 cm^2, 4 308 mg (14.36 mg ink/cm^2 x 300 cm^2) ink is injected. The concentration limit (CL) becomes 0.7 ppm lead (3 µg / 4 308 mg) x 1/1000 = 0.00000007 = 0.00007 % (w/w)). In (JRC, 2015a) higher concentrations of Pb has been reported by JRC (up to 401.5 ppm). Based on this calculation, for lead a concentration limit (CL) of 0.00007 % equals RCR = 1. Consequently, a concentration limit of 0.00007 % is suggested.

Consequently, the concentration limit (CL) for maximum exposure (DNEL) 0.05 µg Pb/kg is:

$$CLi < \frac{0.00005 \frac{mg_{substance}}{kg_{bw} \times d}}{72.00 \frac{mg_{ink}}{kg_{bw} \times d}} = 0.00000069 \frac{mg_{substance}}{mg_{ink}} \approx 0.7 \frac{mg_{substance}}{mg_{ink}} = 0.7\,ppm$$

According to the calculations, the proposed concentration limit for reprotoxic "only" substance (this means classified as Repr. 1A/B without being simultaneously classified as a carcinogen, a mutagen or a sensitiser) is is 0.7 ppm (mg/kg) and it is a factor of 2.85 lower than the maximum allowable concentration according to ResAP(2008)1.

Concentration limit for substances on Table 3 of the CoE ResAP(2008)1

Industry consultations conducted during the development of the second CoE resolution (ResAP(2008)1) led to the recommendation to limit the concentration of selected impurities to 2 ppm. The limits are demonstrated to be technically achievable as a large share of tattoo inks and PMU currently on the market in Member States with national legislation are compliant with them. For selected impurities – arsenic, barium, copper, lead, and zinc – the Dossier Submitter's risk assessment has suggested the need for different concentration limits than those recommended by ResAP(2008)1. The proposed concentration limit for lead proposed in this paper is 0.00007% w/w.

Concluding remarks

Lead substances are included in the restriction proposal based on their non-threshold effect of developmental neurotoxicity (EFSA, 2013), acknowledged by RAC in the lead in jewellery and consumer article restrictions. EFSA (EFSA, 2013) concluded that there is no evidence for a threshold for a number of critical endpoints including developmental neurotoxicity (including from *in utero* exposure), increases in systolic blood pressure and renal effects (e.g. changes in proteinuria, glomerular filtration rate (GFR) or creatinine levels and clearance) in adults).

EFSA concluded that protection of children and women of child-bearing age against the potential risk of neurodevelopmental effects should be protective for all other adverse effects of lead, in all populations. EFSA also recommended work should continue to reduce exposure to lead, from both dietary and non-dietary sources. Therefore as it cannot be excluded that women of childbearing age would have tattoos and taking into account the non-threshold effects of lead, the Dossier Submitter proposes these lead compounds to be restricted in tattoo inks and PMUs.

A maximum exposure limit of 0.05 µg Pb/kg bw/d has been supported by RAC and EFSA and using this value in risk assessment shows lead compounds at a maximum concentration allowed by ResAP(2008)1 (2ppm) and the maximum concentration found in tattoo inks (400 ppm) would not be properly controlled. A new concentration limit of 0.00007% w/w (0.7 ppm) for lead impurities found in tattoo inks is proposed.

Appendix B.11. Risk assessment of zinc (Zn)

Risk evaluation of Zn^{2+} in tattoo inks

Background

A wide range of different metals and other elements, including zinc, have been found in tattoo inks (JRC, 2015b) (DEPA, 2012). According to Table 3 in the CoE ResAP(2008)1, which the national tattoo legislation in Italy, Sweden, Spain and Slovenia is based upon, the maximum allowed concentrations of impurities of zinc in products for tattoos and PMU is 50 ppm (mg/kg). It should be noted that the specific national tattoo legislation in some other MS (Belgium, France, Germany and the Netherlands) and Norway is based on CoE ResAP (2003)2, which does not specify specific concentration limits for many impurities (including zinc).

Zinc oxide (ZnO; CAS no 1314-13-2; Pigment white 4; CI no 77947) is one of four white colourants (all being inorganic pigments) reported to be used in tattoo inks. The presence of zinc (Zn; CAS no 7440-66-6) and zinc ferrite brown spinel (CAS no 68187-51-9; Pigment Yellow 119; CI no 77496) in tattoo inks has also been reported (JRC, 2015b).

Zinc oxide is included in the list of colourants allowed in cosmetic products (Annex IV, entry no 144) of the cosmetics regulation (EC No 1223/2009). The substance is also allowed to use as an UV filter in cosmetic products (Annex VI, entry 30 (non-nano) and 30a (nano)) with a maximum concentration in ready for use preparation of 25% for both forms, except in applications that may lead to exposure of the end-user's lungs by inhalation.

In the elements' analysis described in the JRC Report on Work Package 2 (JRC, 2015b), zinc (Zn) was detected in 459 samples within the range of 0.3 - 1690 mg/kg, equivalent to 0.00003 – 0.17%. Ninety-nine of these samples (21%) showed concentrations higher than the recommended limit value of 50 ppm (mg/kg) set in the CoE ResAP(2008)1.

The reported concentrations refer to the total content of the element after complete digestion of the samples, as set in the CoE ResAP(2008)1 (JRC, 2015b).

ZnO in nanoform is under Substance Evaluation in REACH in 2017. It was therefore agreed not to address ZnO as pigment or in nano particle (NP) form in this document and rather to refer to Zn^{2+} ions as impurities only. Risk assessment of ZnO particles in tattoo inks may be performed after substance evaluation is completed this year. In general, ZnO as nanoparticles are considered less toxic than Zn^{2+}-ions due to low solubility and lower absorption rate in humans (Wegmüller, et al., 2014). The toxic effects of ZnO NP are mainly due to their solubility, resulting in increased intracellular Zn^{2+}. ZnO NP exposure via inhalation poses the most important hazard, while for skin exposure hazard can be considered rather minimal in view of the limited uptake via the skin and the absence of local effects (Vandebriel & De Jong, 2012).

Hazard evaluation

Introduction

$Zinc^{2+}$ is an essential mineral for growth and development, testicular maturation, neurological function, wound healing and immune function. Over 300 zinc enzymes have been discovered covering all six classes of enzymes and in different species. Zinc has structural, regulatory or catalytic roles in many enzymes. Additionally, it maintains the configuration of a number of non-enzymatic proteins such as pre-secretory granules of

insulin, some mammalian gene transcription proteins and thymulin. Well known zinc containing enzymes include superoxide dismutase, alkaline phosphatase and alcohol dehydrogenase. In biological systems, zinc exists as Zn^{2+} and is present in all tissue and fluids in the body. Total body content of zinc is between 2 and 4 g and plasma concentration is between 11 and 18 µM (approximately 0.1% of total body content). Urinary zinc excretion is between 300 to 700 µg/day. Zinc content in the most common single nutrient supplements on the market is 30 mg per capsule, range 15-50 mg and in the most common multiple nutrient supplements is 10-15 mg, range 2-20 mg (SCF, 2003). Consumption of excess Zn can cause copper deficiency.

Classification

Zn EC.: 231-175-3 / CAS no.: 7440-66-6

Water solubility (mass/vol.) 100 µg/L @ 20 °C and pH 6.93 - 8.57 [1]

No harmonised classification for human health.

Toxicokinetics - ADME

Once in plasma, zinc is carried by a number of proteins that include albumin, transferrin and caeruloplasmin. Most of the absorbed zinc is excreted in the bile and eventually lost in the faeces. There appears to be no specific zinc "store" in the body. Tissue content and activity of zinc-dependent processes are maintained over a wide range of dietary zinc intakes. When zinc intake is increased, the fractional absorption decreases and intestinal excretion increases while urinary losses remain fairly constant. Endogenous faecal zinc losses may increase several fold to maintain zinc homeostasis with high intakes. When these primary homeostatic mechanisms are not sufficient to handle large dietary excesses of zinc, the excess zinc is lost via the hair. The kinetics of zinc absorption and elimination follow a two-component model. The initial rapid phase has a half-life in humans of 12.5 days and the slower pool turns over with a half-life of approximately 300 days (SCF, 2003).

In rats, dietary zinc intakes up to 1 g/kg body weight have been well tolerated, but dietary zinc intakes above 2 g/kg body weight have usually led to death (SCF, 2003). The mechanism whereby high zinc intakes antagonise copper status has been clarified (Cousins, 1985). High zinc intakes increase the synthesis of metallothionein in intestinal mucosal cells. Metallothionein avidly binds copper and when mucosal cells are rich in this protein, little copper is able to traverse the cells into the body. Studies in rats have shown that high levels of zinc supplementation (0.5-2 g/kg body weight) can affect iron storage and encourage depletion, interfere with iron uptake in the liver and cause anaemia as a result of higher iron turnover (SCF, 2003).

Irritant/corrosive/ sensitisation

In a study on skin-irritating effects of different zinc compounds (water soluble zinc-salt, (SCCS, 2017)), open-patch test were performed with rabbits, guinea pigs and mice. Skin-irritating effects (epidermal hyperplasia, erythema and ulceration) were reported after application of zinc chloride (1% in water). Application of zinc acetate (20% in water) revealed acanthosis, hyperkeratosis and parakeratosis. In addition, mildly irritating effects were found with zinc sulphate (1% in water), including slight epidermal hyperplasia. Furthermore, zinc sulphate was considered to induce ocular irritation (such as corneal injury, epithelial damage and conjunctival irritation) (ATSDR, 2005).

Despite a wide range of possible exposures to water-soluble zinc salts from cosmetics and pharmaceuticals, skin sensitisation has been reported only in a few individual cases. Animal studies with zinc sulphate have revealed negative results.

Genotoxicity

The weight of evidence from the *in vitro* and *in vivo* genotoxicity tests supports the conclusion that zinc, notwithstanding some positive findings at chromosome levels at elevated doses, has no biologically relevant genotoxicity activity (Walsh, et al., 1994) (WHO, 2001).

There is an indication of genotoxic/mutagenic/clastogenic potential of zinc ions (released from zinc oxide nanoparticles) *in vitro* and *in vivo* acting most likely via secondary mechanisms, e.g. via oxidative stress and inflammation and thus considered threshold-dependent. However, the evidence is still limited to conclude on a genotoxic potential of Zn-ions from ZnO (Ghosh, et al., 2016) (Khan, et al., 2015) (Pandurangan, et al., 2015) (Pati, et al., 2016)**.**

Mutation/Carcinogenicity

There are no indications of carcinogenic effects of Zn in rodents after oral intake. However, for some of the studies on zinc and its inorganic compounds it was not possible to assess the carcinogenicity from the available studies (DFG, 2010).

In human studies, no indication of a significant increase in cancer mortality was found in a prospective mortality study comprising 4 802 workers in nine American copper and zinc refining plants (DFG, 2010). In a further study, the association between supplementary zinc intake and the occurrence of prostate cancer was investigated in 46974 male US Americans. During the 14-year observation period, 2901 new cases of prostate cancer were diagnosed, including 434 in an advanced stage. Approximately 25% of the participants took zinc supplements (24% up to 100 mg/day, 1% above 100 mg/day). Supplemental zinc intake of up to 100 mg/day was not associated with an increased prostate cancer risk. With high zinc intake (> 100 mg/day), the relative risk for advanced prostate cancer was 2.29 (95% CI: 1.06 to 4.95). However, the authors noted that residual confounders by supplemental calcium intake or unmeasured zinc supplement use cannot be excluded (DFG, 2010).

A nested case-control study was conducted within the European Prospective Investigation into Cancer and Nutrition cohort. Serum zinc and copper levels were measured in baseline blood samples by total reflection X-ray fluorescence in cancer cases (HCC n=106, IHDB n=34, GBTC n=96) and their matched controls (1:1). Pre-diagnostic circulating levels of copper, zinc and their ratio (Cu/Zn) in relation to hepatocellular carcinoma (HCC), intrahepatic bile duct (IHBD) and gall bladder and biliary tract (GBTC) cancers were assessed. The Cu/Zn ratio, an indicator of the balance between the micronutrients, was computed. Multivariable adjusted odds ratios and 95% confidence intervals (OR; 95% CI) were used to estimate cancer risk. For hepatocellular carcinoma (HCC) in humans, the highest vs lowest tertile showed a strong inverse association for zinc (OR=0.36; 95% CI: 0.13-0.98, Ptrend=0.0123), but no association for copper (OR=1.06; 95% CI: 0.45-2.46, Ptrend=0.8878) in multivariable models. The calculated Cu/Zn ratio showed a positive association for HCC (OR=4.63; 95% CI: 1.41-15.27, Ptrend=0.0135). For IHBC and GBTC, no significant associations were observed. Zinc may have a role in preventing liver-cancer development, but this finding requires further investigation in other settings (Stepien, et al., 2017).

In a multicentre hospital based case-control study on prostate cancer, an association between high zinc intake and prostate cancer risk, particularly for advanced cancers was evaluated. The study was conducted between 1991 and 2002 with 1294 cases and 1451 controls. Zinc intake was computed from a valid and reproducible food frequency questionnaire, with the use of an Italian food composition database. Odds ratios (OR) of dietary intake of zinc and the corresponding 95% confidence intervals (CI) were estimated by unconditional multiple logistic regression models, after allowance for several covariates, including total energy. Compared with the lowest quintile, the OR for the highest quintile was 1.56 (95% CI, 1.07–2.26), with a significant trend in risk (p = 0.04). The trend in risk was significant for advanced cancers only, the OR being 2.02 (95% CI, 1.14–3.59) for prostate cancers with a high Gleason score. In this case-control study, a direct association between high zinc intake and prostate cancer risk, particularly for advanced cancers was observed and thus the favourable and protective effect of zinc on prostate carcinogenesis seen in other studies may be questionable (Gallus, et al., 2007) (Kristal, et al., 1999).

The evidence for carcinogenicity is still limited, which makes it difficult to conclude.

Reproduction

Zn (7440-66-6): An OECD Guideline 416 study was conducted to evaluate the reproductive toxicity potential of test material ($ZnCl_2$,) in rats for two generations. Male and female rats were administered test material at the doses of 7.50, 15.00 and 30.00 mg/kg/d over two successive generations. Control group animals received deionised water. Exposure of F0 and F1 parental rats to test material showed significant reduction in fertility, viability (0-20 and 12-24% mortality in males and females respectively at the lowest to highest dose, days 0 and 4), and the body weight of F1 and F2 pups from the high-dose group but caused no effects on litter size, weaning index, and sex ratio. Significant reduction in body weights of F0 and F1 parental males and postpartum dam weights female rats was observed. Exposure of test material to F0 and F1 generation parental animals resulted in a non-significant change in clinical pathology parameters (except the alkaline phosphatase level). Reduction of brain, liver, kidney, spleen and seminal vesicles weights of males and in the spleen and uterus of females was observed in F0 and F1 rats. Gross lesions were observed in gastro-intestinal (GI) tract, lympho-reticular/ hematopoietic and reproductive tract in parental rats in both generations. Reduced body fat was also recorded in F1 parental rats (Khan, et al., 2007).

Under the test conditions, administration of test material to adult male and female rats throughout maturation, mating, gestation and early lactation resulted in significant effects on adults and offspring at 30 and 15 mg/kg/d. Maternal effects cannot be excluded (Khan, et al., 2007). Although effects were seen at 7.5 mg/kg bw/day, these were considered to be biologically non-significant and are therefore considered to be the "No Observed Adverse Effect Level" (NOAEL).

A study reported to ECHA dissemination website (ECHA, 1986c) showed that dietary zinc (Zn) supplementation at 4,000 ppm zinc as zinc sulphate, $ZnSO_4$ (4mg/kg/day) reduced male fertility in rats under the conditions of the study. A study was conducted to determine the effects of dietary zinc supplementation on male fertility in Charles-Foster rats. 4 000 ppm zinc as zinc sulphate was fed to 18 test males in diet for 30-32 days. 15 control males were fed normal diet for the same duration. All animals mated with individual non-treated females once between day 30 and 32. After mating, males were sacrificed for sperm characterization and zinc concentration analysis in different reproductive organs. Mated females were allowed to have full term gestation. Mating by treated males caused

significant lowering of incidence of conception and number of live births per mated female. However, no stillbirth or malformed litter was observed. Motility of the sperm was significantly reduced in the treated rats but viability was unaffected. Zinc content was significantly increased only in the testis and sperm of the treated rats.

The results indicate that dietary zinc supplementation at 4 000 ppm reduced male fertility in rats under the conditions of the study (ECHA, 1986b). In a repeated dose toxicity 90 day study in rats fed zinc monoglycerolate up to 1% in the diet (dose range was 0, 0.05, 0.2 and 1%), equal to ca 335 mg Zn2+/kg bw/day for 58 days, after which the concentration in the feed was decreased for one week to 0.5%, equal to ca. 300 mg Zn2+/kg bw/day. Subsequently, the animals had to be killed at day 64 because of poor health and compromised food consumption (note also the non-linearity in the Zn2+-doses). The testes of all these males showed hypoplasia of the seminiferous tubules to a varying degree and in addition the prostate and seminal vesicles showed hypoplasia. In all but one female the uterus was hypoplastic. All other rats exposed to 0.05 or 0.2% (ca. 13 or 60 mg Zn2+/kg bw/day, respectively) survived to the end of the 13 weeks treatment, without showing detrimental effects on sex organs. NOAEL was set to 60 mg/kg bw/day (ECHA, 1986a).

In a human exposure study, 250 women (before 20 weeks of gestation) were given 20 mg elemental zinc daily until the end of pregnancy. The control group received placebo. Various adverse outcomes were tested, including maternal bleeding, hypertension, complications of delivery, gestational age, Apgar scores, and neonatal abnormalities. The main endpoint under study was the birth weight. There were no differences in the outcomes studied, as well as in birth weight of babies, between mothers receiving zinc and controls (Bingham, et al., 2001).

Specific Target Organ Toxicity, repeated exposure (STOT-RE)

In the 90 day study described above (guideline 408 study, key study used in EU risk assessment report for Zinc metal was performed), groups of 20 male and 20 female Sprague-Dawley rats were fed zinc monoglycerolate at dietary levels of 0, 0.05 or 0.2% (equal to 0, 31.52 or 127.52 mg/kg/day for males and 0, 35.78 or 145.91 mg/kg bw/day for females, respectively) for a period of 13 weeks in a study performed according to OECD 408. A similar group was fed 1% (equal to 719 and 805 mg/kg bw/day for males and females, respectively) of zinc monoglycerolate up to day 58 of the study when a deterioration in their clinical condition (poor physical health and reduced food intake) necessitated reducing the dietary level to 0.5% (equal to 632 and 759 mg/kg bw/day for males and females, respectively). However, as no improvement occurred, these rats were killed on humane grounds on day 64 of the study. These rats developed hypocupremia manifested as a hypochromic microcytic regenerative type anaemia (low haemoglobin and haematocrit, decreased MCV and MCH, and increased MCHC, red blood cell and reticulocyte count). Enlargement of the mesenteric lymph nodes and slight pitting of the surface of the kidneys were noted. Severe pancreatic degeneration and pathological changes in the spleen, kidneys, incisors, eyes and bones were observed. The testes of all males showed hypoplasia of the seminiferous tubules to a varying degree and in addition the prostate and seminal vesicles showed hypoplasia. In all but one female the uterus was hypoplastic. All other rats survived to the end of the 13 weeks treatment. This dose level (0.05%) is equal to 31.52 or 35.78 mg zinc monoglycerolate/kg bw for males and females, respectively, so the NOAEL in this study is 31.52 mg/kg bw/day (»13.26 mg Zn2+/kg bw) (ECHA, 1986a).

Oral long-term intake of 150 mg zinc per day (zinc equivalent about 2.1 mg/kg body weight and day) produces copper deficiency and anaemia (SCF, 2003). In women, a zinc intake of

2.5 mg/kg body weight/day causes a significant decrease in ceruloplasmin and erythrocyte superoxide dismutase activity after six weeks (Samman & Roberts, 1987). Significantly reduced activities of erythrocyte superoxide dismutase, though with no effect on ceruloplasmin concentration, were found after a zinc intake of 0.71–0.83 mg/kg body weight per day for several weeks (Yadrick, et al., 1989) (Milne, et al., 2001) (Fischer, et al., 1984). In another human study, zinc intake of about 0.43 mg/kg body weight per day for 14 weeks in men produced no significant change in whole blood superoxide dismutase activity or ceruloplasmin concentration (Bonham, et al., 2003a) (Bonham, et al., 2003b). The reduction in superoxide dismutase activity marks the range where effects on copper balance begin. A NOAEL of 0.43 mg zinc/kg body weight/day after oral intake can thus be derived at which no effects on parameters of the copper metabolism occur (DFG, 2010).

ATSDR (Agency for Toxic Substances and Disease Registry) has derived an intermediate-duration oral MRL (Minimal Risk Levels) of 0.3 mg Zn/kg/day for zinc based on decreased erythrocyte superoxide dismutase, a sensitive indicator of body copper status, and changes in serum ferritin in women given supplements containing zinc gluconate for 10 weeks (Yadrick, et al., 1989). It should be noted that the MRL is calculated based on the assumption of healthy dietary levels of zinc (and copper), and represents the level of exposure above and beyond the normal diet that is believed to be without an appreciable risk of toxic response. The MRL is based on soluble zinc salts; it is less likely that non-soluble zinc compounds would have these effects at similar exposure levels. The intermediate oral MRL has been adopted as the chronic oral MRL (ATSDR, 2005).

US EPA has derived an oral reference dose (RfD) of 0.3 mg/kg bw/day for zinc (IRIS, 2005).

A key 003 study report with human exposure, registered in ECHA under Zn (CAS 7440-66-6) (Unnamed, 1989): A 10-wk single-blind study was conducted to determine the response of iron, copper and zinc status to supplementation with oral zinc or a combination of zinc and iron supplement. 18 female volunteers were randomly assigned to the two treatment groups and consuming either 50 mg Zn/day as zinc gluconate (Group Z) or 50 mg Fe as ferrous sulfate monohydrate in addition to the Zn (Group F+Z). Blood and saliva samples were analysed for Fe, Cu and Zn levels before treatment (pre-treatment) and after 6 and 10 week of supplementation. For Group Z, serum ferritin, hematocrit, and erythrocyte Cu and Zn-superoxidedismutase (ESOD) were significantly lower ($p < 0.05$) after 10 wk supplementation compared with pre-treatment levels. Serum Zn increased ($p < 0.01$) but no change occurred in serum ceruloplasmin, hemoglobin, or salivary sediment Zn levels. For Group F+Z, ESOD and salivary sediment Zn ($p < 0.05$) decreased with treatment. Serum ferritin and serum Zn increased significantly, but hemoglobin, hematocrit, and ceruloplasmin were not affected by the combination treatment. Under the conditions of the test, zinc supplementation significantly lowered iron and copper status, as assessed through serum ferritin, hematocrit and ESOD levels and inclusion of iron with zinc ameliorates the effect on iron but not on copper status. The NOAEL in this study is less than 0.83mg Zn2+/kg bw/day (based on a body weight of 60 kg).

The Opinion of the Scientific Committee on Food (SCF) on the Tolerable Upper Intake Level of Zinc (expressed on 5 March 2003) reported that high exposure to Zn in humans, gives systemic toxicity after repeated exposure on the Cu-balance, lowering copper status. The NOAEL was found to be 0.83 mg Zn/kg bw/day (SCF, 2003). The European Food Safety Authority (EFSA) has subsequently confirmed this NOAEL established by SCF in two opinions from 2006 (EFSA, 2006) and 2014 (EFSA, 2014). In addition, the SCCS opinion on Zn^{2+} used in oral hygiene products have used the same endpoints as the EFSA report from 2014

for their safety evaluation (SCCS, 2017). They concluded that the ULs (upper limit) should be 7, 10, 13, 18 and 22 mg/day for children aged 1-3, 4-6, 7-6 10, 11-14 and 15-17 years, respectively, and an UL of 25 mg/day for adults.

Derivation of DNELs

Zn (7440-66-6)

STOT:

The EFSA report from 2006 (EFSA, 2006) and supported by the SCCS opinion from 2017 (SCCS, 2017) adopted a NOAEL of 50 mg/day or 0.83 mg Zn^{2+}/kg bw/day which is based on the absence of any adverse effects on a wide range of relevant indicators of copper-status as critical endpoint. They applied an UF (uncertainty factor) of 2 owing to the small number of subjects included in relatively short-term studies but acknowledging the rigidly controlled metabolic experimental conditions employed. Thus, a Tolerable Upper Intake Level (UL) of 25 mg/day was recommended.

In this report, a human dietary zinc supplementation study that significantly lowered iron and copper status was used to identify the NOAEL. The lowered iron and copper status was assessed through serum ferritin, hematocrit and ESOD (Erythrocyte superoxide dismutase levels) and inclusion of iron with zinc ameliorates the effect on iron but not on copper status (A key 003 study report, human exposure, (Unnamed, 1989). The NOAEL in this study is less than 0.83 mg Zn2+/kg bw/day. The population in the study reflects only healthy volunteers and it was thus suggested to add an AF of 5 in order to cover the young and more vulnerable population.

A DNEL from this study is estimated to be 0.83/5 = 0.166 mg Zn2+/kg bw/day. Total amount of Zn that may be tolerable can be estimated to be: 0.166 mg Zn/kg bw/day x 60 kg bw = 10 mg Zn^{2+} per day.

Risk evaluation

Risk = Exposure/DNEL >1

Exposure:

In order to assess the risk of Zn^{2+} in tattoos the exposure must be estimated. Following the exposure scenario described in the "Proposed risk assessment approach for chemicals in Tattoo inks and Permanent Make Up (PMU)" (by DK), the amount of chemical applied in a large tattoo (300cm^2) is 4.308 g ink/tattoo session (14.36 mg ink/cm^2 x 300cm^2).

In a worst case scenario according to information from the JRC Report on Work Package 2 (JRC, 2015b) there will be 1 690mg Zn/kg tattoo ink, giving 0.17% Zn (1.69g/1000g=0.0017) in a tattoo ink. Thus, a large tattoo will give a total amount of:

4.32 gram/100*0.17% = 7.3 mg Zn^{2+} applied.

When applying the DNEL from the STOT-study on Zn^{2+}:

RCR= 7.3/10 =0.73 which is <1 and thus the risk is controlled

Specific Concentration limit

If RCR = 1, then Zn exposure equals to 10 mg zinc in 4.32 gram of ink (0.166 mg/kg bw x 60 kg =9.96 mg zinc) and that gives a limit of zinc in tattoo ink of 0.23%

(9.96 mg/1000)/4.32 g x 100% = 0.23%

Converted to ppm: 2300 ppm

In conclusion, a limit value of 2300 ppm or 0.23% for Zn^{2+} in tattoo ink equals RCR = 1

Conclusion

In conclusion, for dissolved zinc the RCR appear to be below one and thus there is no risk due to the content of zinc in tattoo inks. Thus, it may be concluded that zinc should not be restricted in tattoo inks.

A recommended limit value would be 0.23%.

Annex C. Baseline

In order to assess the impacts of the proposed restriction options over the study period, it is important to understand the current and future amount of tattoo inks placed on the EU market will change as well as the number of people exposed to tattoo ink.

The study period – entry into effect (assumed for analytical purposes to be 2021) plus 20 years – is selected on the basis of the time anticipated for the costs and benefits of the proposed restriction options to fully develop, in particular those quantified and monetised. The selection was also influenced by best practices for similar assessments. (See other restriction dossiers submitted to ECHA.)

The geographical boundaries for the assessment are the territories of Member States of the European Union (EU28) and the European Economic Area (EEA31). In addition, selected statistics are presented for those Member States who currently do not have national legislative measures for tattoo inks (EEA22). These include: Austria, Bulgaria, Croatia, Czech Republic, Cyprus, Denmark, Estonia, Finland, Greece, Hungary, Ireland, Latvia, Lithuania, Luxemburg, Malta, Poland, Portugal, Romania, Slovakia, United Kingdom, and Iceland (EEA). Italy is also included, as the ResAP recommendations are not enforced in all parts of the country.

The "business as usual" scenario is defined as the current and predicted future use of the substances in scope in tattoo inks without the proposed restriction. No pending legislative changes of relevance have been identified. The only uncertainty related to the volume tattoo inks and number of people exposed to tattoo ink in the EU stems from the uncertainty of the status of the UK within the EU and the EEA following their activation of article 50 of the Lisbon Treaty. Therefore, where possible, the statistics for the UK are presented separately. EU27, EEA30 and EEA21 thus refer to the EU/EEA excluding the UK.

A. Number of people with tattoos and PMU

a) Tattoos

For the purpose of assessing the impacts of the proposed restriction options, an important component of the baseline would be the number of people exposed to tattoo inks and PMU. According to estimates for 2014, about 12% of the EEA population has at least one tattoo. This is an increase from 4-8% prevalence as of 2003. The prevalence for younger age groups is much higher and in some Member States it is more than double the national average, exceeding 30% for some groups under 40-years-old. While in the early days of popularity (prior to the 1990s), tattoos were more typical for men, figures show that this trend is changing and in certain cases the tattoo prevalence is higher for women, particularly for younger generations. The first tattoo is usually obtained at a young age, often much younger than 25 years old, e.g., in Denmark, 37% get their first tattoo before the age of 20, while in Germany, 17.6% before they are 18. Adolescents also get tattoos even though in some countries the minimum age for obtaining a tattoo is 18. (JRC, 2015b)

Table 128 shows the total number of people in the EEA31 who are estimated to have a tattoo (excluding removals) over the study period. The future population with at least one tattoo is estimated on the basis of incidence and current and anticipated trends of getting a tattoo.

The incidence of obtaining a tattoo (getting tattooed for the first time) in the population is estimated on the basis of EuroStat population projections and JRC data (JRC, 2015b) that show that people with tattoos in EEA31 increased from 30 million in 2003 to 62 million in

2014. By 2014, this number increased to more than 62 million people. This implies that, annually, on average, about 0.5% of the EEA31 population got tattooed for the first time. Assuming the trend between 2003 and 2014 continues in the future (main scenario), more than 81 million people in EEA31 will have at least one tattoo by 2021 – the assumed year of entry into effect of the proposed restriction options. Of those, about 43.5 million would be living in a Member State where there is currently no national regulation on the chemical composition of tattoo inks and PMU (EEA22). By the end of the study period, the population of people with at least one tattoo is expected to double under the main scenario. This implies a prevalence rate for EEA31 of 26% by 2040.

Table 127 Estimated number of people with tattoos

Geographic Area	Prevalence over study period				Average incidence 2021-2040
	2014	2016	2021	2040	
EU28	61 363 400	66 788 900	80 431 900	133 032 300	2 766 900
EEA31	62 025 600	67 510 600	81 309 500	134 603 900	2 803 200
EEA22	33 221 200	36 156 200	43 535 800	71 972 400	1 495 900
UK	7 722 100	8 413 800	10 181 200	17 365 100	377 100
EU27*	53 641 300	58 375 100	70 250 700	115 667 200	2 389 800
EEA30*	54 303 500	59 096 800	71 128 300	117 238 800	2 426 100
EEA21*	25 499 100	27 742 400	33 354 600	54 607 300	1 118 800
Prevalence rate	12.1%	13.1%	16.2%	26.1%	

Notes: 2014 data based on EuroStat and (JRC, 2015b). 2016-2040 – projected based on EuroStat data. *The data stands for respectively EU28, EEA31, and EEA22, excluding the UK.

Table 128 shows that on average, under the main scenario, 2.8 million new people would get a new tattoo over the study period. This number is not a proxy for the number of visits of tattooists, number of tattoos obtained, or number of tattoo sessions per year. These latter estimates would be much higher, as about half of the people with tattoos have more than one tattoo. Survey data from (Høgsberg, et al., 2013) and (Klügl, et al., 2010) show that close to 83% and 73% respectively have less than three tattoos. In two-thirds of the Member States responding to a survey, the group of people with 2-5 tattoos was the largest (JRC, 2015b). About half of the population have smaller tattoos (Table 129) and this tendency is stronger for women than for men. The total body surface tattooed for about half of the people is less than 1%, while about 15% of the men have an area greater than 20% tattooed (Høgsberg, et al., 2013). These larger tattoos would require more visits to tattooists, sometimes over the course of a year or more, in particular if the tattoo design is complex (e.g., realistic style) and comprised of several colours. A Danish survey of tattoo artists found that the tattoos being performed in the studios ranged in size primarily from 10cm^2 to 450 cm^2, with a median of 176.7 cm^2 (i.e., just over 1% of the average body surface of a woman and just under 1% of that of an adult male) and an average of 150 cm^2 (or 140 cm^2 after removing outliers). The study also found that larger tattoos were also common, often performed over several sessions. (see Appendix F.1)

The number of sessions and the amount of ink – all important components for determining exposure, risk, and the likelihood of developing an adverse effect – are discussed qualitatively in the analysis. Another important factor discussed qualitatively is tattoo removal. Although removal may have an impact on tattoo prevalence, this factor is

considered less important for the purpose of this analysis. Regrets have been reported between 5% and 20% of those with tattoos (JRC, 2015b) but there is insufficient information to translate regret to actual removal and its impact on tattoo prevalence, in particular when a large percentage of the tattooed population has more than one tattoo and tattoo removal can be followed by a new tattoo. Furthermore, the indicator, number of people with tattoos, is primarily necessary for this analysis to estimate the number of adverse reactions to tattoos. Therefore, removals are not expected to lead to an overestimation of these effects as a tattoo may lead to adverse health effects in the period prior to its removal (and in fact, complaints or complications of tattoos can be the reason for such removal). On the other hand, the number of removals is important as some removal techniques have been shown to lead to adverse reactions themselves. (See section D.2.3.2. Human health and environmental impacts)

Table 128 Tattoo size in Europe

Size (% of body surface)	Women	Men	Total
≤0.1%	10.0%	3.4%	7.2%
>0.1–≤1%	45.5%	35.0%	41.0%
>1–≤4%	24.2%	25.1%	24.6%
>4–≤6%	11.7%	18.2%	14.5%
>6%	8.7%	18.3%	12.8%
Total	100%	100%	100%

Notes: A skin surface of 1% roughly corresponds to the area of the palm and fingers of the hand.

Sources: Pooled data from (Høgsberg, et al., 2013), (Klügl, et al., 2010), and (ISS, 2017)

Table 128 also shows the projected prevalence and average incidence on the basis of projections over the study period. These projections are estimated on the basis of anticipated future trends of obtaining tattoos. These are associated with high uncertainty but some indication can be obtained from:

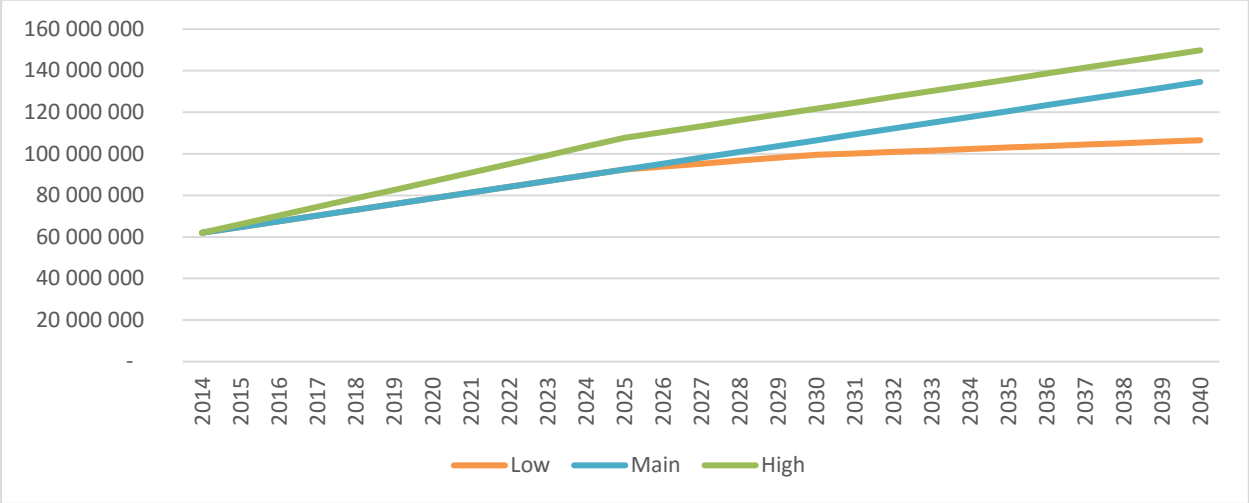
- Trends in other countries: begun in North America. In the US and Canada for example, which led the tattoo revival, currently the prevalence is higher: 20% and 21% respectively. (JRC, 2015b) Considering the cultural similarities between North America and Europe, it can be anticipated that the prevalence in Europe would in the near future reach that of Canada and the US.
- Fashion trends: The change in social perception of tattoos, and substantial growth in the number of people with tattoos and number of tattoos per person, was boosted by the embracing of tattoos by fashion setters (icons) such as performance artists and elite athletes. Similarly, the popularity of PMU has increased thanks to advancements in PMU techniques, plastic surgery and the fashion trend towards more visible (heavy) make-up. This is akin to previous fashion trends, for example in the 1960s and 1970s, which were followed by trends that favour more natural look. Future fashion trends cannot be predicted but according to the tattoo and PMU industry, given the still somewhat rebellious nature of tattoos in particular, it is possible that future generations would not be interested in tattoos like their parents were.
- Other impacts: It is possible that the increased perception of the safety of the tattoos and PMU and the decline in the social stigma would encourage more people in the future to have similar body enhancements.

Therefore, to test this uncertainty, two other scenarios are prepared in addition to the Main scenario:

- Low prevalence scenario: Assumes that the current incidence rate will decline by 50% in 2025 and again in 2030. Under this scenario, the overall prevalence is estimated at 15.7% as of 2021 and 20.3% as of 2040.
- High prevalence scenario: Assumes that more people will choose to get a tattoo for a first time (i.e., 50% higher incidence rate) in the short term. After 2025, the incidence of getting a tattoo will return to current levels. Under this scenario, the overall prevalence is estimated at 17.5% in 2021 and 28.5% in 2040.

The effects of these assumptions are displayed on Figure 5 and further assessed in Annex E: Assumptions, uncertainties and sensitivities.

Figure 5 Population with tattoos – projections



Notes: 2014 data based on EuroStat and (JRC, 2015b). 2016-2042 – projected based on EuroStat data.

b) PMU

There is very limited information on the prevalence of PMU in the EEA. On the basis of data from three Member States, it can be estimated that the PMU prevalence in EEA31 in the general population is between 3% and 20% (JRC, 2015b). Thus, it can be estimated that about 53 million people in EEA31 have had at least one PMU procedure (on the basis of mid-point estimate). The numbers are presented separately in Table 130. Due to the limited information and the possibility that a person with a PMU could also have one or several tattoos, these estimates are not projected and added to the population with tattoos. First PMU procedures are reported after 18 or 25 years of age. (JRC, 2015b) PMUs tend to be more popular with women. Its popularity has increased due to advancements in PMU techniques, plastic surgery and the fashion trends. Industry expects that PMU would continue to replace traditional cosmetics and to be used as a technique for enhancing human features in the long term.

Table 129 Estimated population with PMU in 2016 (number)

Geographic Area	Low	Mid-point	High
EU28	15 274 900	52 613 700	101 832 900
EEA31	15 443 400	53 194 000	102 956 100
EEA21	8 262 800	28 460 800	55 085 400
UK	1 951 700	6 722 600	13 011 600
EU27	13 323 200	45 891 000	88 821 400
EEA30	13 491 700	46 471 300	89 944 500
E20	6 311 100	21 738 200	42 073 800
Prevalence rate	3%	10%	20%

Sources: EuroStat 2014 data and (JRC, 2015b)

For further information on tattoo and PMU prevalence and population characteristics, please see the JRC report (JRC, 2015b).

B. Volume of tattoo inks and PMU

Annex A: Manufacturing and uses discusses the estimated volume of tattoo ink and PMU manufactured, exported and imported to EEA31. Table 1 shows that about 152 000 litres of tattoo ink and 10 750 litres of PMU are placed on the EEA31 market in 2016. No statistics are available specifically for tattoo inks and PMU. Therefore, the estimates were developed on the following basis:

- Tattoo inks: the volume of tattoo ink on the EEA31 market is derived on the basis of information on the amount of tattoo ink used by tattoo artist on average annually: between 0.5 and 3 litres for full-time professional tattoo artist, with amateur artists 25-50% of this. (JRC, 2015b) (industry interviews) The number of tattoo artists was established by the JRC (JRC, 2015b) via questionnaires. The results, presented in Table 1 in Annex A, were verified with industry representatives. Information from the same JRC report provided the share of EU manufactured (20-30% of ink volume), exported (about 5% of EU manufactured ink) and imported (70-80%) volumes for the EEA31 market. (JRC, 2015b) (Michel, 2015)[38]
- PMU: the volume of PMU placed on the EEA31 market was estimated on the basis of information from the JRC report (JRC, 2015b), supplemented by interviews with industry. (See Table 1 in Annex A for results). In contrast to tattoo inks, the majority of PMU placed on the EEA31 market is manufactured in the EU (80-90%). EU PMU manufacturers[39] also export nearly 20% of their production internationally. Less than

[38] The main EU manufacturers of tattoo inks are based in the UK and Germany, other EU Member States include Italy, Spain, Sweden, and Poland, although there is uncertainty of the exact place of origin of some products. (JRC, 2015b) In total, the study suggests that there are about 90 EU-based and international manufacturers of tattoo inks on the EEA31 market.

[39] Germany dominates the EU-based manufacturing of PMU, other EU Member States include Italy, Spain, France, Austria, and the Netherlands, although study notes that the EU and global market is complex and "it is not easy to understand who is producing what", as one manufacturer may produce more than one brand (own or for private label). In total, the study suggests that there are 55 PMU EU-based and international manufacturers on the EEA31 market. (JRC, 2015b)

5% of PMU on the EEA31 market is imported according to estimates, primarily from the US or China. (JRC, 2015b)

There is no historical information regarding the volumes of ink placed on the EEA31 market. Estimation of the tattoo ink and PMU volume on the basis of the projected incidence is hampered by lack of information and the numerous variables that impact the amount of ink used, e.g., style (realistic vs abstract), mono vs multicoloured, size, etc. Therefore, information about future volume can only be inferred on the basis of information available on the overall demand for tattoos and PMU in the future. For the purpose of the analysis of the impacts of the proposed restriction options, similarly to the projections of tattoo prevalence, it is assumed in the Main scenario that the amount of tattoo ink and PMU on EEA31 market is expected to remain at about current levels during the study period. For sensitivity purposes, two more scenarios, in line with the Low and High prevalence scenarios, are prepared and the effects of these changes are assessed in Annex E: Assumptions, uncertainties and sensitivities.

Table 130 Tattoo inks and PMU on the EEA31 market – projections (litres)

Scenario	2016	2021	2040	Average 2021-2040
Low	162 800	164 100	48 600	86 100
Main	162 800	164 100	166 300	166 000
High	162 800	240 800	167 400	184 700

Notes: Estimates based on interviews with selected manufacturers and JRC data (JRC, 2015b). See Annex C: Baseline for further information.

Annex D. Impact Assessment

D.1. Risk Management Options

D.1.1. Proposed options for restriction

Following an assessment of the current Member States' national legislation, the recommendations by the CoE, and an assessment of the substances that can be present in tattoo inks, two restriction options are proposed: Restriction option 1 (RO1), presented in Table 2 and Restriction option 2 (RO2), presented in Table 3 of the report. The options differ primarily in terms of the proposed concentration limits for selected substance groups and how the links with the CPR are managed. The scope and other conditions of the two restriction options are identical. Both options have advantages and disadvantages (discussed in detail in sections Justification for the selected scope of RO1and Justification for the selected scope of RO2 which makes it difficult to weigh one option against the other.

The proposed restriction text makes reference to five tables A-E. Table A is included in Table 4, Table B in Table 5 and Tables C-E in Appendix 1 to the report.

The following section briefly outlines the common aspects of the proposed restriction options. Their differences are discussed in sections Proposed restriction option: RO1 and Proposed restriction option: RO2.

a) Rational for the proposed restriction options

The proposed restriction options are formulated taking into account the following:

- If a substance is not permitted in cosmetic products because it is not considered safe to apply on human skin (in general or under specific conditions listed in the CPR), it is logical to assume that it is also not safe to be applied under the skin, i.e., in a tattoo or permanent make-up where the skin is damaged and the substance is deposited in the dermis for a prolonged period of time.
- The substances classified as CMR, and thereby not permitted to be placed on the market or used for supply to the general public as substances on their own or as constituents of other substances or in mixtures (by virtue of entries 28 to 30 of Annex XVII to REACH), should not be used in tattoo inks that will be applied under the skin of members of the public.
- Substances whose hazard profile suggests that they lead to skin sensitisation, irritation or corrosion or eye irritation and damage, should not be applied under the skin (or in the eye), i.e., in a tattoo or permanent make-up where the skin is damaged and the substance deposited in the dermis or in the eye for a prolonged period of time.
- Conclusions of (semi-)quantitative risk assessment by the Dossier Submitter of substances that can be found in tattoo inks, on the basis of reasonable exposure estimates.
- Industry will find difficult to substitute some substances, in particular selected colorants. Taking into account the hazards and risks of the exposure to the relevant pigments, derogations are proposed for these substances.

b) Concentration limits

The proposed concentration limits are derived on the basis of either the substances hazard classification, presence in the Cosmetic Products Regulation or a quantitative or qualitative

risk assessment carried out by the Dossier Submitter. One of the main differences between RO1 and RO2 are the proposed concentration limits for CPR substances in scope (i.e., Annex II and Annex IV with use restriction in column g) and substances with harmonised classification. Both options aim to discourage intentional use and the rational for the different approaches is explained in section D.1.1.2 and D.1.1.4 for RO1 and RO2 respectively.

The concentration limits for the remaining substances are the same for both RO1 and RO2: PAHs, PAAs, azo colourants, methanol, and impurities listed on Table 3 of ResAP(2008)1. The limits for the remaining substances are derived on the basis of (semi-) quantitative risk assessment (e.g., barium, copper) carried out by the Dossier Submitter, considerations for technically achievable limits (e.g., nickel), limits established under other measures (e.g., PAHs), etc. In some cases, several considerations are taken into account in the setting of the limit. For example, while semi-quantitative risk assessment of PAAs derived a risk-based concentration limit of 0.3 ppm (see Appendix B.2), considerations related to limits of detection, technically achievable concentrations and availability of alternatives necessitated the proposal of a higher concentration limit: 5 ppm. For details on these substance categories, see the relevant sections in Annex B and the respective appendixes.

c) Derogations

i. Selected colourants

The proposed restriction options have been designed taking into account the availability of alternatives for some substances, in particular colorants, which industry will find difficult to substitute. Also taking into account the hazards and risks of exposure to the pigments in Table B of RO1 (see Table 5 in report), a derogation is proposed for these substances. For example, Pigment Blue 15:3 and Pigment Green 7 are two essential colourants in tattoo inks.

To date, there is no information for a possible substitute of Pigment Blue 15:3. Although there are other blue pigments, these have been found lacking in brilliance and change colour (e.g., turn grey) when mixed with white pigments – a common practice to achieve different colour tones. (ECHA CfE, 2016a) Pigment blue 15:3, together with a number of other colourants were added to Annex II of the CPR with the condition 'not to be used in hair colours'. At the same time, Pigment Blue 15:3 and 24 other pigments are on the positive list for colourants allowed in cosmetic products (CPR, Annex IV) without conditions of use. Many of the pigments prohibited in hair colours were included in Annex II of the CPR on the basis of the cosmetic industry not providing relevant information to justify continued use in this application. As tattoo inks and PMU do not fall within the scope of the CPR, the tattoo industry was not able to participate in the process, even though the Annex II requirements applied to them via national legislation. Therefore a derogation is proposed for Pigment Blue 15:3 and for the 24 other pigments prohibited in hair colours in Annex II but allowed in Annex IV of CPR (included Table B).

Pigment Green 7 was used in tattoo inks prior to the introduction of the national legislation based on ResAP, on the grounds that it is banned from use in hair colours (Annex II of CPR) and use in products applied on mucous membranes (Annex IV of CPR, column g). According to industry, this pigment has largely been replaced with pigment Green 36 which is a brominated version of Pigment Green 7 raising questions related to Green 36's hazard and risk. (ECHA CfE, 2016a) No other technically feasible alternatives to Pigment Green 7 have been identified to date. Furthermore, both Pigment Green 7 and Blue 15:3 are

phthalocyanines, which are insoluble in water and stable in most solutions. As shown in Appendix B.9, risk for these substances cannot be demonstrated with the currently available information. Therefore, a derogation is also proposed for Pigment Green 7. (See Supplementary Table B marked as Table 5 in report).

ii. Classified substances for inhalation exposure only: As risks associated with the inhalation route only are not relevant for tattoo and PMU exposure, substances classified as carcinogenic via this route only are derogated (e.g., titanium dioxide).

d) Labelling requirements

RO1 also foresees labelling requirements for tattoo inks and PMU. The CoE resolution contains a number of labelling requirements, in addition to its various bans and restrictions. These requirements are:

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

Some of these requirements may be necessary under the CLP Regulation. However, it is proposed to include a labelling requirement under this restriction, as it is specified under certain Member States national legislation, to require in addition to any information required under the CLP, the following information on the label of the product:

The person responsible for the placing on the market of a tattoo ink shall ensure that the label provides, in addition to that required by Regulation (EC) No 1272/2008, the following information:

- The intended use of the mixture as a tattoo ink;
- A reference number to uniquely identify the batch;
- The name of all substances present in the tattoo ink that meet the criteria for classification for human health in accordance with Annex I of Regulation 1272/2008 but not covered by the current restriction proposal;
- The name of substances covered by the restriction proposal that are present in the ink at a lower concentration limit than the proposed one;
- Any relevant instructions for use.

The labelling shall be clearly visible, easily legible and appropriately durable.

The label shall be written in the official language(s) of the Member State(s) where the substance or mixture is placed on the market, unless the Member State(s) concerned provide(s) otherwise.

Where necessary because of the size of the package, the information labelling shall be included on the instructions for use.

The information on the label shall be made available to any person who will undergo the tattooing procedure before the procedure is undertaken.

The requirement would ensure that substances not covered by the restriction proposal but which may nevertheless present a risk to human health will be listed to inform consumers who intend to undergo a tattoo procedure. This is particularly important in the case of tattoo inks when hazardous substances are deliberately injected under the skin and may have unforeseen consequences due to this route of exposure. It is also important that consumers who are already (cross)sensitised to certain substances can check to see these are not in tattoo inks.

e) Additional conditions

i. Colourants in Annex IV of CPR with conditions on their use

Some colourants used in cosmetic products have been shown to pose a risk to human health when applied to the skin in concentrations exceeding the maximum allowed concentrations specified in Annex IV of the CPR or when not meeting the other conditions in columns "h" to "i" of the Annex (e.g., purity requirements). (See Supplementary Table E.) Therefore, given the similarities in exposure potential (not allowed if not complying with these conditions in cosmetic products which by definition (Article 2 of CPR) are applied, among other, on the external parts of the human body, which include the epidermis), a comparable restriction for use of these colourants in tattoo inks and PMU is proposed.

ii. Restriction on the use of tattoo inks not meeting the requirements by tattoo artists

As it is possible for tattoo artists to stockpile pigments in powered form and mix tattoo inks, the restriction puts the onus on tattoo artists and PMU practitioners to ensure that non-compliant inks are not used for tattoo or PMU purposes by proposing that inks not meeting the restriction requirements are not used in tattoo and PMU procedures.

f) Transitional period

The restriction proposes a transitional period of one year, which will allow sufficient time for actors in the supply chain to meet the proposed requirements:

- Manufacturers (placing tattoo inks on the EEA31 marked) to develop and begin marketing alternative tattoo inks and PMU compliant with the proposed restriction options: It is expected that one year will be sufficient as currently the majority of the inks on the market are compliant with ResAP and the scope of proposed restriction options. Therefore, industry has knowledge and experience to manufacture tattoo inks and PMU compliant with the CoE resolutions and therefore, with the proposed restriction options. Thus, it is expected that the transition will occur faster than the usual period of transition (one to two years);
- The supply chain (including distributors and tattoo artists) to deplete tattoo inks in stock prior to the entry into effect of RO1 and RO2. Expiration dates of sealed pre-dispersed tattoo inks are typically within two years of manufacture, although

industry reports show that more common colours are used sooner than that.[40] Depending on the preservatives used and the sterilisation method, once opened inks expire within a year afterwards.[41] Therefore, it is expected that the time before the entry into effect of the regulation will allow sufficient time to deplete stocks of tattoo inks and PMU.

- The requirements of the restriction measures on the chemical composition of tattoo inks to be communicated in the supply chain: It is expected that the transitional period will be sufficient as the issue of safe use of tattoo inks has been in focus for the past 20 years.
- Enforcement authorities in Member States currently without national legislation to put in place the necessary measures for control and those Member States with national legislation, to amend current national practices. This would also include the development of standardised testing methods for key groups of substances (e.g., PAAs and azo colourants)

g) Definitions and other enforcement considerations

To assist with enforcement, the proposed restriction text includes definitions of tattoo and PMU practices. The dossier also lists the substances included in the scope. (See Supplementary tables A-E.) These lists also include information on whether the substances have been found in tattoo inks and PMU according to surveillance results or literature review as per JRC report (JRC, 2015b). This will assist enforcement authorities to focus their initial efforts checking compliance on the presence of key substances. This list of key substances can be periodically updated on the basis of selected detailed analysis of tattoo inks.

To assist with future risk management measures on tattoo inks, the dossier includes substances listed in Appendix D1 relevant for any future re-evaluation of the restriction. These substances have been identified as problematic for tattoo inks via detailed stakeholder consultations during the development of ResAP by the CoE or during the preparation of this dossier. However, the current level of information available for these substances (as well as workload) did not allow sufficient assessment to include them in the scope of the proposed restriction options. The dossier calls attention to these substances for future investigation of their hazards (in the context of CLP for example) and their risks in the context of future regulation on tattoo inks and PMU.

Furthermore, the establishment of EU-wide registry for tattoo inks and PMU can be considered, to assist with the revisiting of the restriction. The registry, also recommended by some stakeholders (similar to existing national registries), will provide the regulators with relevant information on the substances found in tattoo inks and PMU, which is essential for the assessment of exposure and risk. In addition, by providing photos and other identification features to a centralised database accessible by enforcement authorities, the acute issue of counterfeiting of inks identified by some manufacturers (primarily US-based) may also be addressed. (ECHA CfE, 2016a)

It should be noted that all the aspects not covered by the restriction proposal such as general hygiene requirements or chemicals with no hazard classification are not covered by this proposal and therefore, can continue to be regulated at the Member State level

[40] http://thetattoonerd.blogspot.fi/2016/03/tattoo-ink-expiration-dates.html

[41] https://www.theplasticbottlescompany.com/care-storage-tattoo-ink-hdpe-plastic-bottles/

provided that such national requirements comply with the Treaty provisions on free movement and provision of services.

D.1.1.1. Proposed restriction option: RO1

RO1 is formulated to follow to the extent possible and justifiable, existing national legislation in nine EEA Member States with national legislation on tattoo inks and PMU. Thus, the proposed concentration limits are set as follows:

a) Concentration limits

- Substances on Annex II and IV (column g) of the CPR

Article 14 of the CPR establishes that cosmetic products shall not contain substances listed in Annex II, restricted substances in Annex III and colorants not listed in Annex IV. Article 15(1) and (2) provide that CMRs are prohibited in cosmetic products (except under certain conditions). Under the CPR, the prohibition of Annex II substances is total in the sense that there are no concentration limits; however, Article 17 allows for "non-intended presence of a small amount of a prohibited substance, stemming from impurities of natural or synthetic ingredients, the manufacturing process, storage, migration from packaging, which is technically unavoidable in good manufacturing practice, shall be permitted provided that such presence is in conformity with Article 3" [Safety]. Therefore, in practice, in Member States enforcing the CPR via national legislation, this is a prohibition at the level of detection/quantification of the available analytical methods, taking into account unavoidable impurities (or traces of prohibited substances). Guidance for these limits may be set in some Member States with national legislation on the basis of analytical methods used and best practices. Different Member States may apply different values for trace amounts.

Following the logic of the proposed restriction (i.e., what poses human health risk for application on the skin would also pose risks for injection in the dermis), tattoo inks should not contain prohibited substances in cosmetic products. Therefore, RO1 proposes to enforce Annex II substances under REACH similarly to the CPR.

Substances in Annex IV are also proposed to be enforced in a similar way to Annex II substances in RO1. They are prohibited for use in tattoo inks under national legislation based on ResAP on the premise that they are not allowed in high risk cosmetic applications (i.e., as per column g in Annex IV: in products applied on mucous membranes or in the vicinity of the eye, as well as leave-on products as they are allowed in rinse-off only). This is similar to the Member States enforcing national legislation.

- CMR substances

According to Article 15 of the CPR, CMR substances are periodically added in batches to Annex II, unless industry demonstrates essential use in cosmetics (please see justification for inclusion of Annex II substances in Appendix B.4). As the majority of these substances will be included in Annex II (for category 1A and 1B, this is within 15 months but for category 2, there is no time limit), it would be appropriate to apply the same concentration limit as for Annex II substances, i.e., total prohibition, at least for carcinogenic and mutagenic substances, Categories 1A, 1B and 2.

As threshold effects can be demonstrated for many reprotoxic substances, a concentration limit derived on the basis of quantitative risk assessment is proposed under RO1 for these substances, Categories 1A, 1B and 2.

- Substances with harmonised classification as sensitisers, irritants and corrosives

A practical limit of 0.1% w/w is proposed for the substances with harmonised classification as skin sensitising, corrosive or irritant and eye irritant or damaging to discourage their intentional use in tattoo inks. This will simplify the restriction requirements for stakeholders. (See respective appendixes to Annex B for further justification.)

b) Interlinkages with the CPR

The proposed restriction scope would ideally be linked to Annex II of the CPR to ensure any future updates are reflected in the proposed RO1. This would ideally avoid frequent updating of an appendix to Annex XVII to REACH mirroring Annex II to the CPR. Therefore, the text of RO1 refers directly to CPR Annex II and Annex IV.

See introduction of section D.1.1 for information on other conditions and elements of RO1 that are the same as RO2.

D.1.1.2. Justification for the selected scope of RO1

The proposed RO1 follows existing national legislation in Member States to the extent possible and equalises the level of protection of people in EEA31 who seek to get a tattoo.

The main advantages of RO1 are that it:

- follows national legislation to the extent possible and it will therefore, provide similar level of protection currently applied by national rules in seven EU Member States (and two additional EEA members) that are based on the recommendations of the CoE ResAP;
- is easy to communicate as the proposed restriction scope follows to the extent possible existing current legislation based on the recommendations of ResAP. Tattoo ink manufacturers are already aware of these requirements (although some substances are added). This will facilitate compliance with the proposed restriction;
- will ideally be dynamically linked to Annex II and IV to the CPR and Annex IV of the CLP to ensure future changes to those annexes apply directly to the restriction;
- proposes concentration limits that are derived on the basis of the argumentation for risk.

The main concern with RO1 is that the unavoidable presence of some impurities, not intentionally added to the inks, could result in some inks currently allowed on the market to not be allowed due to the proposed restriction. These unavoidable traces are dealt with in a practical manner in national legislation (on the basis of Article 17 of the CPR), which will be difficult under the setting of Annex XVII of REACH. This could lead to costs to society that are difficult to estimate on the basis of the currently available information.

It is difficult to enforce a restriction without a specific limit value as the default enforcement may be the limit of detection which is linked to the performance of the available analytical methods. Therefore, manufacturers may face some difficulties complying with the restriction and possibly be subject to different treatment in different Member States, depending on the analytical method used by the enforcement authorities. On the other hand, it is not the first time that Annex XVII to REACH includes an entry without a limit value. It is expected that the development of a guideline or harmonised analytical methods will overcome this disadvantage.

The remaining sections of this annex demonstrate that RO1 is effective, practical and monitorable.

D.1.1.3. Proposed restriction option: RO2

The scope of RO2 differs from that of RO1 only in terms of concentration limits (for substances with harmonised classification and those on Annex II and IV of CPR) and the management of the interlinkages with the CPR.

a) Concentration limits

i. Substances with harmonised classification

The maximum concentration of substances with harmonised classification as CMRs, skin sensitisers, corrosives or irritants or eye corrosives or damaging is proposed to be limited to the generic or specific concentration limit of the substances set in the CLP Regulation.

ii. Substances on Annex II and IV (column g) of the CPR

For substances on Annex II, a practical limit of 0.1% w/w is proposed. (See Supplementary Table C.) Similarly, the substances on Annex IV with a restriction on their use in cosmetic products specified in column g of the CPR (i.e., not to be used on mucous membranes, in the vicinity of the eye, or only allowed in rinse off products) are proposed to be restricted in tattoo inks with a practical limit of 0.1% w/w. (See Supplementary Table D.) This will simplify the restriction requirements for stakeholders.

b) Interlinkages with the CPR

While RO1 proposes that any future changes in Annexes II and IV of the CPR are taken up in the proposed restriction automatically, RO2 proposes that only substances on Annex II and Annex IV (columns g-i) at the time of the writing of this restriction dossier are included in the scope.

The other conditions and elements of RO2 are the same as for RO1. See introduction of section D.1.1 for further detail.

D.1.1.4. Justification for the selected scope of RO2

The main rational for considering a restriction option with different concentration limits than RO1 is that colorants in particular are often of low purity and therefore, a number of currently unknown impurities could potentially be contained in tattoo inks. As explained previously, the Member States that currently have national legislation on tattoo inks in place, enforce prohibition on substances on Annex II, CMRs and Annex IV substances (column g) similar to cosmetic products whose use is regulated by the CPR. This means if these substances are found in trace amounts in tattoo inks (i.e., due to, as stated in Article 17 of the CPR), they would not be considered non-compliant. As pigments are not manufactured by the formulators of tattoo inks, many such impurities of the manufacturing process could also be contained in the tattoo inks, which are mixtures of a colorant in a solution of auxiliary ingredients. As it is extremely complex to catalogue all impurities that can be found in tattoo inks, a broad brush approach is taken, where a restriction is proposed on substances which can cause skin and systemic effects in humans in order to encourage the use of higher purity, lower risk pigments and auxiliary ingredients in tattoo inks. However, as the list of impurities is unknown, in particular for those pigments that are currently not widely used in the manufacture of tattoo inks, there is the risk of the regulation to render a great share of tattoo inks currently the market as non-compliant if unobtainable concentration limits are imposed. Therefore, this second – RO2 – restriction option is proposed with higher practical limit (0.1% w/w) for CPR substances in scope and the CLP limits for those with relevant harmonised classification.

Another reason harmonised classification limits are convenient concentration limits for a restriction on tattoo inks is that, according to the CLP Regulation, substances in mixtures with harmonised classification need to be specified on the label and the safety data sheet. This will facilitate industry compliance and lead to lower testing costs. It will also facilitate enforcement by competent authorities.

RO2 is also proposed to decouple the restriction from future updates of Annex II and IV of the CPR. Although there is an advantage to take on board changes implemented in the CPR Annex II and IV (on the premise that what poses human health risk for application on the skin would also pose risks for injection in the dermis), a static list of substances (i.e., those included in the CPR as of the writing of the dossier) evaluated for the purpose of a restriction on tattoo inks would avoid legislative gaps that could arise in cases such as these for example:

- If the restriction is dynamically linked to Annex II of the CPR, tattoo inks containing these substances could not be placed on the market (if intentionally added). The CPR has provisions for CMR category 2 substances to be allowed in cosmetic products if the SCCS concludes they are safe to use, leading to their inclusion in Annex III-VI, instead of II. If the cosmetic industry is not interested in making the case for this substance, it will directly be included in Annex II (even though theoretically safe use can be demonstrated under certain conditions). This is creating a situation, where in order to defend a use in tattoo inks for a CMR category 2 substance, the tattoo industry would have to create a fictitious application for use in cosmetics to be evaluated by the SCCS with a recommendation for inclusion in Annex III-VI instead of Annex II. This does not comply with the objective of good administrative practices of the European Commission.
- If the restriction is dynamically linked to Annex IV of the CPRs, a colorant A allowed for rinse off products only will be restricted in tattoo inks. Following an SCCS evaluation, colorant A is removed from Annex IV (altogether or placed on Annex III for example) because it can no longer be demonstrated that it is safe for rinse off use. The colorant will no longer be banned for use in tattoo inks and its removal from Annex IV on grounds of new evidence of greater hazard and risk could lead to more flexible regulation for tattoo inks, paving the way for its reintroduction in tattoo inks.

Therefore, RO2 is proposed as avoiding legislative gaps as the above theoretic examples can be considered more desirable than the possibility to future proof the restriction by dynamically linking it to analysis of relevant substances, specifically under the CPR. The absence of future proofing of RO2 with respect to the CPR can be overcome by periodic examination of the restriction. This may be warranted given the high complexity of the proposed legislation. See section D.1.1 for possible ways to facilitate this.

The main advantages of RO2 are that it:

- will likely lead to lower testing costs as the safety data sheets contain information on substances with harmonised classifications that are present in concentrations above their classification limits. (According to the CLP Regulation, substances in mixtures with harmonised classification need to be specified on the label. This will facilitate industry compliance and lead to lower testing costs.)
- is easy to communicate to law makers, enforcement and industry that must comply with the restriction;
- proposes concentration limits that are derived on the basis of the argumentation for risk, as they are based on CLP limits;

- will allow greater share of inks currently on the market containing some impurities to continue to be supplied.

The main disadvantages RO2 are that it:

- allows higher concentrations of hazardous substances (including substances of very high concern) to be injected under the skin. Tattooed persons can theoretically have a lower level of protection than persons using cosmetics on the surface of the skin. For some substances, it may result in a lower level of protection in Member States that already have national legislation based on ResAP;
- is less consistent as substances on Annex II of CPR will have different concentration limits even though they have similar concerns with respect to human health risks (i.e., those with various classifications and those without).

On the other hand, there is currently no information suggesting that industry is unable to meet lower concentration limits for some of these substances in particular since many of the substances have not been found (although also possibly not measured) yet in tattoos inks. Higher concentration limits can reduce the incentive for industry to continue to seek ways to reduce exposure to hazardous substances in tattoo inks and may reverse replacement that has taken place or is taking place as a result of national legislation based on ResAP.

The remaining sections of this annex demonstrate that RO2 is effective, practical and monitorable.

D.1.2. Discarded restriction options

The following additional risk management options (RMO) were investigated:

- RMO1: Restriction option based on the recommendations of CoE ResAP(2003)2 for prohibition of certain substances in tattoo inks and PMU

As further explained in the section Other Union-wide risk management options than restriction, four EU (Belgium, France, Germany and the Netherlands) and two EFTA (Norway and Switzerland) Member States have national legislation based on CoE ResAP(2003)2. However, recommended limits for impurities in Table 3 of CoE ResAP(2008)1 have either formally been subsequently included in the national legislation (e.g., Switzerland) or are used as guidance for acceptable levels of selected impurities (e.g., Norway).

The risk assessment concluded that the exposure to impurities in Table 3 of CoE ResAP(2008)1 exceeding certain levels lead to risk to human health. Therefore, this RMO was not assessed further as it does not sufficiently address all risks to human health arising from substances that can be present in tattoo inks.

- RMO2: Restriction option based on the recommendations of CoE ResAP(2008)1 for prohibition of certain substances in tattoo inks and PMU

As further explained in the section Other Union-wide risk management options than restriction, three EU Member States (Spain, Slovenia, and Sweden) and Liechtenstein have national legislation based on the CoE ResAP(2008)1. The risk assessment of substances that can be present in tattoo inks revealed that there are others, not included in the scope of ResAP, that can lead to human health risks, e.g., skins sensitisation, corrosion, or irritation and eye damage or irritation. Therefore, it was concluded that this RMO does not sufficiently address all risks to human health arising from substances that can be present in tattoo inks and was not assessed further.

D.1.3. Other Union-wide risk management options than restriction

a) Background

Tattooing has been practiced for centuries but it has increased substantially in popularity in the last twenty years. With its growing popularity, the frequency of health concerns has also increased and the safety of tattoos and body piercing became the subject of concerns expressed by the Commission, Member States and the European Parliament. In 2000, the Scientific Committee on Cosmetics and Non Food Products (SCCNFP) noted in its 17 February opinion the large number of colourants used in tattooing for which the chemical structure, identity, and toxicological profile are incomplete or unknown, thereby precluding a proper risk assessment. As a result, the SCCNFP called for a systematic information gathering by the Joint Research Centre and DG SANCO at the time, which was done in collaboration with the Council of Europe (CoE). On the basis of this review (Papameletiou, et al., 2003), the SCCNFP concluded in its opinion of 20 October 2003 that tattooing colourants and piercing materials represent a legal paradox in the EU: Although they are used for cosmetic purposes, the route for their administration (injection/skin penetration) in effect puts them outside the scope of the Cosmetics Directive (76/768/EEC). Tattoo inks were therefore to be considered as general consumer products and hence to be regulated under the General Product Safety Directive (92/59/EEC) and possibly under the Limitations Directive relating to restrictions on the marketing and use of certain dangerous substances and preparations (76/769/EEC, today REACH). (SCCNFP, 2003)

In 2003, the CoE published a resolution on requirements and criteria for the safety of tattoos and permanent make-up, which was revised in 2008. In 2014, the European Commission launched a research project to gather and scrutinise all available information for considering the need for a coordinated initiative on tattoo and PMU inks at EU level. The work of the European Commission is summarised in four publications on the Safety of tattoos and permanent make-up: (JRC, 2015a), (JRC, 2015b), (JRC, 2016a), (JRC, 2016b). As a result and after considering the other relevant legislative options, the European Commission requested ECHA to assess whether there is a need to restrict certain substances used in tattoo inks and permanent make up.

b) Existing EU-wide measures

General Product Safety Directive (GPSD)

Currently, there is no EU-wide measure specifically for tattoo inks or PMU. As they can be seen as products intended for, supplied, used, made available in the course of a commercial activity to consumers, they fall in the scope of the General Product Safety Directive (GPSD, Directive 2001/95/EC of the European Parliament and of the Council of 3 December 2001 on general product safety).[42] The Directive puts the onus on producers and distributers to ensure that only safe products are on sale. Member States have the responsibility to monitor whether products available on the market are safe, ensure product safety legislation and rules are applied by manufacturers and supply chains and apply sanctions or other appropriate measures. They also use the Community Rapid Information System (RAPEX) on products posing risks to consumers. Article 3.3 of the GPSD states that in the absence of Community provisions or national legislation and similar provisions, the conformity of the product to the general safety requirements is to be assessed taking into

[42] Article 2(a) of the GPSD defines the type of products covered.

account the following elements: "(d) product safety codes of good practice in force in the sector concerned; (e) the state of the art and technology; (f) reasonable consumer expectations concerning safety." While it may be argued that under these provisions, the CoE ResAP could be enforced EU-wide, national legislation was introduced in close to one-third of EU and EEA Member States, indicating that the provisions in the GPSD are insufficient to adequately control the risks to human health EU-wide. In addition, the RAPEX notifications from EU countries are almost exclusively from those seven Member States (and two EEA members) who have translated the CoE ResAP into national law, suggesting that the GPSD is not widely applied for tattoo inks and PMU in the EU. Furthermore, as shown in Annex B, there are other substances in addition to those in ResAP ((2008)1 and its predecessor) that pose risk to human health if injected intradermally with tattoo inks or PMU.

Council of Europe (CoE) Resolution (ResAP (2008)1)

Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up[43] (superseding Resolution ResAP(2003)2[44] on tattoos and permanent make-up) laid out a number of provisions related to the chemical composition of tattoo inks as well as tattoo practices to ensure that tattoo and PMU products must not endanger the health and safety of human health:

- the composition and labelling of products used for tattoos and PMU;
- the risk evaluation required before products used for tattoos and PMU are placed on the market, i.e., the manufacturer or person responsible for placing the product on the market should perform a risk evaluation based on recent toxicological data and knowledge;
- the conditions of the application of tattoos and PMU;
- the obligation to inform the public and the consumer of the health risks of tattoos and PMU and tattooing practices. (ResAP (2008)1)

With respect to the chemical composition, ResAP (2008)1 specifies the following requirements for tattoo inks and PMU:

- They do not contain or release the aromatic amines (listed in Table 1 of ResAP (2008)1) in concentrations that are technically avoidable according to good manufacturing procedures. The presence or release of these aromatic amines is to be determined using appropriate test methods which are to be harmonised across the member states in order to ensure comparable health protection of the consumer and to avoid divergent enforcement, drawing on existing methods which can serve as models (specified in Tables 4.a-c of ResAP (2008)1);
- they do not contain substances listed in Table 2 of (ResAP (2008)1), i.e., primarily colourants with CMR properties;

[43] Adopted by the Committee of Ministers on 20 February 2008 at the 1018th meeting of the Ministers' Deputies of the CoE https://wcd.coe.int/ViewDoc.jsp?p=&Ref=ResAP(2008)1&Language=lanEnglish&Ver=original&direct=true

[44] Adopted by the Committee of Ministers on 19 June 2003 at the 844th meeting of the Ministers' Deputies of the CoE https://search.coe.int/cm/Pages/result_details.aspx?ObjectID=09000016805df8e5

- they do not contain substances listed in the CPD Annex II (prohibited substances in cosmetic products);
- they do not contain substances specified in the CPD Annex IV not allowed in the vicinity of the eye, on mucous membranes or allowed only in rinse-off products (former columns 2 to 4, current column g of the CPR);
- they do not contain carcinogenic, mutagenic and reprotoxic substances of categories 1a, 1b or 2 which are classified under the CLP Regulation;
- they comply with maximum allowed concentrations of impurities listed in Table 3 of ResAP (2008)1 and the minimum requirements for further organic impurities for colourants used in foodstuffs and cosmetic products as set out in Directive 95/45/EEC;
- preservatives should only be used to ensure the preservation of the product after opening and not as a correction of insufficient microbiologic purity in the course of manufacture and of inadequate hygiene in tattooing and PMU practice;
- preservatives should only be used after a safety assessment and in the lowest effective concentration. (ResAP (2008)1)

The ResAP is not a binding legislative instrument and member states of the CoE need to introduce national legislation in order to make the provisions binding.

c) National legislation

A number of EU Member States have translated the CoE ResAP into national law:

- Seven EU Member States have a specific national legislation in place based either on CoE ResAP(2003)2 (Belgium, France, Germany and the Netherlands), or on CoE ResAP(2008)1 (Spain, Slovenia, and Sweden);
- Three EU Member States – Austria, Denmark and Latvia – have prepared draft legislation based on the CoE ResAP(2008)1;[45]
- EFTA countries have legislations in place: Liechtenstein – based on the CoE ResAP(2008)1, while Norway and Switzerland are based on the CoE ResAP(2003)2, however, the latter has also introduced the recommended thresholds for heavy metals and PAHs of the 2008 ResAP. No data were available on Iceland. (JRC, 2015a), (Hauri, 2016)

In Italy, ResAP is not mandatory but the Legislative Decree # 206/2005, on the basis of Directive 2001/95/CE, confirms its binding power. For this reason, tattoo inks placed on the Italian market must be in accordance with this legal framework. (Renzoni, et al., 2015) However, there are differences at a regional level in Italy. (ECHA CfE, 2016a)

Of all Member States who have incorporated ResAP in their national legislation, only Spain maintains a positive list of tattoo inks that can be placed on the market. Tattoo inks in Spain are covered by the national legislation on cosmetics. In addition to adopting the principles of ResAP in 2008, tattoo inks have to be approved by the Spanish Agency for

[45] Denmark and Austria in 2013 and Latvia in 2014 have notified draft national legislation on tattooing products and services. The proposed drafts are currently put on hold by the Commission as they are in conflict with REACH provisions.

Medicines and Health Products on the basis of toxicological and quality data supplied by the distributor. Approved products are included in the registry. (Laux et al 2015)

No other EU Member States enforce specific requirements for chemical composition of tattoo inks, either those of CoE ResAP or other, even though other aspects of tattoo practices may be regulated. According to JRC reports (JRC, 2015a), Italy, Malta, Romania, and also to some extent the Czech Republic, Finland and Slovakia do regulate tattooing practices and premises safety, in terms of health and hygienic requirements, but they did not transpose the CoE ResAP into their national legislative scheme. Bulgaria, Croatia, Cyprus, Estonia, Greece, Ireland, Luxembourg, Poland and Portugal do not have specific legal texts on tattooing activities.

For further information on EU Member State regulations on tattoo and PMU practices, please consult the JRC report (JRC, 2015a).

d) Other EU-wide measures considered

On 3 December 2015, the European Commission formally requested ECHA to examine the need for a restriction under REACH of selected substance groups present in tattoo inks and PMU. The request was sent as it was determined that REACH is the most suitable measure based on an initial assessment of several EU-wide risk management measures. With that said, ECHA also evaluated the effectiveness, practicality and monitorability of other EU-wide measures in comparison to the proposed restriction:

Cosmetics Products Regulation (CPR)

Regulation (EC) 1223/2009 http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02009R1223-20160812&from=ENdefines cosmetic products as "any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours." As tattoo inks and PMU are injected into the dermis, they do not fall into the scope of the CPR. In addition, the regulation specifies in its annexes a number of substances banned for use or allowed in cosmetics under specific conditions. While many of the substances banned for use on the epidermis likely constitute risk for injection in the dermis, there are a number of substances allowed in cosmetics that cannot be present in tattoo inks. Therefore, the integration of the tattoo inks in the CPR would require substantial changes to the cosmetics legislation. It was concluded that the CPR is a less practical approach than the proposed restriction.

Biocidal Products Regulation (BPR)

The EU Biocides Regulation 528/2012 covers a very diverse group of products, including preservatives. As tattoo inks are not considered cosmetics, the in-can preservative used in tattoo inks are not subject to the cosmetics regulation, and therefore are de facto subject to the BPR rules. This includes rules regarding the placing on the market of the active substance and biocidal products, and since 2012, additional rules on the placing on the market of "treated articles" (as defined in Article 3(1)(l) of the BPR, such as mixtures preserved with in-can preservatives). In practice, it means that:

- since 1 September 2006, only active substances in the Biocidal Review Programme (i.e., listed in Annex II to Regulation (EU) No 1062/2014), or approved, for Product-

type 6 "in-can preservatives" can be made available on the market and used in the EU by EU manufacturers of tattoo inks.

- since 1 March 2017, only tattoo inks preserved with in-can preservatives approved or under assessment on 1 September 2016 (see article 94 of BPR) can continue to be placed on the EU market (also relevant for imported tattoo inks).

The obligations concern the "placing on the market" as defined in Article 3(1)(j) of the BPR, and not the subsequent supplies. Tattoo inks already supplied or further in the supply chain are not concerned by these provisions (i.e., they might still contain preservatives not assessed and approved in the EU). As well, it does not forbid the use of tattoo inks which were preserved with preservatives not assessed and approved in the EU.

The approval decisions on active substances are usually not specific, and do not forbid or put restrictions on the use of active substances unless specific risks have been identified at the approval stage. Therefore, the question of tattoo inks is not likely to be looked at the approval stage of active substances but would rather be assessed at the biocidal product authorisation stage, where the use of each product must be precise enough, as a biocidal product shall only be used in the EU for its authorised used. Therefore, if the use in tattoo inks is not mentioned in the authorisation of the biocidal product, it is de facto not authorised for that. To date, there are no known biocidal product applications for authorisation for tattoo inks.

As the BPR regulates only preservatives as part of the tattoo ink mixture, the use of pigments, additives and fillers in tattoo inks is not in its scope. The proposed restriction would not change the obligations under the BPR but would limit the type of preservatives that can be authorised for the use, i.e., to only those that are not classified as CMRs, skin sensitisers, irritants or corrosives and eye corrosive or damaging.

Classification and labelling

The CLP requires assignment of hazard categories, based on available information, to substances and subsequent labelling provisions to indicate the intrinsic hazard of the substance to the users. These requirements already apply to tattoo inks and do not in themselves restrict the placing on the market of mixtures containing these substances. The proposed restriction is linked to the requirements of the CLP in that substances with certain hazard categories are not allowed in tattoo inks above a certain concentration limit.

EU Ecolabel

The EU Ecolabel applied to textile products were also assigned to tattoo inks thus excluding the use of dyes with harmful properties: Commission Decision of 15 May 2002 establishing the ecological criteria for the award of the community eco-label to textile products and amending Decision 1999/178/EC (2002/371/EC), Official Journal of the European Communities 2002; L133@29-41. Tattoo inks also must not contain carcinogenic azo dyes or aromatic amines in accordance with an opinion of the Scientific Committee on Cosmetic Products and Non-Food Products that refers to cosmetics: Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers Concerning the Safety Review of the Use of Certain Azo Dyes in Cosmetic Products, SCCNFP/0495/01, final 2002. SCCNFP, 2002. This is a voluntary measure and even if expanded will not restrict undesired substances in tattoo inks and PMU in a consistent and harmonised manner.

Other voluntary industry actions

Voluntary measures by industry exist to a varying degree in EU Member States. These may include provision of information by tattoo artists on the risks and after care, including advice on consulting a physician (specialist, tattoo artists) in the event of adverse reactions and complications as well as an exchange of information through the supply chain on tattoo and PMU inks that may have led to adverse reactions. The degree of voluntary measures depends to a large extent on the organisation of the sector for example via professional associations or formal regulation related to tattoo practices and services such as certification, registration, requirements for formal training, etc. Overall, the level of organisation of the sector varies substantially from one Member State to another. Given the high level or variability EU-wide, the large number of often non-organised operators, as well as the high percentage of non-registered tattoo service providers, it is likely that voluntary measures that effectively control the risk to human health will be difficult to agree and implement uniformly within the EU28. Such measures have not been proven fully effective to date.

Separate legislation on tattoo inks

In addition to the chemical composition of tattoo inks, there is an array of other factors that influence the safety of tattoo practices. These relate to hygiene,[46] registration, certification or training requirements of tattoo artists. There are significant differences how and whether these are addressed at national level. An advantage of a standalone EU-wide legislation would be that all these elements (and not only the chemical composition under REACH) can be addressed in concert and applied universally EU-wide. A standalone legislation would also be able to address the specific needs of tattoo products, which some claim can be unique in comparison to similar products such as cosmetics (as, for example, cosmetic products are applied on the surface of the epidermis or mucous membranes while tattoo inks are injected in the dermis).

The main disadvantages of a standalone legislation is that it will be difficult and time consuming to negotiate EU-wide, especially since a number of issues such as business licenses, training and certification, etc. are currently within the jurisdiction of local and regional authorities and some maintain that these issues are best addressed at this level of governance, similar to the regulation of other professions and business licensing. On the other hand, the leadership in the field of chemical regulatory management is centralised and managed via REACH.

[46] Currently, a CEN standard (TC 435) is being developed on the hygienic requirements before and during tattooing. It is expected to contain guidelines for correct procedures for protection of the client and self protection for the tattooist

D.2. Alternatives

D.2.1. Description of the use and function of the restricted substance(s)

Tattoo ink (and PMU) is a suspension of pigment particles (small, solid particles, insoluble in, and normally not affected by, the medium in which they are suspended in a solution of water), glycerine and alcohol. The tattoo is the result of the pigment (also referred to as colourants) in the skin after healing, as the properties of colourants change the appearance of an object by the selective absorption or scattering of light.

The substances in the scope of the proposed restriction belong to three distinct groups: colourants, auxiliary ingredients and impurtities, which are briefly described below. Additional information on the function of the substances and composition of tattoo inks is presented in a JRC report (JRC, 2015a).

a) Colourants

"Colourant" is the commonly used denomination for pigments, lakes and dyes that are coloured molecules. (ResAP(2008)1) Pigments are colourants that are insoluble in its own matrix. (Olsen, 2015) They are responsible for the ink colour and are main constituents of tattoo and PMU inks. JRC (JRC, 2015b) reports that their concentration in tattoo inks can reach almost 60% w/w, although typically they are about 25% of tattoo inks specifically. Pigments used in tattoo inks are distinguished with high light fastness, weather stability, and low-migration properties. (Petersen & Lewe, 2015). These qualities differentiate them from other colourants, i.e., dyes, which due to their solubility and fast biodegradability after application are generally not suitable for use in tattoo inks. In case dyes are used, more often in PMU than in tattoo inks, they are in the form of the so-called "lakes", which are produced by precipitating dyes onto an insoluble base or stratum made by insoluble inorganic compounds, such as barium sulfate and aluminium hydroxide making them more stable to light and other chemicals. (JRC, 2015b) In PMU, often iron oxide pigments (inorganic) are used because they give natural shades. They may fade over time.

Pigments can be grouped in two distinct categories: inorganic or organic substances.

i) Organic pigments

Organic pigments are favoured for tattooing because of their high tinting strength, light fastness, enzymatic resistance, dispersion, and relatively inexpensive production. (Olsen, 2015) Organic pigments are mainly synthetically made and contain carbon (Prior, 2015), although vegetal pigments also fall into this category. Vegetal pigments are often derived from root plants or logwood as well as dried vegetal algae used in some black inks. (Agnello & Fontana, 2015). For example, carbon black, used to produce the popular pigment Indian ink, is obtained by burning bones, tar, pitch, and other substances. Carbon black has historically been used in printing inks and paint. In the past 50 years, it is used in the rubber industry primarily in the manufacturing of tires. Examples of natural pigments used today are curcumine (root extract), brazilin (Brazil wood or Natural Red 24) and santalin (red sandalwood or Natural Red 22/23). (De Cuyper & D'hollander, 2010)

The synthetic organic pigments are the most variable group: According to (Olsen, 2015), approximately 80% of the pigments in tattoo products belong to this group, while (JRC, 2015b) report a slightly higher percentage: 81% of all tattoo ink pigments and 84% of all PMU inks. Synthetic organic pigments fall in the following chemical classes:

- Nitro dye, associated with yellow colours

- Xanthenic dye, associated with various colours, manly shades of yellow;
- Phtalocyannine compounds, associated with blue and green colours;
- Antraquinone dyes, associated with red and brown colours;
- Azo dyes, used for colours such as yellow, red or orange; (Agnello & Fontana, 2015)
- Dioxazine compounds, associated with mostly violet and magenta colours. (De Cuyper & D'hollander, 2010)

Azo dyes represent the largest category of organic pigments: More than 2 000 azo compounds are listed on the Colour Index™ (C.I. ™) (www.colour-index.com) published by the Society of Dyers and Colourists (SDC) and the American Association of Textile Chemists and Colourists (AATCC), promotes a universally accepted dual classification system for dyes and pigments.[47] According to a JRC report (JRC, 2015a), the azo dyes represent 65% of organic pigments used in tattoo inks and 64% of those used in PMU. They are used in wood, plastics, diesel and clothing. Studies have shown that azo dyes can release primary aromatic amines (some of which carcinogenic or mutagenic) under light irradiation. (JRC, 2015b) (See Appendix B.2 for more information.)

A closer look at the CI database reveals a more detailed classification of organic colourants: nitroso, nitro, monoazo, diazo, triazo, polyazo, azoic, stilbene, carotenoid, diphenylmethane, triarylmethane, xanthene, indamine, indophenol, azine, oxazine, thiazine, sulphur, lactone, aminoketone, hydroxyketone, anthraquinone, indigoid and phthalocyanine chemical category. Of these classifications, azo pigments are characterised by the azo group (–N=N–), while the majority of the other categories consist of polycyclic pigments, having aromatic rings in their structure. One exception is represented by the triarylmethane category. (JRC, 2015b)

ii) Inorganic pigments

Inorganic pigments are more frequently used for PMU than for tattoo applications, due to their dull and non-brilliant colour compared to organic ones (JRC, 2015b), which make them more compatible with the natural tones observed on the human body. They are made from minerals such as magnetite (brown and dark colours), cinnabar (red, although rarely used today), various metal oxides and sulphides (for a range of colours, e.g., titanium dioxide and barium sulfate for white pigments or to brighten darker shades, iron oxide for red, brown and black as well as colours similar to the shade of the skin). (Agnello & Fontana, 2015) (Prior, 2015) According to (Prior, 2015), barium sulfate, titanium dioxide, and iron oxides are the main inorganic pigments used. Studies have shown that the latter two groups undergo oxidative reductive changes under laser light, resulting in paradoxical darkening. (De Cuyper & D'hollander, 2010)

A substantial concern regarding both organic and inorganic pigments is their purity. (See below section on Impurities)

[47] The Colour Index™ (C.I. ™) lists approximately 31 000 dyes and pigments under 11 691 Generic Names (CIGN) and the corresponding Colour Index™ (C.I. ™) Constitution Number (CICN). For further information, see: http://www.colour-index.com/ and http://colour-index.com/assets/files/upl/CI_leaflet.pdf

Table 131 Overview of colourants used in tattoo and PMU inks

Colour	Tattoo ink colourants							PMU inks						
	Total # of colourants	Organic		Azo		Inorganic		Total # of colourants	Organic		Azo		Inorganic	
		#*	% of total	#	% of total	#	% of total		#	% of total	#	% of total	#	% of total
Red	44	41	93	30	68	2	5	42	40	95	27	64	2	5
Yellow	27	25	93	21	78	2	7	25	24	96	20	80	1	4
Orange	10	10	100	7	70	0	0	8	8	100	6	75	0	0
Blue	7	5	71	1	13	2	29	5	3	60	0	0	2	40
Green	4	3	75	0	0	1	25	5	3	60	0	0	2	40
Violet	9	7	78	0	0	2	22	6	5	83	0	0	1	17
Brown	3	1	33	1	33	1	33	2	1	50	1	50	1	50
Black	6	0	0	0	0	5	83	4	0	0	0	0	4	100
White	3	0	0	0	0	3	100	3	0	0	0	0	3	100

Note: *Number

Source: Table 4.20 and 4.21 (JRC, 2015b)

b) Auxiliary ingredients

According to (JRC, 2015b) additives are used to modify certain characteristics and are usually added in a concentration lower than 5% by weight. Those can include:

- Surfactants: They are used to adjust surface tension thereby promoting dispersion and stabilisation of pigments. Pigment dispersions tend to agglomerate to reach the smallest possible surface in a given volume, and surface-active substances help to reduce or avoid this phenomenon by facilitating the wetting of pigments by binder solution. In order to inhibit the sedimentation of pigment dispersions during long term storage, thixotropic agents, e.g. silica, are part of the formulation of inks. They increase the viscosity and thixotropy of the product.
- Binding agents: They consist of non-volatile compounds, whose function is to bind pigment particles to each other and to the tattooing needle with the aim to facilitate the injection of tattoo and PMU ink in the skin. As reported by (Dirks, 2015), the binders most frequently used in tattoo inks consist of polyethers, polyvinylpyrrolidone, block copolymer and Shellac. They are of high molecular mass (generally in the range of thousands of g/mol).
- Solvents: Water is often used to solubilise and solvate binders. Alcohols, for instance ethanol and isopropyl alcohol, can be used to modify the drying properties, viscosity and dispersability of inks. Their concentration should be limited to avoid skin irritation. Glycerine can be added as an ingredient as it acts as humectant and helps increasing viscosity, while propylene glycol can be used as humectant and to increase dispersability.
- Fillers: Those include primarily inorganic substances, which influence dispersability properties promoting re-dispersion of pigments after long term storage. Silica and barium sulfate are mainly employed for this aim. (JRC, 2015b)

Another group of auxiliary ingredients are preservatives. They are used to avoid the growth of microorganisms in the product after opening. Preservatives can be common allergens. ResAP(2003)2, which is the basis for the national legislation in Belgium, France, Germany, the Netherlands, and Norway, recommended that preservatives should not be used. Out of these Member States, only Norway has a positive list of preservatives, allowing the use of 26 substances with low sensitisation potential.

The revised version (i.e., ResAP(2008)1), which has been adopted by the remaining EU and EFTA Member States with national legislation, specified that preservatives should only be used after a safety assessment and in the lowest effective concentration to ensure the preservation of the product after opening and by no means as a correction of insufficient microbiologic purity in the course of manufacture and of inadequate hygiene in tattooing and PMU practice.

The growth of microbiological organisms in inks is possible due to the high quantity of water present in the formulation. Reducing the water content can be an alternative way to prevent the proliferation of microorganisms in tattoo inks. (JRC, 2015b) Another possibility for maintaining sterility (and reducing the need for using preservatives) is that already sterile inks are supplied in a container which maintains the sterility of the product until application, preferably in a packaging size appropriate for single use. In case multi-use containers are used, their design would need to ensure that the contents would not be contaminated during use. (ResAP (2008)1)

Preservatives in tattoo inks are under the scope of the biocides regulation, this category of substances will therefore not be further assessed in this restriction as the continuing use of these substances is subject to the authorisation regime of the Biocides regulation. However, it should be noted that certain preservatives may be restricted for use in tattoo inks due to their harmonised classification of hazardous properties (e.g., formaldehyde, 2-phenoxyethanol, triclosan, 3-iodo-2-propynyl butylcarbamate).

c) Impurities

Many of the tattoo ink impurities are due to the manufacturing process and some can be unavoidable. For example, impurities can be the result of the use of mediums, e.g., stainless steel, that leave traces in the formulated mixture or as a result of the degradation/reaction of the substances contained in the tattoo inks, e.g., primary aromatic amines.

Other impurities are found as manufaturers formulate tattoo inks from previously synthesised substances containing these impurities as colourants are not exclusively manufactured for tattoo ink and PMU, but for other sectors for which greater purity of the pigment is not required, e.g., plastics, paint manufacturing, etc. Tattoo ink manufacturing is not a substantial pigment use. The colourants used in tattoo or PMU inks are often manufactured for other purposes, e.g., industrial applications (printer inks, car paints) or food and cosmetic uses, the latter two representing smaller market segments than the industrial use. In the EU, the purity of pigments is differentiated according to cosmetic, food and medical requirements specified respectively in EU directives 76/768/EOF, 95/45/EC and 78/25/EC. These higher purity pigments usually command higher market price. As stated in section Manufacture, import and export, the main constituents of tattoo inks, pigments, have low purity: between 70 and maximum 90%, depending on the source reviewed by (JRC, 2015b). Surveillance results of colourants show the presence of impurities such as

chromium VI in chromium oxides; nickel, chromium, copper and cobalt in iron oxides; aromatic amines in azo colourants and polycyclic aromatic hydrocarbons in carbon black. (JRC, 2015b)

Although impurities do not perform particular functions in tattoo inks some stakeholders have stated that the small presence of certain metals can contribute to the permanence and brightness of the tattoo ink. (Asaff, 2017).

D.2.2. Identification of potential alternative substances and techniques fulfilling the function

The Colour Index™ (C.I. ™), www.colour-index.com, published by the Society of Dyers and Colourists (SDC) and the American Association of Textile Chemists and Colourists (AATCC), promotes a universally accepted dual classification system for dyes and pigments. It lists approximately 31 000 dyes and pigments listed under 11 691 Colour Index™ (C.I. ™) Generic Names (CIGN) and the corresponding Colour Index™ (C.I. ™) Constitution Number (CICN). The Colour Index is split into two parts: Part 1 covers pigments and solvent dyes, widely used in the paint, plastics, ink and other colouration industries. Part 2 covers dyes and related products in the following main dye classes: acid, basic, direct, disperse, food, fluorescent brightener, mordant, reactive, sulphur and vat, plus several other classes of minor or historical importance. The colourants are registered by, e.g., textile, paint, plastic, printing ink, and cosmetic manufacturers and suppliers.

Table 132 Number of colourants by main colour

Main Colour	Registered CI generic names (#)	Registered commercial products (#)
Black	815	2 434
Brown	1 226	1 357
Blue	1 947	5 889
Green	557	1 584
Orange	989	2 638
Red	2 292	8 348
Violet	665	1 969
White	39	181
Yellow	1 651	6 163

Note: Numbers exclude historic data.

Source: Colour Index database: www.colour-index.com

Identification of potential alternative tattoo inks and PMU is primarily focused on identifying alternative colourants which are mixed in a solution of auxiliary ingredients such as fillers and additives. Not all of the 31 000 colourants listed on the colour index database have been used in tattoo inks ((JRC, 2015b) reports information about 154 that have been) but they can be potential candidates for use in tattoo inks and PMU if they meet strict technical requirements.

The main qualities of importance for tattoo inks and PMU are the colour hue (of particular importance for PMU where tones close to natural complexion and features are essential), brilliance (maintained even after mixing with other colours), permanence (the colour does not change over time), as well as good workability (viscosity) and healing properties.

Particle size is also of importance. The optimal particle size is 1-5 microns. If the pigment particle size is smaller, they can be removed from the tattoo site. If they are larger, the body can reject them as foreign matter (e.g., via granulomatous reactions). (stakeholder consultation)

D.2.3. Risk reduction, technical and economic feasibility, and availability of alternatives

D.2.3.1. Availability of alternatives

Given the sheer number of substances that are found or can be used in tattoo inks, it is impractical (and extremely difficult) to investigate the availability of alternatives for each substance included in the scope of the proposed restriction options (in excess of 4 000). Therefore, to assess the availability of alternatives and the share of alternative tattoo inks on the market, the dossier uses as a proxy national surveillance results ascertaining compliance with national legislation or ResAP recommendations. National legislation and ResAP recommendations are seen as a good basis for comparison due to the similarities with the proposed restriction options. RO1, which proposes somewhat stricter concentration limits for some substances than RO2, is similar to the recommendations in ResAP(2008)1 (and national legislation based on those in four EEA Member States), although there are notable differences with respect to: sensitising, irritant, corrosive and damaging substances, azo dyes and selected impurities in Table 3 of ResAP(2008)1. The latter is the major difference with the earlier version of the CoE recommendations (ResAP(2003)2), which is the basis of national legislation in five EEA Member States. However, many of the Member States with legislation based on ResAP(2003)2 use the limits in Table 3 of ResAP(2008)1 as a guideline for enforcement of what is technically achievable and what can be considered non-intentional presence of traces of prohibited substances according to Article 17 of CPR (although there are national differences for, e.g., nickel). Therefore, in general terms, it can be stated that RO1 is similar to national legislations and it can be expected that similar share of tattoo inks that are shown to be compliant with national legislation can be expected to be compliant with RO1. RO2 (which has higher limits for several of the substance groups) is less strict than national legislation in the nine Member States. Therefore, more tattoo inks than those reported by national surveillance results can be expected to be compliant with RO2. These "alternatives", i.e., tattoo inks and PMU that are compliant already with ResAP requirements, are not expected to require changes due to the proposed restriction options, such as reformulation, material costs etc. leading to higher prices for downstream users.

Overall, surveillance results are seen as a practical, an alternative assessment approach given the complexity of the market situation despite limitations: Although, no enforcement authority has tested the presence of all substances with restrictions on their use for practical reasons, surveillance projects tend to focus on the most problematic groups of chemicals that may be present in tattoo inks: restricted colourants, PAHs, other impurities, PAAs, and list of substances with hazardous properties previously found in tattoo inks. To overcome these limitations, priority in the discussion below is given to those surveillance results that demonstrate the compliance of tattoo inks (and PMU) over the majority of most problematic groups of substances, although it is important to note that not all groups are relevant for all colours. Additional evidence that the proposed individual concentration limits are achievable is also provided.

Surveillance results in general are seen as a good indication of the share on the market as alternative tattoo inks as they are often based on random sampling. However, in some cases, it is explicitly stated (e.g., (Hauri, 2017)) that the surveillance studies are targeted

at problematic inks. The estimation of the share of compliant inks on the EEA market using these results would likely lead to underestimation.

Overall, surveillance by enforcement authorities have shown that in general there are tattoo inks currently on the market that meet the ResAP recommendations and requirements of several national regulations in EU Member States for the specific substances investigated.

All colours

Impurities with hazardous properties can be a concern for potentially all colours of tattoo inks and PMU. Despite the challenges in the supply chain and manufacturing process brought up by some stakeholders, there are tattoo inks on the market that meet the requirements of ResAP(2008)1 related to chemical composition. As demonstrated by the results of different surveillance programs, ResAP compliant inks historically marketed in the EU comprised more than 50% of all inks and PMU on the market, with more recent studies placing the compliance rate higher than 70%:

- Monitoring in the German federal state of Baden-Wuerttemberg found in 2012 non-compliance with requirements for chemical composition in 30% of the inks originating from USA, Japan, Mexico, Brazil, Italy, UK, and Germany. (CVUA, 2015)

- Monitoring in the German federal state of Baden-Wuerttemberg found in 2010 non-compliance with requirements for chemical composition in 42% of the tattoo inks (out of 38). (CVUA, 2015)

- In 2014, according to Istituto superiore di sanità (ISS), half of tattoo inks subject to control in Italy included heavy metals and PAAs in excess of the ResAP(2008)1 limits. (ECHA CfE, 2016a)

- In 2014, the Italian enforcement authority found that 85% of tattoo inks placed on the market are compliant with ResAP(2008)1 requirements for PAAs and impurities (CoE ResAP(2008)1, Table 3). Of the non-compliant inks, 45% warranted serious risk notification via RAPEX (as per GPSD). In 2015, the share of non-compliance was slightly higher (28%) but the deviations were smaller and only 23% represented serious risk. (ISS 2017)

- In 2015, 52% of tattoo inks for tattoo or permanent makeup on the Swedish market contained banned substances or excessive levels of impurities. Of the 29 products tested, 6 inks contained one or more banned aromatic amines and 13 products contained excessive levels of one or more impurities. (SMPA, 2015) This is an improvement of surveillance results in 2010 and 2011, which showed less than 30% compliance with chemical composition requirements. (KEMI, 2010) (ECHA CfE, 2016a)

- In 2012, the Danish competent authority surveyed tattoo inks used in Denmark. A total of 65 tattoo inks were analysed from 10 different colour series. It was concluded that there are inks on the market in all colours that meet ResAP. (DEPA, 2012a)

- 2013 national surveillance program in Switzerland analysed a total of 229 samples (206 tattoo inks and 23 PMU) from 32 tattoo ink and eight PMU brands and found that PMU comply with the chemical composition requirements of Swiss legislation

and ResAP(2008)1.[48] A number of tattoo inks (50% of tattoo samples, or 45% of all samples) were banned due to content of prohibited substances, including preservatives. (Hauri, 2014)

- In 2015, 19 tattoo ink samples were collected from tattoo studios in the Canton of Basel in Switzerland. Of those, 32% were banned due to concentration of prohibited substances exceeding ResAP recommendations and requirements of national legislation. (Hauri, 2016)
- In 2016, 37 tattoo ink samples were collected from tattoo studios in the Canton of Basel in Switzerland. Of those, eight contained prohibited colourants, six prohibited preservatives, four PAH, and one carcinogenic aromatic amines not meeting ResAP recommendations and requirements of national legislation. (Hauri, 2017) The total non-compliance rate with chemical composition can be estimated at 30% assuming that each objection (i.e., non-compliance with serious risk) is associated with unique tattoo ink and the objections related to preservatives are excluded. The report concludes that the compliance rate is likely not representative, as "risk colours were collected in a targeted manner." (Hauri, 2017)

Sensitising, irritant and corrosive substances

Concerning the restriction of chemical substances with a harmonized classification as sensitizers, Danish EPA (DEPA, 2017a) identified that of the 1 159 substances with a harmonized classification as sensitizing 1A or 1B, 22 chemical substances have been found in tattoo inks. (JRC, 2015b) Of these nine do not have CMR harmonized classifications, although six of them are also classified as irritants or corrosives. Since CMR classified substances are already restricted in those Member States with national legislation, the additional measure is seen to impact mainly these nine substances.

Six of these nine sensitisers are preservatives. As Annex V of the CPR contains close to 150 preservatives allowed in cosmetic products, it can be assumed that alternative preservatives are available. The remaining three substances are:

- Para-phenylenediamine, p-Phenylenediamine and its salts, p-Phenylenediamine (EC: 203-404-7, CAS: 106-50-3): The substance also has a harmonized classification as an eye irritant and belongs to the PAA group of substances and as such is evaluated specifically in the dossier in Appendix B.2. It is used as a black colourant. The most commonly used black colourant is carbon black, thus inks based on alternative black colourants are available on the EEA market.
- Cobalt (EC: 231-158-0, CAS: 7440-48-4): It is used in blue colourants. RO1 and RO2 propose a limit value of 25 ppm: the same as in Table 3 of ResAP(2008)1. A survey by the Danish EPA in 2012 found cobalt in seven blue inks in concentrations between 0 and 0.48 ppm and in three violet inks between 0 and 0.068 ppm. (DEPA, 2012a) Thus, it is concluded that alternative blue inks meeting the concentration requirements for cobalt are available on the market. (See also section on blue inks below.)
- Rosin (CAS: 8050-09-7): It is a viscosity regulator with no other relevant classification. Since no information on this substance has been submitted during the

[48] While Swiss national legislation is based on the CoE ResAP(2003)2, the introduced thresholds in the ResAP(2008)1 for heavy metals and PAH were incorporated. (Hauri, 2014)

public consultation, it is assumed that alternative viscosity regulating agents are available (e.g., glycerin).

The additional requirement for tattoo inks not to contain irritant or corrosive substances (in excess of 0.1% w/w in RO1 and in excess of their harmonised classification for mixtures in RO2) would impact 15 substances which have been found in tattoo inks and which do not belong to any of the other substance groups in the scope of the proposed restriction options. Five of those substances are on Annex V of CPR of allowed preservatives with numerous substitutes. Another substance is also reportedly used in tattoo inks as a preservative (2-Amino-2-methylpropanol). Of the remaining 9 substances, seven (isopropanol, methyl ethyl keton, thymol, potassium or sodium hydroxide, sodium hydroxide, hydrochloric acid, ammonia, and ethylhexyl glycerine) have been found in tattoo inks as additives with several alternatives. The remaining two are: Metheneamine (CAS 74-89-5) was found in 14 samples by Lehner 2011 in concentrations from 0.08 mg/kg to 21.64 mg/kg and Strontium (Sr, CAS 7440-24-6) in 10 samples out of 31 measured by KEMI 2010 in concentrations from 0.174 mg/kg to 120 mg/kg, i.e., all samples meet the stricter concentration requirement by RO1. (JRC, 2015b) Therefore, it can be concluded that there are tattoo inks meeting the requirements regarding irritant and corrosive substances in RO1 and RO2.

The following paragraphs demonstrate through surveillance results the availability of inks on the EEA market meeting the specific requirements for black and some colour inks. The availability of inks meeting the specific concertation requirements for common impurities is also discussed.

Black tattoo inks

Black tattoo inks contain from five to more than 50 organic (Jacobsen & Clause, 2015) and inorganic components. Some tattoo inks have been shown to contain even larger number of substances. Specifically for black inks, the main concern is that some contain PAHs with carcinogenic and mutagenic properties. However, it has been demonstrated that there are black inks already on the market that do not contain PAHs at levels above those recommended by ResAP(2008)1: sum of all PAHs to not exceed 0.5 ppm and Benzo(a)pyrene (BaP): 5 ppb. A study of 19 black inks supported that conclusion by showing that the total PAHs content varied from 0.14-201 µg/g in the inks. (Regensburger, et al., 2010) Similar results were shown by a study of 11 inks: 0.46-29 µg/g of total PAHs in the inks. (Høgsberg, et al., 2013a)

Surveillance results in other Member States support the conclusion that there are black inks currently present on the EU market that meet the chemical composition requirements of ResAP. In 2015/2016, the Netherlands Food and Consumer Product Safety Authority concluded that 52% of sampled black tattoo inks meet the chemical requirements in ResAP for PAHs and impurities. The results were similar to previous surveillance outcomes. About 13% of the sampled inks (54 in total) showed levels of impurities constituting serious risk (as per RAPEX guidelines). According to the label these inks were manufactured in the US and China. (NVWA, 2017) In 2014, monitoring in the German federal state of Baden-Wuerttemberg found all tattoo ink and PMU samples (22 in total) were in compliance with the PAH recommendations in ResAP and requirements in German national legislation. (CVUA, 2015)

Of the 19 black, grey and dark colours analysed by the Danish EPA, three black inks had a sum of PAH in the scope of this restriction dossier of 1.0, 1.1 and 13 ppm respectively. Of

these one had a content of Benzo(a)pyrene (BaP) of 5.3 ppm. The other 16 did not contain any PAHs in the scope of this restriction dossier. In total 10 black inks were analysed. This provide a compliance ratio for total PAHs of 70% for black inks. The samples showed a correlation between the content of carbon black and PAH (DEPA, 2012a)

RO1 and RO2 propose less strict requirements than those of ResAP(2008)1: only tattoo inks with individual concentration of PAHs with carcinogenic and mutagenic classification exceeding 0.5 ppm are restricted. Thus, from recent surveillance results it appears that a large share of inks currently on the EEA market would meet these requirements.

Colour tattoo inks

Of primary concern for colour tattoo inks are impurities as well as the presence of PAAs with low light fastness (leading to photodegradation) or of azo groups which could cleave to PAAs with hazardous properties. The latter is of particular concern for colourants belonging to the azo groups. These would include red and yellow and their nuances. Azo colourants are the largest colourant group in use today due to their high lightfastness, cheap production cost, etc. According to *Table 132*, containing information gathered by the JRC, azo colourants represent 60 out of the 113 colourants used in tattoo inks (in red, yellow and orange inks) and 54 out of the 100 colourants used in PMU. Appendix B.2 described that in total 67 azo colourants are used in tattoo inks and PMU. However, in general, azo colourants can be replaced with a number of other groups of substances, such as polycyclic pigments, etc. (ECHA CfE, 2016a)

Primary aromatic amines (PAAs)

The CoE resolution recommends that tattoo inks "should not contain" (which in effect is no intentional presence in tattoo inks above the limit of detection) PAAs in Table 1 of ResAP(2003)2 and ResAP(2008)1. RO1 and RO2 propose similar requirements in terms of the concentration limit: 5 ppm. Surveillance results suggest that a large share of the tattoo inks currently on the market are compliant with these requirements. It is further recommended that the analysis is performed without reductive cleavage due to the difficulties with replicating the process of reductive cleavage. This dossier also proposes to impose a concentration limit on a larger number of azo colourants: 32, which is approximately half of the pigments currently in use. However, 35 colourants are still available (See Appendix B.2 for further details.)

- 2014 surveillance project by the Italian enforcement authorities found seven (out of a 72) non-compliant samples with ResAP requirements on PAAs. Two of those were considered of serious risk (as per RAPEX). In 2015, 16 out of 94 samples showed presence of PAAs, with four of those deemed of serious risk. The concentration of PAAs ranged from 8.3-377 ppm. (ISS 2017)
- The 2013 monitoring results for PAAs in Germany demonstrated that most inks are below the ResAP recommendations for aromatic amines. In general, the amount found was minimal and in compliance with German legislations (BVL, 2014). In 2010, monitoring in the German federal state of Baden-Wuerttemberg found non-compliance related to aromatic amines in less than 16% of the analysed inks (140 in total) (CVUA, 2015).
- In the 2012, the Danish EPA analysed 14 inks without reductive cleavage. The results showed contents of single PAAs from 1.6 to 190 ppm. The sample contained six red, three purple, two blue, one black, one brown and one yellow ink. It was not possible to conclude whether certain colours contain specific PAA as well as if the

content in the colours differs a lot with regard to concentration and type of PAA found. About 60% of red tattoo inks tested met the ResAP recommendations. (DEPA, 2012a)

Blue and green tattoo inks

To date, industry has named only two colourants which could not be replaced with suitable alternatives (technically feasible and leading to lower risks) in the short to medium term: Pigment Blue 15:3 and Pigment Green 7. (ECHA CfE, 2016a). The latter is currently not allowed in tattoo inks and PMU in Member States with national legislation due to its restriction for use in cosmetic products under Annex IV of the CPR (not to be used in the vicinity of the eye). The former is not consistently enforced in all Member States with national legislation as it is banned under Annex II but allowed under Annex IV of the CPR. As stated in section Proposed options for restriction derogations are proposed for these two colourants in both RO1 and RO2, together with 11 other colourants which are in a similar situation: prohibited under CPR, Annex II (hair dyes) but allowed in all products with Annex IV of the CPR.

According to information gathered by JRC (JRC, 2015b), 13 blue and green pigments have been reportedly used in tattoo inks. Of those, ResAP(2008)1 recommends that three colourants – Blue 15, Blue 86, Green 7 – are not used in tattoo inks, although there are national differences in the interpretation of the recommendation regarding Blue 15. Both RO1 and RO2 propose that:

- Blue 15 and Green 7 are derogated due to industry concerns and the difficulty to demonstrate risk with the current level of information
- Blue 86 is restricted under RO1 (“shall not contain”) and RO2 (0.1% w/w), as it is not allowed to be used in cosmetic products with prolonged contact to skin (i.e., allowed in rinse-off products only according to Annex IV of the CPR)
- Blue 27, Acid Blue 9, Green 17 and Green 18 are allowed in tattoo inks subject to purity or composition requirements (as specified in Annex IV of CPR, column i) under RO1 and RO2
- The remaining six pigments in Table 134 are not restricted (i.e., are allowed in tattoo inks)

Therefore, under RO1 and RO2, eight of the 13 colourants found in tattoo inks could continue to be used without restrictions, while four could continue to be used subject to purity or composition requirements. The use of Blue 86 is proposed to be restricted with a concertation limit discouraging intentional use.

Table 133. Blue and green colourants used in tattoo and PMU inks and their regulation in the CPR and the proposed restriction options

Colourants	CAS numbers	Annex II of CPR entry #	Annex IV of CPR entry #
Pigment Blue 15 (PB 15)*†	147-14-8	1367 - when used as a substance in hair dye products - Column b	105 – allowed in all cosmetic products
Pigment Blue 17 (PB 17)*	71799-04-7		
Direct Blue 86 (DB 86)*‡	1330-38-7	1368 - when used as a substance in hair dye products – column b	106 – rinse-off products only – column g
Pigment Blue 27 (PB 27)¥	12240-15-2		138 – free from vyanide ions – column i
Pigment Blue 29 (PB 29)	57455-37-5		120 – allowed in all cosmetic products
Acid Blue 9 (AB 9)¥	2650 18-2		63 purity criteria as set out in Commission Directive 95/45/EC (E 133) - column i
Pigment Blue 25 (PB 25)	10127-03-4		
Y Pigment Blue 60 (YPB 60)	81-77-6		95 – allowed in all cosmetic products)
Pigment Green 7 (PG 7)*†	1328-53-6	1369 - when used as a substance in hair dye products – column b	107 – not to be used in eye products – coloumn g
Pigment Green 36 (PG 36)*	14302-13-7		
Pigment Green 17 (PG 17)¥	58591-12-1, 1333-82-0		129 – free from chromate ion – column i
Pigment Green 18 (PG 18)¥	12001-99-9		130 free from chromate ion – column i
Acid Green 25 (AG 25)	4403-90-1		92 – allowed in all cosmetic products

Source: use in tattoo inks and PMU as reported in JRC report (JRC, 2015b)

Notes:

* Phthalocyanines

† Derogated under RO1 and RO2

‡ Restricted under RO1 ("shall not contain") and RO1 (0.1% w/w)

¥ Allowed in tattoo inks subject to purity or composition requirements (Annex IV, column i) under RO1 and RO2

Copper

ResAP(2008)1 recommends concentration of soluble copper to be limited to 25 ppm w/w, while RO1 and RO2 propose a less strict limit: 500 ppm. The presence of soluble copper has

often been associated with blue, green or violet inks. While the analytical method used by the Danish EPA in their 2012 study did not make it possible to differentiate between soluble and non-soluble copper content, the ResAP recommendations were met by 42% of the tested blue, green and violet inks. Green was the most problematic colour. (DEPA, 2012a)

According to the survey by the Danish EPA in 2012 (see Table 135) all inks not based on phthalocyanines are far below the limit value of both 25 ppm and 500 ppm.

It is assumed that it is possible to measure dissolved copper without dissolving copper incorporated in the colourant and thus, assuring that the restriction on copper will not indirectly restrict pigments based on copper. According to the survey made by the Danish EPA 14 out of 16 green and blue inks are based on pigments containing copper. (DEPA, 2012a)

Table 134 Content of copper measured in tattoo inks on the Danish market

Colour	Concentration range (ppm)	Number of tested inks
Black	0.24 – 18	11
Red	0.17 – 8.0	12
Orange	0.64 – 100	3
Peach	0.68 – 3.4	3
Violet	0.69 – 1,020	3
Brown	140	1
Blue	5,300 – 20,000	7
Green	1.1 – 17,000	9
Yellow	0.36 – 13	7
White	0.52 - 12	5

Notes: Analytical method used did not make it possible to differentiate between soluble and non-soluble copper content.

Source: (DEPA, 2012a)

White tattoo inks and Barium

In their 2012 study, the Danish EPA tested five white colour tattoos. Of those four met ResAP recommendations. (Danish EPA, 2012a) Barium sulfate is a commonly used in white colourant. It is also often mixed with other colourants to obtain different nuances. RO1 and RO2 propose a concentration limit for soluble barium of 84 ppm which is less strict than the ResAP(2008)1 limit of 50 ppm. Soluble barium is another substance which is difficultly measured. However, a review by JRC (JRC, 2015b) demonstrated that the barium limit is achievable: of the 886 samples analysed, barium concentration ranged from 0.015 ppm to 401.5 ppm, with only 20% of samples exceeding 50 ppm.

Mercury

RO1 and RO2 propose the same concentration limits for mercury as ResAP(2008)1: 0.2 ppm. In a study by the Danish EPA (DEPA, 2012a), mercury was only detected in two colours, peach (in one out of three inks tested) and blue (in one out of seven). For peach, the concentration was 0.11 ppm and for blue it was 0.038 ppm. In total, 65 ink were investigated for mercury content. No mercury was found in black, red, orange, purple, brown green, yellow or white inks. Thus, it can be concluded that the inks on the market in

Denmark in 2012 complied with the limit value of mercury. Similarly, a review by JRC (JRC, 2015b) demonstrated that the mercury limit is achievable: of the 809 samples analysed, mercury concentration ranged from 0.2 ppm to 0.253 ppm, with only 2.5% of samples exceeding 0.2 ppm. The proposed restriction is relevant since historically pigments based on mercury were widely used in red or nuances of red, which were later replaced by azo colourants. Thus, the restriction on mercury will prevent undesirable substitution.

Lead

RO1 and RO2 propose a stricter concentration limits for lead (0.7 ppm) in comparison to ResAP(2008)2 (2 ppm). In a study by the Danish EPA (DEPA, 2012a), lead was detected in all colours. Nevertheless, Table 136 shows that inks with a content below 0.7 ppm are available. Similarly, a review by JRC (JRC, 2015b) demonstrated that the lead limit is achievable: of the 2 175 samples analysed, lead content ranged from 0.015 ppm to 401.5 ppm, with only 8.5% of samples exceeding 2 ppm.

Table 135 Content of lead measured in inks on the Danish market

Colour	Concentration range (ppm)	Number of tested inks	Number > 0.7 ppm
Black	0.017 – 1.5	11	2
Red	0.039 – 1.34	12	1
Orange	0.21 – 1.6	3	1
Peach	0.11 – 0.19	3	0
Violet	0.016 – 0.092	3	0
Brown	0.21	1	0
Blue	0.052 – 5.7	7	2
Green	0.11 – 9.3	9	2
Yellow	0.019 – 0.8	7	2
White	0.049 - 10	5	1

Source: (DEPA, 2012a)

Tin

RO1 and RO2 propose the same concentration limits for tin as ResAP(2008)1: 50 ppm. In a study by the Danish EPA (DEPA, 2012a), tin was detected in all colours. However, the concentration level was in the range of 0 to 4.1 ppm. The highest concentration was found in orange inks, where two out of three contained tin. Thus, it can be concluded that the inks on the market in Denmark in 2012 complied with the limit value of tin. Similarly, a review by JRC (JRC, 2015b) demonstrated that the tin limit is achievable: of the 277 samples analysed, tin content ranged from 0.5 ppm to 101 ppm, with only 1.4% of samples exceeding 50 ppm. Due to the frequent occurrence of tin in tattoo inks the proposed restriction is considered relevant.

Other impurities

Further information on other impurities is compiled by the JRC (JRC, 2015b). A summary of this information is also presented in Consumer exposure in Annex B.

Conclusion

As demonstrated by the results of different surveillance programs, ResAP compliant inks historically marketed in the EEA comprise between 50% and in excess of 70% of all tattoo inks and PMU on the market. Concentration limits for key substances are also achievable. As both restriction options propose concentration limits that are similar or higher than those enforced by Member State national legislation based on the CoE ResAP recommendations, it is expected that a higher proportion of tattoo inks and PMU currently on the EEA market meet the proposed requirements. Therefore, it can be concluded that manufacturers of these tattoo inks (and society as a whole) would not incur additional costs and other impacts due to the proposed restriction options. This conclusion is valid for all colours and assumes that the derogations proposed in RO1 and RO2 (for Pigment Green 7, Pigment Blue 15:3 and similar) and described in section Proposed options for restriction are in place.

D.2.3.2. Human health and environmental risks of alternatives

Risk assessment of tattoo inks is not a very well developed area. No specific guidelines on the evaluation of the risk of inks for tattoos and PMU are well-developed and used. Several European authorities have established procedures to evaluate the risk derived from the daily or continuous low dose exposure to chemicals. Examples are the European Food Safety Agency (EFSA) for food ingredients and the US-based Product Quality Research Institute (PQRI) for the evaluation of leaching substances from plastics in medical devices. Although recently guidance documents were published, there still remain uncertainties regarding the appropriate methodology for assessing risks due to intradermal exposure and risks arising from mixtures. Two such guidance documents include the BfR guidance discussing validated, regulatory approved toxicological test methods which form the basis for risk assessment of other products like cosmetics, and which might be suitable also for the testing of tattoo inks or their ingredients, respectively. The toxicological endpoints discussed include irritation, sensitization, genotoxicity, carcinogenicity and systemic toxicity. (BfR, 2012) Building on the BfR work is the recently published guidance by the CoE. (CoE, 2017) Some tattoo ink manufacturers have expressed that pigments used today are selected on the basis of experience gathered during decades of tattooing. Testing to obtain toxicological data is not affordable for tattoo ink manufacturers, the majority of whom are micro or small enterprises. Therefore, identification of new pigments presenting less risks to human health and the environment would most likely be on the basis of a review of their hazardous properties, in order to identify less risky alternatives. This process may be impeded as the toxicological properties of many of these pigments are not extensively studied.

As it is not practical to discuss the human health and environmental hazards and risks of all possible colourants on the Colour Index database, the discussion in this section is concentrated on those for which there is historical information that have been found in tattoo inks and PMU.

On the basis of literature review, industry surveys and surveillance information, 154 colourants have been used in tattoo inks and PMU to date. (JRC, 2015b) Of those, only one substances has harmonised classifications falling in the scope of the proposed restriction options: Pigment green 17 (CAS 1333-82-0: carcinogenic 1a, mutagenic 1b, reprotoxic 2, skin sensitiser 1, and skin corrosive 1) and 2-(propyloxy)ethanol (eye irritant 2). Of the remaining substances, five are proposed to be restricted because they are on Annex II of the CPR:

- Colouring agent CI 12075 (Pigment Orange 5) and its lakes, pigments and salts, Pigment Orange 5, Pigment orange 5, EC 222-429-4, CAS 3468-63-1, Registered, ResAP(2008)1 Table 2 entry #24, Annex II entry #397

- Colouring agent CI 45170 and CI 45170:1 (Basic Violet 10), Basic Violet 10, Basic violet 10, EC 201-383-9, CAS 81-88-9, ResAP(2008)1 Table 2 entry #1, Annex II entry #398
- Colouring agent CI 15585, Pigment red 53:1, EC 225-935-3, CAS 5160-02-1, Registered, Annex II entry #401.0
- Solvent Red 1 (CI 12150), when used as a substance in hair dye products, Solvent red 1, EC 214-968-9, CAS 1229-55-6, Registered, Annex II entry #1231
- 2,2'-[(3,3'-Dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[3-oxo-N-phenylbutanamide] (Pigment Yellow 12) and its salts, when used as a substance in hair dye products, Pigment yellow 12, EC 228-787-8, CAS 6358-85-6, 15541-56-7, Registered, Annex II entry #1263

Of the remaining substances with information on use in tattoo inks and PMU, 15 are restricted due to their use restriction via Annex IV of the CPR (i.e., for eye products, mucous membrane or in rinse-off products only). In addition, six pigments are specifically derogated as they are prohibited in hair dyes under Annex II but allowed in all cosmetic products via Annex IV of the CPR. Twenty-five substances have restrictions on concentration limits or purity via Annex IV of the CPR or due to quantitative risk assessment of the substance which concludes that the maximum concentration limit of the substance need to restricted (e.g., zinc oxide). This leaves in total of 101 colourants historically used in tattoo inks which will not be affected by the restrictions. As seen in Table 4, these colourants represent a broad range of the colour palette used in tattoo inks. None of these 101 substances are classified for human health and only seven can be considered candidates for classification under the CLP for categories in the scope of the proposed restriction options, if the percent notifiers (i.e., the percent notifiers listing the classification category exceeding 50%) is considered a good indicator for a possible harmonised classification. These are listed below and in Appendix D.X. for further investigation:

- 1,4-dihydroxyanthraquinone: Eye Irrit. 2 (96.1%), STOT SE 3 (95.9%), Skin Irrit. 2 (95.9%) – the substance is registered under REACH
- 2-hydroxy-1,4-naphthoquinone: Eye Irrit. 2 (89.3%), Skin Irrit. 2 (89.3%), STOT SE 3 (82.1%)
- 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride: Eye Dam. 1 (76.7%), Aquatic Chronic 1 (61.0%), Aquatic Acute 1 (56.9%), Acute Tox. 3 (51.1%)
- Carminic acid: Eye Dam. 1 (56.5%), Skin Corr. 1A (56.5%),
- N-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-3-oxo-2-[[2-(trifluoromethyl)phenyl]azo]butyramide: Eye Irrit. 2 (56.0%) – the substance is registered under REACH
- Methyl 1-methyl-4-[(methylphenylhydrazono)methyl]pyridinium sulphate (Pyridinium, 1- methyl-4-[(methylphenylhydrazono)methyl]-, methyl sulfate, Pigment yellow 87), EC269-503-2, CAS 68259-00-7, 14110-84-6: Acute Tox. 4 (95.3%), Aquatic Chronic 2 (57.8%), Eye Irrit. 2 (39.1%), Skin Irrit. 2 (31.2%), Aquatic Acute 1 (28.9%), Not Classified (3.1%), STOT SE 3 (3.1%), Aquatic Chronic 4 (0.8%)

- C.I. Pigment Yellow 36: EC 609-398-6, CAS 37300-23-5: Acute Tox. 4 (92.0%), Aquatic Acute 1 (92.0%), Aquatic Chronic 1 (92.0%), Carc. 1A (92.0%), Skin Sens. 1 (92.0%), Not Classified (8.0%)

In general, the substances in tattoo inks or PMU do not lead to substantial environmental exposure. It is estimated that only a small amount of ink is disposed of and can find itself in ground or influent waste water. As seen in Table 4, only four substances found in tattoo inks and PMU have relevant environmental classification. Of the 221 substance without any proposed restrictions on the basis of their relevant human health classification or inclusion in Annex II or IV of the CPR, only one has harmonised classification for environmental hazards: copper oxide (Aquatic Acute 1, Aquatic Chronic 1). Some of the remaining substances are potentially good candidates for harmonised classification under the CLP Regulation for environmental hazard judging by the number of notifiers who have identified these hazards. (See Table 4) As the rational for this restriction proposal is human health, the environmental risks arising from substances in tattoo inks and their alternatives are not discussed further.

Conclusion

Overall, it can be concluded that there are large number of colourants outside the scope of the proposed restriction options which have more benign human health hazards and therefore, risks assuming similar level and conditions of exposure. This statement should be taken with caution as a large number of these substances (more than half) have not been registered yet under REACH and many have not been assessed in detail.

D.2.3.3. Technical and economic feasibility of alternatives

While there are a number of pigments on the global market, according to stakeholders, whether a pigment is suitable for tattoo purposes can only be determined via tattoo tests. Some of the main technical characteristics sought in a tattoo ink and a PMU are: colour hue, brilliance, permanence, good workability, healing properties, particle size, etc.

Stakeholder consultations have revealed that alternative tattoo inks and PMU can be available but at a higher costs. Pigments with higher purity, e.g., cosmetic, food or medical grade colourants, are available sometimes at higher costs than industrial grade. For cost differences, economic and other impacts on industry as a result of transitioning to alternatives due to the proposed restriction options, see section Substitution costs.

To date, only two pigments have been named which could not be replaced with suitable alternatives by industry in the short to medium term: Pigment Blue 15:3 and Green 7. (ECHA CfE, 2016a) Both are phthalocyanines and as such their crystalline structure leads to low solubility in organic solvents. They are insoluble in water, and stable in neutral, acidic or alkaline solutions. As shown in Annex B, the risks of the use in tattoo inks are adequately controlled.

Both pigments are examples of colourants restricted in hair dyes under Annex II of the CPR. According to industry claims, these colourants were primarily restricted in hair dyes as the cosmetic industry did not defend their application in cosmetic products. Under Annex IV of the CPR both colourants are allowed in cosmetic products, although there is a restriction for Pigment Green 7 for use in products in the vicinity of the eye.

Pigment Blue 15:3 is reportedly the best blue colourant on the market for tattoo inks. Other examined blue colourants, outside the scope of ResAP and the proposed restriction options lead to higher risks (due to degradation products) or lead to colour change when blended

with white pigment (a desirable quality as colourants are often mixed to obtain different colour shades). (ECHA CfE, 2016a) As shown in Annex B, risks of the use of Pigment Blue 15:3 in tattoo inks could not be demonstrated. The substance has been investigated in both in vitro and in vivo studies. A complete REACH evaluation dossier is available containing experimental toxicity data on acute, subchronic and chronic toxicity, skin sensitization, genotoxicity, reproductive toxicity, developmental toxicity and carcinogenicity. The substance is not classified.

Pigment Green 7 is also considered the best green pigment on the market from a technical standpoint. Although Pigment Green 36 has been identified by some as a technical equivalent to Pigment Green 7, industry has expressed that on the basis of available hazard information on both green 7 and 36, it can be concluded that Pigment Green 7 has better hazard and risk profile for human health.

Conclusion:

Technically feasible alternatives with similar or better hazard and risk profiles exist. Notable exceptions are Pigment Blue 15:3, Pigment Green 7 and other pigments prohibited for use in hair dyes under Annex II of the CPR, listed in Supplementary Table B of the proposed restriction options. (See Table 5 in report and the justification for derogation of these pigments in section D.1.1.2.) Therefore, on the basis of technical feasibility and hazard and risk considerations, a derogation is proposed for these colourants.

The transition to alternative tattoo inks and PMU will lead to higher substitution costs for ink manufacturers due to the need to invest in R&D and due to higher material costs. Further analysis on substitution costs, impacts to manufacturers and downstream users, as well as affordability of the restriction options are present in the forthcoming sections.

D.3. Restriction scenario(s)

The two restriction scenarios differ mainly in terms of concentration limits for part of the substances in scope and how the link with the CPR are managed. Therefore, RO1 and RO2 impacts will differ slightly in terms of risk reduction capacity, substitution costs,

enforceability and impacts on industry. The following sections focus on the impacts of RO1. The differences between RO1 and RO2 are highlighted in the respective below. The impacts of the two options are summarised in section For RO1 to break even, between 320 (calculated using cost of illness (COI) plus higher WTP values) and 1 060 (COI plus lower WTP values) cases of chronic allergic reactions (i.e., requiring surgical removal) need to be avoided on an annual basis. This is between 0.02-0.06% of the estimated number of people getting tattoos for the first time each year (19-63 avoided removals for every 100 000 tattooed people) in EEA22 – the Member States currently without national legislation.

It is reasonable to expect that these cases would be avoided as a result of the proposed restriction measure as:

- the estimated average prevalence rate of tattoo complications is 1.7% (see point d) in section D.6.1. Human health impacts
- removal of tattoos due to an allergic or papulo-nodular reaction is just one group of the health outcomes. As stated in section D.6.1. Human health impacts, a number of people experience complications that require topical or systemic corticosteroids as well as experience mild ongoing complaints from their tattoos and PMU. This is in addition to the potential contribution of tattoo ink and PMU exposure to carcinogenic, reproductive, developmental and other systemic adverse effects.
- Therefore, although full cost-benefit comparison it is not possible, it is reasonable to assume that the benefits would outweigh the costs, **as very few cases of only** one type of adverse effects (non-infectious, inflammatory) are necessary for the restriction to break even. Quantification and monetisation of other adverse effects (systemic, carcinogenic, reproductive or developmental) would lead to higher overall value of benefits from RO1.
- As the concentration limits of RO2 are higher than RO1, it can be hypothesised that RO2 offers a lower level of protection and therefore, fewer benefits. However, as costs for RO2 are also lower than RO1, it is difficult to determine the overall proportionality of RO2 in comparison to RO1.

Comparison of Restriction Options.

D.4. Economic impacts

D.4.1. Substitution costs

In the event the proposed restriction options come into force, tattoo inks not meeting the requirements of the proposed restriction options (non-compliant) would no longer be available. Therefore, the market would have to transition to compliant tattoo inks which tend to have similar or slightly higher market price than non-compliant. (stakeholder interviews) This price difference is seen to reflect the higher costs tattoo ink and PMU

manufacturers would incur to comply with the proposed restriction options: research and development costs for manufacturers to develop compliant tattoo inks and PMU, increased testing costs to ensure compliance with the proposed regulatory requirements and potentially higher costs to procure the necessary purity colourants. Their magnitude would depend on the degree of their current compliance with ResAP recommendations incorporated into national legislation of Member States.

The incremental substitution costs estimated to be incurred by downstream users of tattoo ink and PMU as a result of RO1 are about €4.4 million annually during the temporal scope of the analysis (in 2016 values). The estimation is based on the following inputs and assumptions:

- Between 30-70% (50% as a mid-point in the main scenario and 30% and 70% in the High and Low share of alternatives scenarios shown in Annex E) of tattoo inks on the EEA31 market do not meet the requirements of the proposed restriction options. As shown in section *Risk reduction, technical and economic feasibility, and availability of alternatives*, surveillance results of national campaigns in Member States with national legislation and other countries in EEA31 have shown that in excess of 50-70% of inks are compliant with the ResAP recommendations. As the requirements of RO1 and RO2 are similar to the ResAP recommendations, and in some cases less strict (in particular for RO2), it is expected that those inks compliant with ResAP would take over the share of non-compliant inks after the entry info effect of the proposed restriction options. It is assumed that the proportion of ResAP compliant inks in the remaining EEA22 Member States is similar, as some Member States without national legislation enforce ResAP recommendations to a degree (e.g., Italy, Denmark), while others are vigilant with respect to RAPEX notified products. Furthermore, surveillance is often targeted at high risk suppliers and products, therefore, the 50-70% compliance rate of tattoo inks is likely a conservative assumption. In addition, interviews with manufacturers revealed some of those that are compliant with ResAP recommendations do not have separate product lines for jurisdictions with and without national legislation (e.g., due to for example economies of scale some manufacturers do not use different formulations for sales in countries with or without national legislation based on ResAP). Furthermore, interviews with industry have shown that the majority of EU-manufactured tattoo inks are compliant with ResAP, and that non-compliant are primarily imported products, largely from China. Therefore, it can be concluded that the assumptions that 30-70% (or 50% on average) would not be substituted is considered a reasonable assumption.

- Up to 20% of PMU (10% in the main scenario) currently on the EEA31 market are not compliant with the proposed restriction options. The reasons for making this assumption are similar to those described above for tattoo inks, i.e., similarity between ResAP and the requirements under RO1 and RO2, surveillance results that show generally better compliance for PMU in comparison to tattoo inks, and low product differentiation for markets without national legislation. Interviews with industry have revealed that PMU on the EU-market are largely compliant, although there are national differences when it comes to treating some impurities (e.g., nickel). Manufacturers explain this with the more demanding customer base for PMU in comparison to tattoo inks.

- Projected volumes of tattoo inks (and PMU) on the EEA market as shown in Table 1 in Annex A.

- The price difference between compliant and non-ResAP-compliant tattoo inks and PMU currently on the EEA31 market is about 15%. The price difference is derived on the basis of the average retail price per 30 ml tattoo ink and 15 ml PMU bottle reported by stakeholders,

excluding average value added tax (VAT). The price difference is seen to reflect the main difference in the costs of manufacturers of compliant inks in excess of those incurred by non-compliant formulators: higher pigment, testing, research and development costs. With respect to the latter, stakeholders have reported that these can range from €100 000 to €400 000 for materials and testing of the newly developed product. As colourants can be of lower purity (60-80%) (JRC, 2015b), a number of tattoo ink and PMU manufacturers are testing their input materials in order to meet national regulations or to ensure consistent product. These testing costs for compliant tattoo inks have been reported up to €80 000 per year.

As RO2 imposes less strict requirements than (ResAP and) RO1, it is anticipated that more tattoo inks and PMU on the market are already compliant with RO2. Therefore, lower substitution costs are anticipated to comply with RO2 requirements.

D.4.2. Enforcement costs

To estimate the costs that can be anticipated to be incurred by enforcement authorities, jurisdictions with national legislation were contacted (i.e., Germany, Norway, Sweden). On the basis of the information received, the following can be deduced about the enforcement of current national legislation:

- Enforcement of tattoo ink legislation is closely integrated with enforcement of the CPR at Member State level. This is natural as the basis for national legislation - ResAP - is linked to the CPD and its successor, the CPR.
- While a number of other aspects of the legislation involve ongoing monitoring (e.g., inspection of tattoo parlours, national registry of tattoo inks and PMU), surveillance of the chemical composition of tattoo inks and PMU occurs less frequently (the highest frequency reported was every 4-5 years). This is because national legislation competes for a limited national budget for surveillance which is allocated in terms of risks and priorities among various projects.
- Based on past experience, it can be assumed that about 100 tattoo inks and PMU are tested for the presence of a broad range of substances with combined cost of these tests of €500/sample. Extrapolating to EEA22 results in an annual average incremental cost for analytical testing of about €200 000. Member States with national legislation are anticipated to continue having the same level of spending on analytical testing to ensure compliance with the proposed restriction options. They are not anticipated to have incremental testing costs associated with the proposed restriction options.

In addition to the analytical costs, Member States are expected to incur administrative costs for enforcing the proposed restriction. These costs constitute opportunity costs as Member States with predominantly fixed enforcement resources, would need to reallocate budget for the enforcement of a new restriction from already existing restrictions. These total opportunity costs are estimated at €53 800 annually for EU28. (ECHA, 2017i) Member States already with national legislation are anticipated to have some costs to restructure their enforcement administration in accordance with the proposed restriction options. These are assumed to have a minor impact.

Therefore, the total incremental enforcement costs to be incurred over the temporal scope of the analysis are estimated at €235 000 annually. This is likely an overestimation as it assumes that the same level of enforcement efforts will be required over the entire

temporal scope, while in reality enforcement efforts decline with industry compliance, and industry compliance improves as familiarity of the restriction requirements increase over time.

D.5. Other impacts

D.5.1. Social and distributional impacts

a) Tattoo ink and PMU formulators

Regulations of this scale can be challenging for smaller businesses. Many formulators are small (10-50 employees) or micro (less than 10 employees) enterprises on the basis of number of employees. Few can be considered truly global scale companies, although via Internet direct sales their products can reach all parts of the world. As such, many companies may lack the resources to keep abreast on regulatory issues or to invest in extensive research and development and hazard and risk investigation of their products.

The highest regulatory burden from the proposed restriction options would likely be on micro or small businesses which do not have compliant inks. Those most likely are located and conducting business in Member States and international jurisdictions without legislation on the chemical composition on tattoo inks and PMU and where the tattoo industry and cosmetic practitioners are not well organised. It is likely that those companies that currently do not have compliant tattoo inks (and to a lesser extent, PMU) on the market would likely bear the lion's share of these costs. It is expected that these additional costs would not lead to closures and lay-offs.

To date, industry concerns have been primarily associated with inconsistencies in ResAP recommendations, their different interpretation nationally and diversity in analytical methods used, leading to different treatment of the same products in different Member States, all with national legislations based on ResAP. Larger, US brands are also particularly concerned with the counterfeiting of their products. The establishment of an EU-based registry may assist with this problem.

b) Tattoo artists

The proposed restriction options are not expected to impact employment or the ability of tattoo artists to perform their profession and art, although it is possible that the available colour palette could become less diverse in the short term. Not all artists work with a broad palette of colours (usually those specialising in realistic tattoos primarily do so), although with experience tattoo artists grow accustomed and develop preferences for particular colour (or brand) due to its brightness, permanence, viscosity, healing properties, etc.

As a result of RO1 or RO2, many artists would have to ensure that the inks they continue to use are compliant with the regulatory requirements. This will be of particular importance for those who buy directly from manufacturers or internationally, via internet based resellers, as opposed to EEA31-based distributors, some of whom reportedly take measures to ensure sales of safe, genuine brands. The latter may be challenging in particular for home-based tattoo artists who are not often members of associations, are not engaged in industry information exchanges on regulatory issues, and sometimes cannot purchase from distributors who may sell to registered artists only. In general, participation in industry associations varies greatly in EEA31 and so does the level of engagement on regulatory issues.

c) Pigment manufacturers

The tattoo ink industry is a small market segment for large pigment manufacturers, therefore any changes in the tattoo ink business would likely not lead to significant impacts on the pigment industry. Currently, another concern of some tattoo manufacturers is having

to purchase pigments using separate legal name as some pigment manufacturers do not sell to the tattoo ink industry. It is possible that as a result of the more transparent requirements for tattoo inks and PMU, more pigment manufacturers may increase their sales to the tattoo industry.

D.5.2. Wider economic impacts

A significant share of tattoo inks (about 70-80%) on the EEA31 market is imported from jurisdictions without regulation on the content of tattoo inks. Import of PMU is lower: 20-30%. (JRC, 2015b) Therefore, it is possible that as a result of the proposed RO1 and RO2, some imported products may no longer be available. By the same token, some EEA31 manufactured tattoo inks and PMU also may not be available. From that perspective it is not expected that the proposed restriction options would distort the trade balance but no historical information is available about the trade in tattoo inks and PMU to ascertain their impact on extra-EEA31 trade (although any historical information would be difficult to interpret due to the inconsistent application of ResAP recommendations across EEA31).

D.6. Human health and environmental impacts

D.6.1. Human health impacts

a) Absorption, distribution, metabolism, transportation and excretion of pigments and other ink constituents and impurities in the human body

This is an area still largely unexplored. The tattoo inks are injected in the human body with rapidly oscillating needles. The epidermis is punctured and about 2.5 mg/cm^2 of pigment is deposited into the dermis, leading to several grams of pigment being injected into the skin to produce a decorative image, substantially exposing extensively tattooed people in particular. While some inks can be injected sub-dermally due to improper tattooing technique, tattoo pigments reside mainly in the dermis between collagen bundles or within fibroblasts. (Kluger & Koljonen, 2012) Following tattooing, the pigment particles are encapsulated in the dermis. They are found in the cytoplasm of cells in the membrane-bound structures identified as secondary lysosomes. Macrophages may also contain phagocytosed pigment particles. (Bäumler, 2015)

Not all pigment remains in the dermis indefinitely. Studies show that the pigment, initially rapidly, decreases over time with 30% being removed within the first 6 weeks (and up to 60% if exposed to UVR), (Engel, et al., 2008) with only 1-13% remaining in the skin after several years, which causes a fading of the tattoo. (Lehner, et al., 2011) Because of the refractory properties and the colour strength of the pigments, this substantial decrease of the pigment is not easily gauged with the human eye.

Bäumler suggests that the reduction of the pigment in the dermis is due to three main mechanisms: part of the colourant may leave the skin with the bleeding during or directly after tattooing; part of the colourant may be transported away from the skin via the lymphatic or blood vessel systems; and part of the colourant decomposes months or years after tattooing due to repeated exposure to solar radiation. Furthermore, any process that reduces the size of the particles assists in the reduction of the pigment concentration in the skin. The larger pigment particles (that stay in the dermis because they cannot pass the lymph nodes) undergo a process of disintegration due to light-induced decomposition of pigment molecules. Other mechanisms such as enzymatic activities or recurring activities of the macrophages also contribute to the transport off the tattoo site. (Bäumler, 2015)

Sepehri et al confirmed that tattoo pigments distribute within the body via the blood in addition to the lymphatic pathway. The blood stream is reached either via translocation of the particles directly from the skin to the circulation or indirectly via release from the lymph nodes as part of the normal lymphatic drainage. (Sepehri, et al., 2017a) Translocation of tattoo particles in the nano- and micrometre range from skin to lymph nodes was confirmed in the human body in a recent study of human cadavers. (Schreiver, et al., 2017) Soluble ingredients are likely to be metabolised and excreted from the body within weeks, although their pattern of systemic bioavailability will be variable depending on the solubility of the individual substance/compound (Serup, et al., 2015).

Depending on the route of transportation (lymph or blood system) and their physical structure, pigments can circulate through the human body, prior to excretion or deposition in other organs. Studies demonstrated that the transportation via the lymph system leads to deposition in the regional lymph nodes, which appear efficient in holding back particles (Dominguez, et al., 2008). Circulating particles above 10 nm (the maximum value for glomerular particle filtration) cannot be excreted in the urine and must recirculate in the blood. (Haroldsson & Sörensson, 2004) Most pigment particles (before degradation in the human body) range in size of 60-800 nm with black pigment more in the nanometre range and other colours in the micrometre range. (Høgsberg, et al., 2011)

Whether the pigment particles target other human organs has been the subject to very few recent studies. Sepehri et al observed red and black tattoo pigments in Kupffer cells in the liver of mice one-year post tattoo. The authors explain the observation with the pigment circulation in the blood and the body's detoxification mechanisms, as the Kupffer cells have a gatekeeper function, serving to encapsulate and inactivate particulate elements, which have reached the blood and passed through the liver. The study did not demonstrate tattoo pigment deposits in other internal organs. The authors examined the spleen and lungs, in addition to the lymph nodes and kidneys, and pointed to the deficiencies in light microscopy (limited resolution which allows only large pigment aggregates to be visualised) and transmission electron microscopy (or TEM, which can analyse a minute element of a composite organ, while deposits can occur spontaneously anywhere in the organs) applied in the analysis. (Sepehri, et al., 2017a) Other studies of, e.g., intradermally injected quantum dots (Gopee, et al., 2007), demonstrated the deposition of the nanoparticles in the liver, regional draining lymph nodes, kidney, spleen, and hepatic lymph nodes. This suggests that the physical form of the particles can have an important role in which internal organs are targeted. As mentioned previously, tattoo inks contain nanoparticles (Høgsberg, et al., 2011) and larger pigment particles can be broken-down due to light-induced decomposition of pigment molecules. (Bäumler, 2015)

b) Classification of adverse effects related to tattoos and PMU

A review of literature (Papameletiou, et al., 2003) concluded that generally, the pigments used for tattooing seem to be well tolerated by the skin. Nevertheless, adverse reactions have been published in the literature. Further, it is very likely, that a great number of skin reactions on tattoos is not reported. The lack of centralised tracking of this information has impeded the aggregation of information on the frequency of these effects, association between specific pigments and other ink constituents with certain human health conditions, and other clinical and epidemiological data that can facilitate the treatment and regulation of the tattoo inks and PMU. E.g., in Europe, tattoos, whether they are normal or complicated are coded using the same international diagnosis number, L81.8E, Morbus Cutaneus Pigmentosus Alia. (CHDP, 2015)

With that said, the number of reported adverse effects associated with tattoos and PMU has been increasing with the growing number of tattooed people. (Wenzel, et al., 2013) Tattoo reactions have been reported in the literature for almost every ink colour.

The composition of modern tattoo ink, however, is poorly understood. Black ink is composed of soot derivatives and carbons, including polycyclic aromatic hydrocarbons, and this has not changed radically over the last several decades. The composition of colour inks, however, has changed since the 1970s. Whereas heavy metals – such as mercury, cadmium, and lead – were previously key ingredients in tattoos, since the 1970s, they are no longer common components of tattoo inks, primarily as a result of the US FDA banning their use for cosmetic purposes. Synthetic organic pigments, such as azo dyes and polycyclic compounds, are now more frequently used. (Brady, et al., 2015)

Adverse effects that have been observed in relation to tattoos can be classified in a number of different ways:

- According to the length of their evolution: acute and chronic reactions (Kluger, 2016a). E.g., acute processes can include delayed healing and infection, while keloids, allergy, autoimmune responses, and malignancy are referred to as chronic events (Brady, et al., 2015)
- According to the delay of onset after tattooing: early during the healing phase or delayed, after tattoo healing. (Kluger, 2016a) Various complications may occur soon after tattooing, from benign complications such as transient limb edema, palpable lymph nodes, and contact eczema, to more severe such as infection by virulent micro-organisms, cellulitis, necrotizing fasciitis or cutaneous vasculitis. These are described in detail in (Kluger, 2012). Those that can be associated with exposure to the substances in tattoo inks and PMU are discussed below.
- According to the type of reaction: i) infectious,[49] ii) tumours, and iii) granulomatous, lichenoid or hypersensitivity allergic reactions (Wenzel, et al., 2013). Some authors refer to the latter as inflammatory/immune reactions (Brady, et al., 2015), while others combine the second and the third categories under the title "non-infectious", e.g., (Serup, et al., 2015b). While infectious adverse effects continue to be observed despite the significant headway in hygiene in tattoo parlours (e.g., some authors report them as 0.5% of reactions (Klügl, et al., 2010), while others, in excess of 10%, citing underreporting as most infection cases are handled by general practitioners (Serup, et al., 2016)), of primary concern for this dossier are the reactions that can be associated with exposure to the substances contained in tattoo inks and PMU, i.e., of those reported above: inflammatory/immune reactions and malignant tumours. Other reactions of interest are systemic reactions that indicate damage to internal organs and reproductive and developmental effects. These categories, the latter in particular, have seen less attention in the reviews of tattoo adverse effects as the majority of these reviews are by medical specialists in dermatology, their long-term and multifactorial nature makes it difficult to be clearly associated with tattoo ink exposure, etc.
- According to the severity of the reaction: discomfort (or complaints as referred to by some authors) or complications. Discomfort in connection with tattoos is defined as

[49] Viral infections (e.g., hepatitis B, C or D; papillomaviruses; etc.), bacterial (e.g., streptococcus, staphylococcus, mycobacterium, etc.), and fungal infections.

local or general objective or subjective discomfort that the tattooed person has incurred by being tattooed and which is the result of the tattoo. Discomfort may be acute or immediate in connection with tattooing, but temporary concurrently with the healing of the tattoo, or chronically as permanent or continuously recurring discomfort. Clinical complications in connection with tattoos are defined as serious adverse side effects in tattoos (or resulting from tattoos), which manifest themselves as objective pathological/clinical changes, as well as subjective symptoms to a degree that they have the nature of disease or disablement, and which typically make the tattooed person seek medical treatment. (CHDP, 2015)

- Whether they are associated with the normal healing process or otherwise: The tattoo machine makes approximately 5 000 punctures per minute in the epidermis, injecting tattoo pigments and auxiliaries in the skin. Acute septic inflammatory reactions of variable intensity appear immediately after tattooing due to the needle trauma releasing histamine. The reaction is characterised with erythema, induration and what some authors describe as "peau d'organge" with dilation of the hair follicles of the tattooed skin (Kluger, 2016a), while others as a nettle-rash-like reaction (CHDP, 2015). The skin is infiltrated with white blood corpuscles, resulting in inflammation, during which superficial crusts on the skin are formed and the ink retained in the epidermis is shed as the epidermis peels away. The healing phase ends with a period of dryness and cracking. (CHDP, 2015) Such reactions are not considered as complications, as they occur to all tattooed individuals. They rather are considered as part of the natural healing process or the "natural history" (Kluger, 2016a) of the tattoo. The reaction usually disappears after 2-4 weeks. (Oantă, et al., 2014)
- Whether they are associated with the tattoo procedure or otherwise: These may include effects due to the technique applied by the tattoo artist, e.g., scarring due to needle trauma, pigment overload or blow-out, but also psycho-social impacts as a result of having a tattoo. Social disability can be a consequence of tattooing due to the negative impact of an unwanted tattoo on the psyche and quality of life. (Serup, et al., 2015b) Tattoo removal is costly, and therefore, inaccessible to all and itself can lead to severe scaring, darkening of some colours due to improper laser treatment, which can lead to severe disfigurement. These psycho-social effects could lead to psychological trauma (again especially if the disfigurement affects the face) to the individual, some with long-term effects, potentially leading to psychological disorders. These effects are also considered the result of the personal choice to get a tattoo and regulation related to the chemical content of tattoo inks and PMU is not expected to have an impact on them. Therefore, these are not discussed further.
- Whether associated with tattoo removal or not: these may include laser treatment induced adverse effects, results of self- or non-medical professional treatment (e.g., lactic acid, water tattooing, scarring, etc.). Tattoo removal is often not entirely effective. The removal can itself lead to severe scaring, darkening of some colours (e.g., titanium dioxide) due to improper laser treatment, which can lead to severe disfigurement and psycho-social effects, again especially if these affect the face, due to removal of PMU for example. Similarly to the previous point, these effects likely will not be impacted by a regulation on the chemical composition of tattoo inks, and therefore, are not discussed further.

- Whether the health impact is due to the tattoo/PMU or it aggravates an underlying condition, e.g., atopic dermatitis, psoriasis, sarcoidosis.
- Typical for tattoo inks or for PMU or for both. While many adverse health effects have been reported as a result of both tattoo and PMU procedures, some have been associated exclusively with one or the other. For example, loss of eyelashes, eyelid necrosis, and ectropion[50] have been reported primarily for PMU. (De Cuyper, 2015)

For detailed review of infectious and other adverse human health effects observed in relation to tattoos and PMU consult (JRC, 2016a) and (JRC, 2016b). The effects as described in these reports are briefly summarised in *Table* 137.

Table 136 Adverse health effects

Category health effect	Description
1. Acute aseptic inflammation	Individuals getting a tattoo experience immediate discomfort, swelling and erythema during the procedure and the days after, together with transient bleeding and lymphadenopathy. This acute inflammatory reaction of variable intensity remains in principle aseptic, unless cases of bacterial contamination. During the healing phase lasting 1 to 4 weeks, a superficial crusting and induration takes place in the tattooed area and patients may complain about pain, itching, blistering and burning sensation, like after sun exposure.
2. Infectious risks	The source of infection may be the tattooist, the instruments, the ink or the tattooed individual himself. Infections may occur if tattoo instruments are not properly sterilized and from tattoo inks microbiologically contaminated at the manufacturing phase or after the opening of the bottle, due to deficient hygienic conditions and e.g. by diluting inks with non-sterile water. Infection can further take place during the healing phase of a tattoo.
2.1. Bacterial infections	Skin infections in the form of papulo-pustules provoked usually by pyogenic strains, such as staphylococcus aureus or streptococcus, may appear quickly within the first few days after the tattoo procedure. Both acute superficial pyogenic infections, such as folliculitis, impetigo or ecthyma, and deep regional pyogenic infections, like furunculosis, erysipelas and cellulitis of the entire limb, are seldom, while systemic involvement and life-threatening outcome (by gangrene, osteomyelitis, epidural abscesses, septicaemia, toxic shock syndrome, etc.) remains exceptional under correct hygienic circumstances. Infective endocarditis has been mostly documented in patients getting extensive and repeated tattoos.
2.2. Viral infections	Isolated cases of viral warts caused by the human papilloma virus (HPV) or molluscum contagiosum (MCV) transmitted during the tattoo process or due to the presence of HPV in the tattoo ink have been observed, after an incubation period of 2 weeks to 10 years, but these events rarely take place within professional settings. These may also be due to Kobner phenomenon. Blood-borne viruses, such as Hepatitis B (HBV), Hepatitis C (HCV), and

[50] Ectropion (eversion of the lower eyelid) symptoms are tearing (due to poor drainage of tears through the nasolacrimal system, which may no longer contact the eyeball) and dry eyes. (MSD, 2017)

	HIV, have also been associated with tattoo practices, although the latter remains theoretical as transmission of HIV requires massive and prolonged bodily fluid contact, which is unlikely to happen during a standard tattoo session.
3. Non-infectious risks 3.1. Allergic or hypersensitivity reactions	
3.1.1. Acute or chronic eczematous dermatitis (A/CED)	A/CED presents usually as an itchy and scaly erythema in sensitized patients following any topical application during the healing phase, e.g. antibiotics, disinfectants, or by contact with gloves' latex, etc. The papulovesicular rash is typically localized at the tattoo site, but can secondarily spread as an urticarius to the whole body.
3.1.2. Photosensitivity	Photosensitivity (or sun allergy) is an immune system reaction triggered by sunlight. Photosensitivity reactions include solar urticaria, chemical photosensitization, and polymorphous light eruption are usually characterized by an itchy eruption on patches of sun-exposed skin. (MSD, 2017)
3.1.3. Lichenoid & granulomatous	Lichenoid (papules or plaques) and granulomatous (firm indurated nodules) pruritic lesions are generally confined to the red portion of the tattooed area
3.1.4. Lymphomatoid	Lymphomatoid reddish indurated nodulo-papules and plaques, sometimes pruritic, and much similar to cutaneous lymphomas at clinical and histologic examination, though without malignant evolution in the vast majority of cases. Pseudolymphomatous infiltrates, which are thought to be a delayed reaction to chronic antigen stimulation-albeit without conclusive patch-test, are not always confined to the tattooed area. A case of malignant transformation of a long-standing pseudolymphomatous tattoo reaction into a cutaneous lymphoma has been reported.
3.1.5. Pseudoepitheliomatous hyperplasia (PEH)	Pseudoepitheliomatous hyperplasia (PEH) appears rarely as verrucous nodules or plaques, within weeks or months after tattooing. They are difficult to distinguish from tumours, hence skin biopsy is advisable, as they can also be linked to various infections.
3.2. Coincidental pathologies 3.2.1. Concomitant underlying dermatoses reactivated by tattooing	
3.2.1.1. Köbner phenomenon	The Köbner (Koebner) phenomenon describes the appearance of new skin lesions on areas of cutaneous injury in otherwise healthy skin. It is also known as the isomorphic response. (DermNet, 2017) Therefore, tattoo putative clients known to suffer from such chronic pathologies should be warned against tattooing, which might precipitate their underlying disease.
3.2.1.2. Sarcoidosis	Sarcoidosis is a disorder resulting in noncaseating granulomas in one or more organs and tissues; aetiology is unknown. The lungs and lymphatic system are most often affected, but sarcoidosis may affect any organ. (MSD , 2017) Lesions on tattoos consist mainly of asymptomatic, itchy or sometimes tender papules, nodules, plaques or infiltrations on the tattoos, with sometimes scaling, ulcers or blisters. Granulomatous reaction to tattoos, even

	of the not sarcoidal pattern, may reveal or complicate systemic sarcoidosis.
3.2.1.3. Lichen planus	It is challenging to determine if a lichenoid eruption following a tattoo represents a generalized lichenoid tattoo reaction or a true lichen planus.[51] Other anecdotal cases of lichen sclerosus and atrophicus,[52] perforating granuloma annulare (commonly with red pigments),[53] perforating collagenosis[54] occurring in red tattoos, Darier's disease (genetic Keratosis follicularis), erythema multiforme[55] and scleroderma-like reaction[56] restricted to the red parts of a tattoo have also been reported
3.2.1.4. Lupus erythematosus	LE is a chronic, multisystem, inflammatory disorder of autoimmune etiology, occurring predominantly in young women. Common manifestations may include arthralgias and arthritis, malar and other rashes, pleuritis or pericarditis, renal or CNS involvement, and hematologic cytopenias. (MSD, 2017)
3.2.1.5. Vasculitis	Cutaneous vasculitis refers to vasculitis affecting small- or medium-sized vessels in the skin and subcutaneous tissue but not the internal organs. Purpura, petechiae, or ulcers may develop. Diagnosis requires biopsy. Treatment depends on etiology and extent of disease. (MSD, 2017)
3.2.1.6. Pyoderma gangrenosum	Pyoderma gangrenosum is a chronic, neutrophilic, progressive skin necrosis of unknown etiology often associated with systemic illness. (MSD, 2017) PG is a rare complication of tattooing, particularly on the lower extremities, and has been described in only two patients, one of whom had an underlying blood cancer [57]. PG-like ulcers might be also elicited by bacterial infections.
3.2.2. Tumours 3.2.2.1. Pseudoepitheliomatous Hyperplasia (PEH)	Rapidly growing after tattooing (between 1 week and few months), albeit benign lesion, it presents as nodules, large verrucous plaques or ulcerated lesions, mostly confined to red areas. It can be associated with various infectious, inflammatory or neoplastic processes, and its clinical and histologic features are

[51] Lichen planus is a recurrent, pruritic, inflammatory eruption characterized by small, discrete, polygonal, flat-topped, violaceous papules that may coalesce into rough scaly plaques, often accompanied by oral and/or genital lesions. Diagnosis is usually clinical and supported by skin biopsy. Treatment generally requires topical or intralesional corticosteroids. Severe cases may require phototherapy or systemic corticosteroids, retinoids, or immunosuppressants. (MSD, 2017)

[52] Lichen sclerosus is an inflammatory dermatosis of unknown cause, possibly autoimmune, that usually affects the anogenital area. The earliest signs are skin fragility, bruising, and sometimes blistering. Lesions typically cause mild to severe itching. (MSD, 2017)

[53] Granuloma annulare is a benign, chronic, idiopathic condition characterized by papules or nodules that spread peripherally to form a ring around normal or slightly depressed skin. (MSD, 2017)

[54] Of the group of perforating dermatoses. (DermNet, 2017)

[55] Erythema multiforme is an inflammatory reaction, characterized by target or iris skin lesions. Oral mucosa may be involved. Diagnosis is clinical. Lesions spontaneously resolve but frequently recur. Erythema multiforme usually occurs as a reaction to an infectious agent such as herpes simplex virus or mycoplasma but may be a reaction to a drug. Suppressive antiviral therapy may be indicated for patients with frequent or symptomatic recurrence due to herpes simplex virus. (MSD, 2017)

[56] The term scleroderma refers to hardened skin. There are various conditions that are affected by scleroderma or appear similar to it. (DermNet, 2017)

	hard to differentiate from keratocanthoma or verrucous carcinoma. Ten cases have been reported for the last 40 years.
3.2.2.2. Keratoacanthoma (KA) and Squamous Cell Carcinoma (SCC)	KAs are considered by some physicians malignant SCCs, whereas others debate about its malignancy. Biopsy does not always differentiate precisely between PEH, KA and SCC; but time lag after tattoo may help to distinguish KA (which grow usually within a week to a year and resolve spontaneously over some month) from SCC (whose first reported case occurred on a 21-year-old tattoo). Red tattoo ink was associated with 9 out of 11 (82%) of the KAs diagnosed in 8 patients. There is no specific proof for a causative link between tattoos and SCC.
3.2.2.3. Basal cell carcinoma (BCC)	BCC has been rarely found to appear after trauma, for example in surgical scars. In the one case documented, the BCC originated in the adjacent non-tattooed skin and overgrew secondarily the tattoo.
3.2.2.4. Malignant melanoma	Both benign nevi and malignant melanoma can arise de novo in a tattooed area. While it is uncertain whether tattooing is a significant risk factor for the development of malignant melanoma, it can delay its diagnosis and treatment
3.2.2.5. Non-Hodgkin lymphoma	One case of B-cell lymphoma was reported in a patient with a long history of pseudolymphoma (a benign lymphocytic infiltration) on tattoos on both arms, the lymphoma developed on both tattooed and non-tattooed areas. Anecdotal cases of rare skin malignant lesions (two cases of dermatofibrosarcoma protuberans (Darier-Ferrand) occurring 1 and 2 years after tattooing, and a leiomyosarcoma which appeared 9 years after tattooing) for which a true link with the tattooing event is highly speculative. One case of dermatofibrosarcoma protuberans (uncommon, locally aggressive cutaneous tumour of intermediate grade malignancy) arising in a tattoo.
4. Medical diagnosis and treatment interference	Dark coloured pigments used in tattoos make it a bit more difficult to distinguish possible growth and malignant transformation of pre-existing nevi or metastatic invasion of a lymph node by a melanoma. False-positive results may occur in mammography when tattoo inks contain metals, and especially iron oxides able to blur diagnostic imaging such as MRI and PET scan. In rare cases MRI exams may also lead to complaints of pruritus and burning in tattooed individuals. Spinal anaesthesia in the lumbar region is more difficult to carry out if the skin area is tattooed, in particular with dark colours, but the potential risks of such a procedure are still under debate.
5. Contraindications to tattooing	Include skin disorders and some pre-existing systemic conditions. Patients are advised to consult a medical professional prior to tattoo procedure.
6. Adverse health effects linked to tattoo/PMU removal 6.1. Thermally induced acute	Blistering is reported being one of the major transient effects of epidermal thermal damage induced by removal treatments. According to some authors, this side effect is expected in most cases and is linked both to incorrect parameters applied to the laser device and to an unexpectedly high level of absorption of

inflammation	laser energy by epidermal melanin. Local development of crusting is an additional effect caused by epidermal thermal stress. It requires 7-10 days of appropriate post-intervention care. Even though modern laser therapy drastically reduced the development of scars with respect to the earlier procedures, the formation of permanent scars is still possible when the type of laser and/or applied conditions are not correct and the damage is deeper. In case of particularly resistant tattoos (multi-coloured tattoo containing iron oxide or titanium dioxide), which require a more intense treatment for removal, it is more likely to develop permanent scars. Erythema formation and/or pinpoint bleeding are due to photo acoustic damage of dermal capillary walls as a result of the high peak of laser energy. This promotes extravasation of blood into the surrounding tissue. Erythema is reported healing after few days from the laser treatment with adequate cooling Additional acute effects include scaling, induration and fibrosing. Transient textural changes may also be observed and are reported self-resolving in 1-2 months
6.2. Allergic and systemic reactions	Similar reactions to those during/after tattoo procedure have been described following laser removal. In this case, it is not only the original dye that triggers a reaction, but also its degradation products that are considered as new antigens scattered by laser treatment.
6.3. Pigment disorders 6.3.1. Hypopigmentation	Hypopigmentation has been attributed to the presence of epidermal melanin, which is known to compete for laser light absorption. This interaction eventually leads to the destruction of melanocytes, according to the same mechanism that applies to tattoo pigments. As a chromophore, melanin is able to absorb energy throughout the whole range at which QS lasers operate with peaks of absorption lying in the ultraviolet range and decreasing at the longest wavelengths. However, some colours such as red, yellow and orange require (shorter) 532 nm wavelength to be removed and side absorption by melanocytes with consequent hypopigmentation is unavoidable. Most of the time, the loss of melanin pigment is transient, but it may persist up to years or even become permanent especially after repeated treatments. The incidence of permanent hypopigmentation in different studies has been estimated to be up to 10% of the studied population. Time of onset is reported being 4-6 weeks up to several months after treatment.
6.3.2. Hyperpigmentation	Hyperpigmentation is considered a result of an increased UV sensitivity of the skin after laser irradiation. Again, it is related to the patient's skin type, with darker skin being more prone. The incidence is 5-10% of the population who underwent QS lasers, with higher occurrence in individuals subject to multiple laser treatment, and it is considered a transient effect
6.3.3. Paradoxical darkening	Can take place due to the removal of multicolour tattoos. It is strongly linked to the chemical composition of some colours, e.g., some metal oxides. Titanium dioxide, which is contained in white inks and is often used to add brilliance to other tattoo inks, is

	responsible for darkening when light colours are present. The same complication can appear in tattoos containing iron pigments often used in flesh-toned colours for PMU. This is explained with the reduction of ferric oxide to jet black ferrous oxide. In a study of 184 patients who underwent QS laser removal of non-black tattoos, 18% experienced colour shifts, ranging from mild greying to complete blackening of the white, flesh-coloured, red, brown, yellow and crimson parts of their tattoos.

Source: (JRC, 2016a), (JRC, 2016b)

c) Effects related to the chemical composition of tattoo inks and PMU

The sections below focus on chemical-related adverse effects only as these could be directly influenced by regulation on substances that can be contained in tattoo inks. The remaining effects may increase the severity of chemical-related effects by, e.g., increasing the metabolism of the pigment particles in the body, others can abate them by leading to faster expelling of the pigment particles from the body. However, as these adverse effects are not considered triggered by the substances present in tattoo inks, therefore, they are not discussed further in this dossier. Although, there are various categorisations of tattoo reactions, the effects below are grouped in: non-infectious inflammatory, systemic, malignant tumours, and reproductive and developmental.

Non-infectious inflammatory reactions

Clear classification of tattoo and PMU reactions is challenging because clinically, the manifestations are often non-specific and there is much variability, while histological patterns often overlap. Earlier reviews of these effects group them according to histological patterns in granulomatous, lichenoid, or hypersensitivity allergic reactions (Wenzel, et al., 2013), also referred to as inflammatory/immune reactions (Brady, et al., 2015). More recent reviews of adverse effects ((CHDP, 2015), (Serup, et al., 2015b), (Serup, et al., 2016)) have grouped them on the basis of clinical descriptive assessment and have submitted the classification to the World Health Organisation (WHO) as a proposal to the 11th revision of the International Classification of Diseases. As (JRC, 2016a) and (JRC, 2016b) present a detailed overview of various adverse health effects categorising them to an extent on the basis on their histological pattern, for completeness, this dossier presents the categorisation of these effects on the basis of clinical appearance proposed by Serup and various co-authors. A summary of the different adverse health effects categorised on the basis of histological pattern is presented in *Table* 137 (as per (JRC, 2016a) and (JRC, 2016b)).

The non-infectious inflammatory reactions can be separated into allergic and primarily non-allergic inflammatory reactions.

Allergic reactions

According to (Papameletiou, et al., 2003), an allergic reaction is an acquired, abnormal immune response to a substance (allergen) that does not normally cause a reaction. Sensitisation, or an initial exposure to the allergen is required; subsequent contact with the allergen then results in a broad range of inflammatory response. Allergic conditions include eczema, allergic rhinitis or acute catarrhal inflammation of the nasal mucous membrane, hay fever, bronchial asthma, urticaria (hives) and food allergy. Allergens may be introduced by contact, ingestion, inhalation or injection.

The traditional method for diagnosing allergic dermatitis requires the manifestation of typical clinical symptoms and a positive patch test to substances that the person is exposed to. The latter are rarely obtained for skin reactions observed in relation to tattoos. In the absence of valid test reference, Serup et al. define the diagnosis of contact allergy based on the following criteria:

- the reaction is monomorphic, i.e., uniformly manifested in one particular colour and everywhere this colour is used in the tattoo. According to (Kluger, 2016a), red and nuances of red, such as purple and violet, are the most common colours involved but reactions have been described with almost all colours, except white, although limited cases have been observed by (Serup, et al., 2016). In that study, allergic reactions to red, red nuances, violet and purple comprised 85% of all allergic reactions.
- there is a latency period, i.e., a sensitisation period of weeks, months and maybe years from tattoo application until the appearance of the reaction. According to (Kluger, 2016a), the latency can range from immediate to 45 years later, but usually is several weeks to several years after tattooing.
- the reaction is chronic, constant in appearance, degree of discomfort, and resistant to treatment with topical steroids;
- the active reaction results in a similar reaction in an older tattoo with the same or nearly the same colour, which had not demonstrated prior allergic symptoms.

According to (Kluger, 2016a), allergic (hypersensitivity) reactions to tattoo pigments are currently the most common complication observed in relation to tattoos. They comprised 37% of all tattoo associated adverse effects found in the study of 493 tattoo complications (Serup, et al., 2016). (Kluger, 2016a) describes the symptoms as tenderness, swelling, asymptomatic or itchy papules or nodules, isolated pruritus (itching), and complete infiltration of the colour. Necrosis (dead tissue) can occur very rarely, while photosensitivity may be an associated or the only symptom. The itch is often severe. On the basis of clinical severity, Kluger et al. describes chronic allergic reactions as:

- Minor: e.g., a mild or moderate degree of swelling in the tattoo with no or only light and barely visible keratinisation;[57]
- Moderate: e.g., inflammation with plateau-like swelling, reddening and sensitiveness of the tattoo, with a secondary reaction in the epidermis in the form of scales of keratinisation with a skin surface dominated by condensed horny material;
- Strong: deeply ulcerating (developing an open sore) or massive keratinisation (swelling as far as 6-8 mm above the level of the surrounding skin), which completely dominates the appearance, and covers or masks the allergic reaction in the underlying dermis like armour. (CHDP, 2015)

According to (Serup, et al., 2016), the allergic tattoo reactions can be grouped as follows on the basis of their clinical appearance: plaque-like, ulcerating, excessive hyperkeratotic, photosensitivity and urticarial-like patterns.

Plaque-like

[57] Organic process by which keratin is deposited in cells and the cells become horny (as in nails and hair). (Source: Mnemonic Dictionary)

Plaque elevation is the most common tattoo reaction, comprising 32.2% of all tattoo associated adverse effects found in the study of 493 tattoo complications, associated with red and colour tattoos (Serup, et al., 2016). The clinical appearance is thickening and elevation of the tattoo, with or without large adherent scales. Inflammation with lymphocytes concentrated in the outer dermis is observed, also sometimes extending to the non-tattooed skin. Histologically, a tattoo with plaque elevation may show interface dermatitis. (Serup, et al., 2015b) Skin elevation was one of the persistent skin problems reported by 0.7% of respondents in (Klügl, et al., 2010).

Ulcerating patterns

These adverse effects have been seen in 1.4% of the reactions, primarily associated with red and colour tattoos (Serup, et al., 2016). Histologically, these reactions show in the periphery severe dermal inflammation, e.g., interface dermatitis or inflammation as part of necrosis, which is followed by the rejection of the dead tissue creating an ulcer. (CHDP, 2015) The ulceration may invade the dermis entirely and approach the subcutaneous fat. Necrosis may extend further into deep tissues (e.g., muscle connecting tissue) and regional lymph nodes where the pigment has migrated. The allergy may progress to autoimmunity, with an attack on non-tattooed skin, presenting itself as vasculitis, bullous reactions and generally delayed wound healing thought the skin. (Serup, et al., 2015b)

Hyperkeratotic

A thickening and elevation of the epidermis resembling sand paper due to keratinisation or cornification of the surface of the skin is observed in 3.7% of tattoo reactions in (Serup, et al., 2016), usually associated with red pigments. The histology shows interface dermatitis but with an especially strong formation of new epidermis cells, which increases the thickness of the epidermis, in severe reactions as 6-8 mm above the surrounding healthy skin. Excessive hyperkeratosis may be interpreted as pseudoepitheliomatous hyperplasia by pathologists. (CHDP, 2015)

Photosensitivity

Light (solar or laser) induced reactions range from minor (swelling, itching, stinging, redness) to pain or thickening in the tattoo. They are associated primarily with darker coloured tattoos, e.g., black, red and blue. The symptoms can begin immediately after light exposure to the following day, lasting 20 min minutes to several weeks. (Hutton Carlsten & Serup, 2014) The results of the beach study by Hutton Carlsten & Serup showed that about 24% of complaints were sun-related.

Some urticarial (wheal-and-flare or hives) reactions can be light-induced. These can be acute and of short duration, sometimes with spontaneous healing, and occasionally lasting days. In the study (Serup, et al., 2016), in total 11% of reactions were provoked by light. Red colours were more frequently associated with light-induced reactivity, indicating to the authors that azo pigments and their photochemical decomposition products may play a role in photosensitivity reactions.

Other urticarial-like reactions

Urticarial-like reactions – wheal-and-flare reactions or hives – can be acute or chronic, often triggered by external factors in addition to light (as described above), e.g., heat, stress (including from the needle trauma of tattooing or possibly connected to a pre-existing urticaria factitia), or an activity (e.g., intake of alcohol). In (Serup, et al., 2016), widespread urticaria lasted several months and was not provoked by light. The authors

postulate that it is possibly due to allergy to some constituents or impurities of the tattoo inks or pigment metabolites.

Therefore, it can be concluded that not all urticarial-like reactions can be associated with the chemical composition of tattoo pigments, and therefore cannot be expected to be impacted by a regulation on tattoo ink substance. However, light-induced and widespread urticaria are the two notable exceptions.

Primarily non-allergic inflammatory reactions

Other important reactions that can be associated with the chemical composition of the tattoo inks include: papulo-nodular, lymphopatic, and neurosensory reactions.

Papulo-nodular pattern

Papulo-nodular reactions are the main example of non-allergic, non-infectious inflammatory reactions. They are the second largest group, accounting for 13% of all tattoo reactions according to (Serup, et al., 2016) and are associated primarily with black tattoos and pigment agglomeration. Skin papules was one of the persistent skin problems reported by 0.4% of respondents in (Klügl, et al., 2010). The papules and nodes appear clinically as round or elongated papular or nodular thickening or elevation in sections of the tattoo with high concentration of pigment. The nodules appear as an agglomeration of black (carbon black) pigment nanoparticles, which the skin regards as foreign body and attempts to eliminate transdermally; however, the basement membrane holds back most of the material in the dermis. Scratching may release these agglomerations enabling the skin to heal, leaving a white spot. (Serup, et al., 2015b)

Papules and nodules may have the histology of pain inflammation and foreign body reaction, granulomatous inflammation or sarcoidosis granuloma (isolated to the tattoo or widespread involving lungs and other organs). (Serup, et al., 2016) The production of Reactive Oxygen Species (ROS) in the agglomerated black pigment may be the reason for the inflammation. (CHDP, 2015) The study (Serup, et al., 2016) associates also papulo-nodular patterns with pigment overload, which may also be a result of the technique used by the tattoo artist. Therefore, it can be concluded that not all cases of papulo-nodular reactions can be attributed to the chemical composition of tattoo inks and thereby, may not be impacted by the proposed restriction.

Lymphopathic pattern

Pigment particles are often transported to the regional lymph nodes causing visible pigmentation and sometimes damage and blockage leading to an enlargement, swelling or tenderness of the lymph, i.e., a lymphedema. The chronic condition is an example of a rare regional tattoo reaction according to (Serup, et al., 2016), although swollen lymph nodes were seen in a number of patients with spontaneous regression. These can sometimes be associated with an allergic reaction, in particular, if they do not occur soon after the tattoo process.

Pseudolymphomatous reactions to tattoo inks, benign reactive T or B cell lymphoproliferative processes simulating cutaneous lymphoma, have been reported. (Islam, et al., 2016) Histologically they have the features of Spiegler-Fendt pseudolymphoma and are characterised by red and violet indurated nodules and plaques that are clinically similar to the cutaneous B cell lymphoma. Some pseudolymphomas may regress spontaneously, although biopsy is needed to exclude lymphoma and treatment with corticosteroids, laser therapy or surgical incision may be indicated. (Islam, et al., 2016)

Neurosensory reactions

Neurosensory complications with discomfort and invalidating pain with little or no inflammation histologically were observed in 2.2% of tattoo reactions by (Serup, et al., 2016). The study authors hypothesised that metabolites of some tattoo pigments may be neuro-stimulators of C-fibres and elicit pain and itch. Inflammatory tattoo reactions generally result in surprisingly prominent itch and pain, and metabolism in the skin with the formation of neuromodulators from pigment raw materials may be common and not limited to distinct pain conditions and syndromes. Pain can propagate and become regional or segmental following dermatomes and can be invalidating and a "pain syndrome". (Serup, et al., 2016) Another example of a neurosensory reaction is numbness. It was one of the persistent skin problems reported by 0.3% of respondents in (Klügl, et al., 2010). It is also possible that the neurosensory reactions are a result of a trauma of the nerve endings during the tattoo procedure which can be further aggravated by the chemical composition of the injected pigment in the skin. (De Cuyper, pers com) From this perspective, these reactions cannot be solely attributed to the substances present in the skin and the impact of the proposed restriction on these reactions would likely be limited.

Systemic or general clinical complications

Self-reported systemic problems directly after tattooing were mentioned by 6.6% of respondents, while 3% reported persistent problems in other than the skin (Klügl, et al., 2010). However, the data does not provide sufficient detail to conclude on the prevalence of systemic effects after the initial healing process of the tattoo. Furthermore, reviews of tattoo adverse effects are primarily the subject of research of dermatologists and therefore, adverse effects are associated with tattoos if the effects also present themselves cutaneously. The metabolism and diffusion of tattoo inks in the human body is not well-established; although, there is clear evidence that pigments are transported to local and regional lymph nodes of humans. (Schreiver, et al., 2015a) Furthermore, animal studies have revealed the presence of pigments in Kupffer cells in the liver, which suggests that the pigments as well as soluble substances present in tattoo inks enter the blood and can be transported to virtually any human organ but it is unknown whether other internal organs can be targets for deposition of tattoo pigments. (Sepehri, et al., 2017a)

Furthermore, modern-day tattoo inks contain nanoparticles. (Høgsberg, et al., 2011) The extent to which pigment containing nanoparticles may reach internal organs and lead to clinical symptoms has not been studied and is unknown. (CHDP, 2015) Animal studies of nanoparticles in general (quantum dots, silver, and gold, injected respectively intradermally, subcutaneously/orally or intratrecheally) show their deposition in organs such as kidney, liver, spleen, lung as well as brain, leading to the blood-brain barrier destruction. The translocation is shown to depend on the particle size, chemical composition, shape, electrical charge, coating, etc. (Gopee, et al., 2007), (Loeschner, et al., 2001), (Sadauskas, et al., 2009), (Sepehri, et al., 2017a), (Tang, et al., 2009)

Although, some substances historically present in tattoo inks have harmonised classification of STOR RE and STOT SE, indicating their acute or chronic toxicity to various internal organs, it is uncertain whether the human body is exposed to these substances sufficiently to lead to an effect clearly associated with exposure to tattoo inks. The association between organ toxicity and tattoos has not been confirmed by well-designed studies.

The following section describes the systemic reactions with cutaneous manifestations that are associated to a various degree with the chemical composition of tattoo inks: general

eczema, sarcoidosis, and other associated skin diseases. These systemic reactions represented 8% of all primary diagnoses and were associated mainly with black tattoos. (Serup, et al., 2016)

Sarcoidosis

Sarcoidosis is an exaggerated immune response to exogenous or autoantigenic stimuli. (Kluger, 2013) Its immunopathogenesis has not been fully established but it likely represents a heterogeneous spectrum of disorders arising in genetically susceptible individual after a variation in the inherent host immunity, infectious processes and environmental exposure that contribute to a final common pathway resulting in systemic noncaseating epithelioid granulomatous inflammation, most commonly affecting the lungs, lymph nodes, liver, spleen, bones, eyes, and skin (about 25% of cases). (Schiviavo, et al., 2014)

Sarcoidosis in tattoos has been observed for years. A specific antigen in the tattoo may drive a cell-mediated immune response characteristic of granuloma formation in predisposed individuals. (Kluger, 2016a) suspects that the culprit reaction trigger may be a reactive ink by-product that appears during the patient's life, rather than a component that is introduced during the actual tattoo procedure. The author compares this process to the formation of a pencil-core granuloma, during which the graphite of the pencil is degraded until a critical size under which a granulomatous reaction may occur, sometimes 20 years after the tattoo trauma.

On the basis of sarcoidosis in the general population and the prevalence of tattoos, it can be expected that sarcoidosis also appears in people with tattoos in 1 to 2 on every 10 000 tattooed Caucasians. The reported cases in literature have been fewer (although there are reasons to suspect underreporting), occurring from six weeks to 45 year after tattoo procedure and primarily involving males. (Kluger, 2016a) Cases of tattoo sarcoidosis have been less. Sarcoidosis was primarily seen in black tattoos and was a common associated disease, found in 5% of diagnosed tattoo reactions. (Serup, et al., 2016)

While it has been hypothesised that the tattoo may be a trigger for sarcoidosis, others have concluded that granulomatous reaction to tattoos may reveal or accompany systemic sarcoidosis. This is because sarcoidosis has poor or questionable association with trauma-induced processes, it can develop sometimes 45 years after the tattoo, and not all pigments and all tattoos are always affected. (Kluger, 2016a)

Therefore, it is recommended that cases of cutaneous sarcoidosis restricted to one pigment colour raise the question of a true sarcoidal hypersensitivity reaction to the exogenous pigment or the first manifestation of a systemic disease. Any granulomatous reaction should prompt to investigate for sarcoidosis. The presence of other cutaneous lesions or extra-cutaneous granulomas helps in distinguishing sarcoidosis from a hypersensitivity reaction. (Kluger, 2016a) A follow-up is often required to determine whether technically granulomatous inflammation is established in at least two organs before a diagnosis for sarcoidosis is given. In the literature review of (Kluger, 2013), 30% of patients with tattoo sarcoidosis did not have extracutaneous manifestations, although no follow-up was published in most cases. This is somewhat consistent with non-tattoo associated cases of sarcoidosis: 70% of patients with specific cutaneous lesions have concomitant systemic manifestations and 30% develop systemic involvement within months or years.

General eczema

It is a general knowledge that chrome and mercury allergies may cause general eczema in the skin due to the use of respectively chromium salts and mercuric sulphides in tattoo inks in the past. Today, primarily nickel salts are in tattoo ink as industrial contamination, which are often in metallic form. Nickel contamination in modern ink can, in people who are already strongly allergic to nickel, induce widespread allergic contact dermatitis comparable to the reactions to mercury and chrome shortly after a tattoo is made. (CHDP, 2015) Rash (allergic skin reaction) was a primary diagnosis in 1.8% of cases in (Serup, et al., 2016).

Other associated skin conditions

Because of the Köbner phenomenon (or isomorphic response, i.e., the appearance of new skin lesions on areas of cutaneous injury), a lesion can localise within the tattoo of an individual with a pre-existing chronic skin condition. These include mainly psoriasis, vertigo or lichen planus. The risk of localisation is dependent on the genetic background of the individual and the activity level of the disease at the time of tattooing. (Kluger, 2016a) These associated skin conditions comprised 1.2% of all diagnoses in (Serup, et al., 2016).

Malignant tumours

A literature review concluded that it is unclear whether tattoo inks may induce skin or visceral tumours, even though many substances contained in tattoo inks (such as PAHs primarily in black pigments or PAAs in colour) and their degradation products, sometimes with increased solubility properties, are classified as mutagenic or carcinogenic. (JRC, 2016a) (JRC, 2016b)

A review of skin cancer cases on tattoos reported in literature between 1938 and 2011 found 50 cases: 16 cases of melanoma, 11 cases of basal cell carcinoma (BCC), and 23 cases of squamous cell carcinoma (SCC) and keratoacanthoma (KA). (Kluger & Koljonen, 2012) These cases presented themselves with various latencies: from soon after to more than 50 years after the tattoo procedure. Melanoma and BCC cases were primarily associated with darker colours (black and blue), while SCC and KA with red tattoo pigments. (Kluger & Koljonen, 2012) Of note is that there is difficulty to distinguish SCC and KA (JRC, 2016b), the latter considered by some benign and self-limiting (CHDP, 2015) and others as borderline lesions (Kluger & Koljonen, 2012). As the number of skin cancers in tattoos is seemingly low in comparison to the prevalence of skin cancers in the general population, the authors concluded on the basis of literature review the association between tattoos and skin cancers coincidental. (Kluger & Koljonen, 2012) Kluger & Koljonen, however, note that more recent reports pertain to younger patients and have shorter delay of malignancy presentation. This they suggest could be explained with the overall predisposition to malignancies of younger people or due to substances in more recent inks with carcinogenic properties. (Kluger & Koljonen, 2012)

Cancer other than the skin as a result of tattoos has not been documented in medical literature. (CHDP, 2015) This includes cancers in internal organs and or in the lymph nodes (i.e., malignant lymphoma or leukaemia), i.e., the first organ, as noted by (CHDP, 2015), that the substances in tattoo inks reach in their most concentrated form and which in contrast to the dermis contains many proliferating cells, which may be exposed to a carcinogen. At the same time, the association between tattoos and malignancies has not been studied clinically and epidemiologically (Kluger & Koljonen, 2012).

Also, there are no well-designed animal studies which examine the link between tattoo ink exposure and cancer. The three recent studies briefly outlined below of tattooed hairless

mice observed up to 356 days have similar deficiencies: the protocol for carcinogenicity calls for an 18-24-month animal studies, therefore, the timespan and number of animals studied, make it difficult to conclude on carcinogenic effects. In addition, the mouse skin is different than the human skin and mini pigs would have been a more appropriate study animal.[58]

- A study of mice tattooed with black ink with high concentration of benzo(a)pyrene (a PAH with harmonised classification as CMR category 1B) surprisingly found a protective effect from the black tattoo on the UVR-induced skin cancer. The authors hypothesise that this may be attributed to the absorption of UVR by the tattoo pigment in the dermis and thereby reducing the backscattered radiation approaching the proliferating basal cell layer of the epidermis, from where the skin cancer originates. (Lerche, et al., 2015)
- A study with a similar set up but of mice tattooed with red ink with high concentrations of 2-anisidine (a PAA with carcinogenic decomposition products under sunlight) demonstrated that tattoos exposure to UVR showed faster tumour onset regarding the third tumour, and faster growth rate of the second and third tumour indicating red ink acts as a co-carcinogen[59] with UVR. The authors conclude that this effect is however weak and may not be clinically relevant. (Lerche, et al., 2017)
- A continuation of the study of the distribution of tattoo pigments in internal organs by Sepehri et al (Sepehri, et al., 2017a), also examined the presence of internal organ cancer in mice tattooed with high content of potential carcinogens (both banned on the Danish market in 2011 because of high concentrations of 2-anisidine and benzo(a)pyrene). Microscopy did not reveal any malignant tumours but two mice with black tattoos (and no UVR radiation) presented non-malignant while a third, a benign cyst. Systemic biopsy samples of the same organs of all mice (lymph nodes, liver, spleen, kidney, and lung) displayed no microscopic cancer. Light microscopy and systemic search found two mice with black tattoo (and no UVR radiation) presenting abnormal macroscopic findings. One sample showed solitary necrosis, while the second presented normal liver tissue. The authors suggest that the free carcinogens in the ink, including BaP and 2-anisidne are supposed to undergo a fast elimination and the initial peak of carcinogens by tattooing is unlikely to exert a clinically significant carcinogenic effect. (Sepehri, et al., 2017b)

Kluger & Koljonen hypothesise that if there is a potential link between tattoos and skin cancer, trauma, ultraviolet sun exposure, and chemical composition of the inks could be the potentially main influences in the multifactor process of carcinogenicity. (Kluger & Koljonen, 2012) The authors challenge the trauma route as an explanation for melanoma, while (CHDP, 2015) point out that tattoos are not supposed to interfere with the spontaneous cell proliferation in the basal cell layer of the epidermis, physiological renewal of the epidermis and the development of typically sun-induced cancer in the normal epidermis because the

[58] According to (CHDP, 2015), pigs have a skin thickness and a microscopic skin structure that is close to that of human skin. Therefore, pigs are most suitable experimental animals for assessing biokinetics and toxicology of tattoo inks. Pigs also have a distribution volume that is closer to humans and this animal species is, therefore suitable as a model for depositing of substances in and affecting distant organs, including by measuring DNA damage. Pigs would be suitable for studies of local tolerability, wound healing after tattooing and the impact on the skin of the combined effect of multiple needle trauma and dermally injected ink.

[59] Chemical carcinogenesis involves chemicals that are carcinogenic per se, while photocarcinogenesis is carcinogenesis induced by UVR from the sun or other sources. There may also be interaction between chemicals and UVR that alters and enhances carcinogenesis, best described as co-carcinogenesis. (Lerche, et al., 2017)

tattoo pigment is deposited in the dermis underneath the basement membrane, hindering epidermal escape of the pigment and direct exposure of the basal cells and the epidermis. Furthermore, (CHDP, 2015) points out that PAHs and PAAs are eliminated quickly (over days or weeks) and therefore, cannot have long-lasting or chronic impacts on mitotic cells. In their opinion: "any hypothetical segregation of PAA over time from azo colourants in pigments in the tissue may easily be so small and insignificant that they do not comprise a risk of cancer." Both (CHDP, 2015) and (Kluger & Koljonen, 2012) point to the lack of evidence and the need for further research.

Overall, the conclusion on the role of tattoo inks in the development of skin or internal organ malignancies cannot be made on the basis of clinical observations. Cancer is a multifactorial disease, which can take decades to express. Therefore, direct causality between tattoos and malignancies will not be easy to demonstrate and the relationship will need to be established on the basis of the hazard properties of the substances in tattoo inks and the limited information available on the degradation and metabolism of substances in the skin and their diffusion in the human body over time.

Reproductive and developmental effects

The effects of injecting into the skin of tattoo inks containing substances with known reprotoxic effects remains an area for research as several aspects of health consequences of tattoos are unclear. Similar to systemic and carcinogenic effects, there is a theoretic possibility for constituents of tattoo inks to enter the blood stream and impact other organs and the unborn foetus. Some of the chemicals in tattoo inks (heavy metals, amines, etc.) can be transferred via the human placenta. There is limited data regarding breast milk and the potential systemic distribution of tattoo constituents and by-products in the circulation and therefore, possibly through the placenta during pregnancy or in the milk in not known. (Kluger, 2015b) The presence of nanoparticles in tattoo inks increases the uncertainty.

This issue of the effects of tattoos on pregnancy and the unborn child is an issue that has become more relevant because of the large number of women in childbearing age acquiring a tattoo. Currently, no data exist to suggest additional risks for the mother or the baby in the presence of tattoos. (Islam, et al., 2016) Reproductive toxic damage in the form of abortion, deformities and malformations resulting from tattoos of fertile women before, up to or during pregnancy has not been shown. On the other hand, this has not been studied systematically either and evaluations have not ruled out that tattoos and tattoo ink may lead to such complications. (CHDP, 2015) Of particular concern is if the tattoo procedure takes place in the critical development period of sexual differentiation as the bioavailability of tattoo inks at that point is the largest. Additionally, the risk of foetal development in heavily tattooed mothers is not known either. Of note is the experience with 25 tattooed women – professional tattoo artists – in France who have had 36 favourable outcome pregnancies. (Kluger, 2015b) Currently, tattoo artists in many Member States advise against getting a tattoo while pregnant.

c) Incidence and prevalence

It is difficult to estimate the true overall incidence and prevalence of complications because no registry and epidemiological studies are available. Furthermore, direct association with the effects and specific substances is extremely challenging due to variability of the components of inks, pigments, and contaminants that can be injected into the dermis. Also, few patients consult their physician regarding minor cases, opting instead to return to the tattoo parlour. (Høgsberg, et al., 2013) A number of studies have attempted to estimate the

prevalence (in specific sub-populations) of discomfort (complaints) and complications due to tattoo and PMU procedures. The following section gives an overview of the most important incidence and prevalence studies of tattoo related adverse effects in countries in the EU. Information on additional studies is summarised in Table 9.1 in (JRC, 2016b).

One of the larger scale studies of health problems associated with tattoos was performed by (Klügl, et al., 2010). It gathered information from 3 411 German-speaking tattooed individuals, i.e., 93% from Germany (evenly distributed from the 16 federal states), 6% from Austria, 1% from Switzerland. More than two-thirds (67.5%) of the surveyed described skin problems, 6.6% systemic reactions, and 7.7% reported health problems four weeks after the tattoo (and therefore, likely not associated with the expected wound healing process of a tattoo procedure) and 6% persistent skin problems with their most recent tattoo. Three percent described other problem such as psychic problems or light sensitivity. The most frequent problems included: bleeding, crusts, itching edema and pain, followed by a burning sensation, blister formation and puss-filled skin, while the most frequently described systemic reactions included: dizziness, headache, nausea or fever. The persistent problems were more frequent with coloured tattoos than for black inks. The participants described a permanent elevation of the tattooed skin that could be due to the formation of scars or granuloma. The mention of intermittent oedemas, papules and itching of tattooed areas indicated to the authors an activation of the immune system due to pigments, inks or other ingredients of the colourant in the skin. One percent of the surveyed sought medical consultation. The majority of those went to multiple consultations (0.7% of all surveyed) and received drugs (0.8% of all surveyed). (Klügl, et al., 2010)

The characteristics of the surveyed population by (Klügl, et al., 2010) are similar to other surveyed populations of tattooed individuals: The majority of people (comprised of slightly more women – 58.9%) obtained their tattoos in professional studios (96.3%), after reaching legal maturity (although, 17.6% were younger than 18 at the time of their first tattoo), have larger tattoos (61.1% have a tattoo larger than 300 cm^2), have more than one tattoo (64.9%) and slightly more have black only tattoos vs multi-coloured (58.7%). Women tended to report more frequent and more severe problems than men. (Klügl, et al., 2010)

(Høgsberg, et al., 2013) studied 154 patients of the Clinic of Venereology, Bispebjerg University Hospital, in Denmark. The study distinguished between complaints and complications. The latter were defined as more serious adverse reactions in tattoos associated with objective, clinical pathologies of the tattoo in combination with subjective symptoms, i.e. events that would typically make the patient consult a doctor. The study also differentiated between early and late tattoo complaints, i.e., complaints noticed prior to and beyond a 3-month period after the tattoo was acquired – a differentiation made to separate complaints associated with the normal healing of tattoos.

In (Høgsberg, et al., 2013) 27% reported complaints in a tattoo beyond 3 months after tattooing. The complaints were predominantly related to black and red pigments. The participants reported complaints in 16% of their tattoos. Fifty-eight per cent of those complaints were sun induced. The complaints (4% of participants) varied in intensity but skin elevation and itching were most frequent.

As (Høgsberg, et al., 2013) report, a mean age of the tattoos (5.3 years) and an average number of tattoos per individual (2.2), the incidence of the health problems can be estimated: 27% experience complaints three months after tattoo procedure, 15% - experience sun sensitivity and 4% experience complications due to their tattoos.

The study population in (Høgsberg, et al., 2013) was similar to other studies, although the study setting does not allow for seamless comparison: the 154 participants (slightly more male) were 27.5 years old on average, had 342 tattoos, the majority of which contained black (95.6%) and were acquired in a professional setting (93.6%). The most frequent occupations were students and craftsmen, although health sector employees accounted for 8% of participants. Eight percent of the surveyed had more than 10% of their body covered in tattoos, while the remaining participants were split in two, similar size groups with tattoos covering less than 1% and between 1-10% of their body.

(Hutton Carlsten & Serup, 2014) studied 467 sunbathers, 31% of which had tattoos. Forty-two percent of those with tattoos reported clinical symptoms and signs. Of those, 52% experienced photosensitivity, defined by the authors as subjective symptoms appreciated by the individuals and photodynamic events as objective changes of the skin structure and appearance preceded by sun exposure. The majority of these sun-induced complaints were swelling (58%), itching/stinging/pain (52%), and redness (26%). The time lapse from sun exposure to when symptoms occurred varied from a couple of seconds to the following day. Reactions disappeared over a period of 20 min to several weeks.

Forty-eight percent of the complains were non-sun-induced. Of those, 31% were constant swelling and 3.5% - long-term tenderness. The remaining were reactions when warm, "allergic" reactions, acne-like changes, tenderness when cold, swelling after consumption of alcohol or tomatoes. Only two individuals sought medical advice (1.4%), with the vast majority consulting their tattoo artist or asked others tattooed for advice. (Hutton Carlsten & Serup, 2014)

The studied population by (Hutton Carlsten & Serup, 2014) has similar characteristics to others: of the 144 participants with tattoos, 52% were female and 48% male, with 2.1 tattoos on average, black being the predominant colour (in 92.4% of tattoos). The average age of participants was 35 years old.

(Kluger, 2016b) studied the tattoo reactions of the members of the French tattoo union. The study population was more heavily tattooed than other studied populations: from 5 to 91% of the body surface with the mean area tattooed of 32.9%. The participants were primarily male (78.1%), between the ages of 25 and 45 (78%), with more than one tattoo (99.8%), the first one acquired before 2000 (68.6%).

More than 42% self-reported reactions in at least one of their previous tattoos primarily consisting of transient itch and wax-and-waning swelling. A reaction to sun-exposure was reported by 23% of individuals. Permanent itch and swelling were rare. The size of the overall tattooed surface was associated with the occurrence of a tattoo reaction, transient swelling, permanent swelling, and colour allergy. (Kluger, 2016b)

In 2016, the Italian National Health Institute (Istituto Superiore de la Sanita, ISS) conducted a study to assess the prevalence of tattoos in the Italian population, their characteristics and potential complaints. The study surveyed more than 7 600 people, 12.8% of whom had at least one tattoo. The study found that more women (13.8%) had tattoos than men (11.7%). About two-thirds of those tattooed had only one tattoo, smaller than 1% of the body surface. Only 3.3% declared complications or mild reactions to tattoos. The most frequent reactions/complications (both short and long term) included: pain; swelling, blisters or granuloma; dermatitis, eczema or itching; skin thickening; allergic reactions. Of those, 21.3% consulted a medical professional. (ISS, 2017)

Table 137 Selected characteristics of surveyed population of tattooed individuals

Study	Studied population	Gender ratio	Age of 1st tattoo	Size of 1st tattoo	Number of tattoos	Colours used	Type
(ISS, 2017)	Sample of Italian pop: 12.8 total tattooed	men – 11.7% & women – 13.8% of Italian population	25.1 yrs (mean)	71.6% of tattooed ≤1% of body surface	1.7 (average)	monochromatic (67.3%) of those black 81.8% polychromatic (32.7), of those black (47.6%) & red (46.2%)	13.4% of tattooed outside authorised centres
(Kluger, 2016b)	448 tattoo artists members of French tattoo union	Male – 78.1% Female – 21.9%	68.6% before year 2000	Mean surface area tattooed 32.9%	99.8% with >1 tattoo	Black & grey – 14% Coloured – 86%	≥99.8% professional
(Hutton Carlsten & Serup, 2014)	467 (31% with tattoos) sunbathers on beaches in Denmark	Male – 48% Female – 52%	Not reported	Not reported	Average – 2.1	Containing: Black – 92.4% Red – 31.3%	Not reported
(Høgsberg, et al., 2013)	154 patients with 342 tattoos of a venerology clinic in Denmark	Male – 51.3% Female – 48.7%	Not reported	<1% of body - 69.9%	Single – 47.4% Multiple – 52.6% Average – 2.2	Containing: Black – 95.6% Red – 15.2%	Professional – 93.6% Amateur – 6.4%
(Klügl, et al., 2010)	3 411 German-speaking tattooed persons: 93% - German, 6% - Austrian, 1% - Swiss	Male – 41.1% Female – 58.9%	<18 – 17.6% 18 to 35 – 77.3% ≥ 35 – 4.8%	<300 cm^2 – 38.8% ≥300 cm^2 – 61.1%	Single – 34.9% Multiple – 64.9%	Black only – 58.7% Multi-coloured – 40.1%	Professional –96.3% Amateur – 2.7% PMU – 0.8% Medical – 0.1%

Table 138 Prevalence of tattoo complains and complications

Study	Prevalence	Type of effects	Studied population
(ISS, 2017)	3.3% of tattooed with complications or mild reactions, of these, only 21.3% consulted a dermatologist or a general practitioner	pain (39.3%); swelling, blisters, granuloma (27.7%); dermatitis, eczema, itching (26.7%); skin thickening (24.4%); allergic reactions (17.5%); other: pus, bleeding, dizziness, headache, scabs & fever	Sample of Italian population (7 600 people)
(Kluger, 2016b)	In at least one of their tattoos: 42.6% - with reaction Permanent: 4% - mild swelling & 1% - itch During/after sun exposure: 14% - itch & 23% - swelling	Transient or permanent itch and swelling; itch and swelling after/during sun exposure; infectious; allergic (not defined); skin cancer	448 tattoo artists members of French tattoo union
(Serup, et al., 2016)	Of complications: 37% - allergic 13% - papulo-nodular 9% - psycho-social 11% - infectious 30% - other 5% - sarcoidosis	Allergic reactions consisted of plaque elevation (32.2% of all complications), excessive hyperkeratosis (3.7%) and ulceration (1.4%). Other include photosensitivity, pain syndrome and lymphopathy	Patients with tattoo complications, Tattoo clinic, Bispebjerg University Hospital, Denmark (2008 to 2015)
(Hutton Carlsten & Serup, 2014)	Of 144 tattooed individuals: 42% - complaints (after initial healing): 52% sun-induced & 48% other (34% persistent) 1.4% - complications	Sun-induced (swelling, itching, stinging, pain, redness) & other (constant swelling, long-lasting tenderness, heat-induced, "allergic", acne-like, tenderness when cold, swelling after alcohol or tomatoes)	467 sunbathers on beaches in Denmark
(Høgsberg, et al., 2013)	> 3 months after tattoo: 27% of participants - complaints, 4% - complications < 3 months after tattoo: 15% - complaints After sun exposure – 15.6%	Complaints related to itching, ulceration, redness, swelling, prolonged healing, fever and malaise, and local infection. Complications most frequently related to skin elevation and itching.	154 patients with 342 tattoos of a venerology clinic in Denmark
(Wollina, 2012)	Incidence of 0.02% based on the number of treated patients per year	Lichenoid, pruritic, sarcoidal, edema, systemic, ulceration and infectious (30%) reactions. Mild reactions are excluded	Patients of Academic Teaching Hospital Dresden-Friedrichstadt (03/2001-05/2012)

(Klügl, et al., 2010)	67.5% of participant skin problems 6.6% systemic reactions 7.7% health problems after 4 weeks 6% persistent skin problems 3% other	Most frequent problems included: bleeding, crusts, itching, edema & pain, followed by a burning sensation, blister formation & puss-filled skin. Systemic reactions included: dizziness, headache, nausea or fever. Other included, e.g., psychic problems or light sensitivity	3 411 German-speaking tattooed persons: 93% - German (evenly distributed), 6% - Austrian, 1% - Swiss.
(Kazandjieva & Tsankov, 2007)	2.1% with complications	Infectious, allergic, and/or granulomatous complications in connection with tattoo pigment	234 dermatological patients with tattoos

On the basis of the key studies in *Table* 139 (excluding Wollina 2012 due to the different studied population, i.e., clinical patients vs tattooed population), it can be concluded that, on average 1.7% of tattooed people develop adverse reaction of severity that requires a doctor's consultation. As can be seen, the studies are primarily of countries where awareness regarding tattoo practices has increased in the last ten years. Therefore, it can be expected that the prevalence of tattoo complications in Member States, especially in those without any national regulations and awareness campaigns would be higher. On the other hand, the preceding sections demonstrated that the onset of chronic tattoo reactions as well as other health effects can occur from weeks to decades after the tattoo has been made; therefore, the statistics above may not yet reflect the advancements in tattoo practices and inks. In the absence of better information, it is assumed that this is a representative rate of tattoo complications for the EEA31. As no long term studies on tattoo complaints and complications exist, it is assumed that the annual increase of tattoo complications will be the same as the incidence rate of tattoos/PMU in the EU population.

d) Treatment

Non-infectious, inflammatory tattoo complications, although relatively rare, are often persistent (chronic), disturbing daily life as they lead to itching, swelling, and pain. They require prolonged treatment and maintenance to avoid flare up of the symptoms or more invasive intervention such as surgical excision, dermatome shaving, or laser removal. As shown in the preceding sections, the most common non-infectious, inflammatory complications related to tattoos are plaque elevation or papulo-nodular reactions. Of the allergic reactions, excessive hyperkeratosis and ucero-necrotic reactions are very rare. Extremely rare are also cases where hospitalisation of several days is required, accompanied by acute excision (e.g., in severe allergic reactions with deep pigment deposition into the subcutis or the underlying muscle or fat tissue), skin graft, painkillers and antibiotics, as well as several months of aftercare during skin recovery.

The treatment of tattoo complications is individualised to the patient and type of tattoo reaction. The following main treatments to tattoo complications are practiced:

- Topical, intralesional or oral treatment

- Dermabrasion[60] or chemical skin ablation
- Surgical:
    - Excision
    - Dermatome shaving
    - Carbon dioxide laser treatment
- Laser removal (with, e.g., Q-switch laser)

For many inflammatory reactions, very potent topical and, or followed by, intralesional steroids are first-line therapies, assuming a biopsy has been performed to exclude infection. Oral corticoid and immunosuppressive treatments are prescribed for allergic reactions, generalised eczema, or dermatitis. Some chronic reactions can be managed with regular application of topic or intralesional steroids, e.g., allergic reactions of small tattoos with limited amount of pigment concentrated in the outer dermis. However, the rate of recurrence is high and the treatment can be limited in time because of the risk of atrophy (local steroids) or other side effects (oral treatment).

In the event that allergic reactions persist after local treatment with steroids, a removal of the pigment is considered. Older removal treatments include: salicylic acid, chloroacetic acids, phenol, sulfuric acid, nitric acid, silver nitrate, tannic acid, zinc chloride, sodium chloride abrasion, cryosurgery, and dermabrasion. These have proven ineffective due to side effects, particularly scarring and difficulties controlling doze versus effect and hazard. (Serup, 2017)

Today, laser treatment has become increasingly popular for tattoo removal. As lasers lead to chemical decomposition of the pigment in the body, laser treatment bears the risk of evoking an additional allergic reaction. (Aberer, et al., 2010) Local and generalized reactions have been reported to occur as a consequence of laser treatment of previously uninvolved tattoos, which support that laser treatment can alter the antigenicity of tattoo pigment. (Shinohara, 2016) Therefore, the use of lasers for allergic reactions is highly controversial and counterindicated. However, they can be effective in reducing the pigment load in some papulo-nodular reactions. (Serup, 2017) Q-switch lasers (neodymium: yttrium-aluminum-garnet [Nd:YAG], alexandrite, or ruby) and newer picosecond lasers are most often used for laser tattoo removal and have largely replaced older, ablative lasers. (Islam, et al., 2016) The removal occurs as the laser light penetrates the skin and is selectively absorbed by the pigments particles, which leads to heat-up and fragmentation of the particles, their reduced concentration in the skin, which in terms leads to the fading of the tattoo colour. Due to the complexity of the chemical composition of the pigments, the efficacy of fragmentation is frequently unpredictable. Laser therapy is most effective in black tattoos and less effective for coloured tattoos. (Bäumler, 2017) Laser therapy does not always lead to complete removal of the tattoo. In pigments containing titanium dioxide (often used to change the shade of a colour), paradoxical darkening can occur. Hypo or hyper-pigmentation are some of the other common side effects. (See (JRC, 2016a) for further details.) Tattoo removal with laser treatment is dependent on the size of the tattoo,

[60] Dermabrasion uses a wire brush or a diamond wheel with rough edges (called a burr or fraise) to remove the upper layers of the skin. The brush or burr spins quickly, taking off and leveling (abrading or planing) the top layers of the skin. (WebMD)

the colours used as well as the skin tone of the person. It can require 10-20 sessions, priced between €50-250 per session.

After tattoo reactions have been diagnosed unlikely to respond to medical treatment and when there are concerns laser therapy triggering allergic reactions, surgical removal of the pigment can be pursued, i.e., via a surgical excision or dermatome shaving.

Surgical excision is practiced in many Member States to remove recurring tattoo reactions. It can be technically difficult and cosmetically deforming for large tattoos (Islam, et al., 2016), and when the excision reaches the lower one-third of the dermis, scarring is unavoidable. The excision site often shows hyper- or hypopigmentation along with scarring. (Sepehri & Jorgensen, 2017) It may make plastic surgery necessity and cosmetic considerations should be given in the treatment selection. (Aberer, et al., 2010)

Another procedure, which is shown to have good results while producing aesthetically acceptable results is dermatome shaving. This procedure involves consecutive shaving of thin horizontal layers of the skin area where the pigment reaction is occurring. The main goal of the surgery is to remove the culprit pigment. At the same time, the aim of the shave is to be as superficial in the dermis as possible, typically, at the mid-dermal level or just below. If the shaving is too deep, entering the subcutis, prolonged healing and major scarring can occur. (Sepehri & Jorgensen, 2017) A follow-up study of the 52 dermatome shaving operations on 50 patients reported patient symptom severity declining from 3.2 (out of 4) pre-operatively to 1, 0.8 and 0.7 after three, six and 12 months, respectively. The patients rated the burden of operation as low and their satisfaction with the outcome was high. (Sepehri, et al., 2015) According to interviews with dermatologists, the aesthetically pleasing outcome of the surgery is also dependent on the skills of the surgeon.

Both the dermatome shaving and the surgical excision are an outpatient procedure, using a local anaesthesia. Larger tattoos can require regional anaesthesia (peripheral nerve block). General anaesthesia, although uncommon, are used for tattoos on a difficult anatomical site. The surgery is followed up with removal and replacing of bandages, which ensure compression (and limit scarring) for some months. Sun exposure is not advised for at least one year. Pre- and post-operative consultation with dermatologist is necessary to determine the best course of treatment and follow-up which can be necessary if after the procedure pigment remains and continues to cause persistent reactions. (Sepehri & Jorgensen, 2017)

Table 140 shows the recommended surgical treatment for specific tattoo complications by Sepehri & Jorgensen, although as mentioned above laser therapy could be used for some papulo-nodular reactions to reduce the pigment load (Serup, 2017) and for others, where the tattoo has uncovered underlying sarcoidosis, medical and other specialist treatment is pursued. (Serup, pers com).

Table 139 Indications and type of surgery for selected tattoo complications

Tattoo complications:	Recommendations:
Chronic inflammatory tattoo reactions - Allergic, type plaque elevation and excessive hyperkeratosis - Non-allergic, type papulo-nodular reaction - Therapy-resistant tattoo reactions, other types - Severe persistent symptoms, including local pain syndrome	1st line: Dermatome shaving* 2nd line: Excision with/out split-skin transplant†
Pigment overload (overdosed tattoo pigment) with inflammation and intermittent symptoms	Dermatome shaving, depending on severity of the case
Skin tumours in tattoos (benign or malignant)	Excision, possibly by punch excision‡
Critical necrosis, caused by severe and deep infection	Excision without or with split-skin transplant¥

Source: (Sepehri & Jorgensen, 2017)

Notes:

* Beyond a healing phase of three months

† Excision preferred if pigment and inflammation involves the very low part of the dermis

‡ In keratocanthoma, the tumour is benign and normally resolves spontaneously over time.

¥ If the general condition is deteriorated, surgery may be required.

Ablative carbon dioxide laser is another technique of pigment removal. The carbon dioxide laser emits an invisible infrared beam at 10,600 nm, targeting both intracellular and extracellular water. When light energy is absorbed by water-containing tissue, skin vaporization occurs. (Shankar, et al., 2009) With carbon dioxide laser therapy, the pigment, together with the top layer of the skin is incinerated. The procedure is similar as the surgical procedure in terms of use of anesthesia and follow-up. Prior to the full removal itself, a test spot is taken.

Some chronic papulo-nodular reactions, when cutaneous granulomatous reactions reveal or trigger underlying diagnosis of systemic sarcoidoisis, are often treated with oral immuno-suppressive medications for the cutaneous manifestations of the illness. Depending on the other organs impacted, other specialist appointments (e.g., ophthamologist, pulmonologist), CT scans or ex-rays, biopsy, and other medical treatment that can last months to years may be required.

Other systemic, reproductive, developmental or carcinogenic illnesses that can be associated with exposure to chemicals in tattoo inks and PMU may require years of treatment, thousands of euro in direct and indirect treatment costs and can lead to loss of productivity and shorter life expectancy. Table 141 includes a list of the cost of selected relevant illnesses in recent studies as an example of the magnitude of these costs.

Table 140 Examples of costs to society of systemic, reproductive, developmental or carcinogenic illnesses associated with exposure to chemicals in tattoo inks and PMU

Illnesses	Costs to society
Infertility	WTP (Willingness to pay) = €29 700/case (2012 €) Direct & indirect costs = €7 400
Birth of child with very low weight	WTP = €126 200/case (2012 €)(2014 €)
Loss of IQ	WTP = WTP per IQ point = $466 (2007 US$)
Hypospadias	Direct, indirect and intangible costs per case = €21 600 (2014 values)
Cryptorchidism	Direct, indirect and intangible costs per case = €36 800 (2014 values)
Cancer	Value of statistical life= €3.5 million Value of statistical case of cancer = €350 000 Value of cancer morbidity = €410 000 (2012 €)
Testicular cancer	€81 000 of direct, indirect and intangible costs of one testicular cancer case, estimated by Norden (2014)
Obesity	Average direct & indirect costs per case of adult diabetes: €290 000 (in 2010 values) estimated by Legler et al (2015)

Source: (ECHA, 2017b)

e) Costs to society of adverse reactions to tattoos and PMU

As described, adverse effects to the chemical composition of tattoo inks can be non-infectious inflammatory, systemic, malignant, reproductive and developmental. With respect to chronic non-infectious inflammatory tattoo complications, the most common treatment involves topical, intralesional or oral treatment for milder cases and surgical or laser removal for more serious cases where topical treatment has proven ineffective. *Table 142* presents a summary of the costs of illness per case associated with the treatment of a tattoo complication. The medical costs represent the procedures described in section e) and represent an average of the information collected from the following Member States: Belgium, Denmark, Finland, Germany and the Netherlands.[61]

[61] The information on treatments was provided by dermatologists, specialising in tattoo complications: Dr De Cuyper, Department of Dermatology, AZ Sint Jan, Brugge, Belgium; Dr Kluger, Department of skin and allergic diseases, Helsinki University Central Hospital; Dr. Serup, the Tattoo clinic at the Department of Dermatology, Bispebjerg University Hospital, Copenhagen, Denmark; Dr van der Bent, Department of Dermatology, Academic Tattoo Clinic Amsterdam, VU University Medical Centre, Amsterdam, The Netherlands.

Table 141 Cost to society of chronic non-infectious inflammatory tattoo complications per case

Treatment	Total cost*
Medical (topical, intralesional, or oral) treatment (annual/case)	€460
Surgical treatment (one-off costs/case) - dermatome shaving - excision - carbon dioxide laser	€2 350
Laser treatment (one-off costs/case)	€2 250
Willingness to pay (WTP) to avoid symptoms of tattoo reactions, annual/case	€2 000 - €12 000**

Notes: *Costs can differ substantially for Member States and similar treatments. ** 2014 Values (ECHA, 2016f)

The costs in Table 142 include direct cost of treatment and do not include indirect costs such as loss of productivity. They also assume that an aesthetically pleasing outcome of the treatment.

- Medical (topical, intralesional or oral) treatment: includes the costs of visits of general practitioner and dermatologist, a course of first topical, followed by intralesional corticosteroids over one year.
- Surgical treatment: includes visits to dermatologists (before and after the procedure) and other medical personnel (e.g., nurse), the cost of the procedure and aftercare. The costs are an average of the three different surgical methods practiced in different Member States: dermatome shaving, excision and carbon dioxide laser treatment.
- Laser treatment: includes the costs for repeated laser treatment sessions (10 to 20).

In addition to medical costs for treatment, the patients suffer a psychological burden due to their symptoms while awaiting recovery (end of treatment). These symptoms include itching and burning sensations that affect their quality of life. Hutton Carsten & Serup study the extent to which the quality of life of sufferers of tattoo complaints is affected. (Hutton Carlsen & Serup, 2015a) To assess the influence of tattoo reactions on quality of life, they interviewed patients[62] with tattoo problems spanning longer than three months using the Dermatology Life Quality Index (DLQI)[63] and the Itch Severity Scale (ISS)[64] questionnaires. The authors concluded that sufferers of tattoo reactions experienced reduced quality of life (DLQI score of 7.4, i.e., moderate effects on life) and were burdened by itch (ISS score of 7.2). Both DLQI and ISS results showed a level of discomfort similar to known skin diseases such as psoriasis (DLQI scores between 10 and 13.32, ISS score of 7.5), pruritus (DLQI score of 8.8, ISS scores between 7.4 and 9.7), and eczema (hand, DLQI score of 8.0),

[62] Patients who sought treatment in the Tattoo Clinic in Bispebjerg University Hospital in Denmark, September to November 2012.

[63] Dermatology Life Quality Index, introduced by Finlay and Khan, is a validated questionnaire with the following topics and elements: 1 and 2: Symptoms and Feelings; 3 and 4: Daily Activities; 5 and 6: Leisure; 7: Work and School; 8 and 9: Personal Relationships; 10: Treatment. Total maximum score is 30.

[64] Itch Severity Scale (introduced by (Majeski, et al., 2007)) is an instrument to measure both the sensory and affective dimensions of itch, covering the following seven topics: 1: Frequency; 2: Description; 2a: Sensory, 2b: Affective; 3:Body Area; 4:Intensity; 5:Effect on Mood; 6: Effect on Sexual Desire/Function; 7: Effect on Sleep.

albeit the typical tattooed affected areas are smaller (average for the interviewed of 9.8 cm^2). The survey results showed that itching is a problem for almost all interviewed with 50% reporting extreme bouts of itching, leading to anxiety (42.5%) and effects on sleep: difficulties falling asleep for 55%, specifically, 22.5% reported problems falling asleep practically every evening, 12.5% had sleep disturbances almost always and 5% needed sleeping medication. Furthermore, 55% experienced varying degrees of embracement/self-consciousness due to their tattoo reactions. The reactions had influence on daily activities for 37.5% and choice of clothing for 53%, while the treatment was a discomfort for 25% of the interviewed.

The results of Hutton Carsten & Serup study suggest that the quality of life impacts of the severe chronic dermatitis estimated by ECHA (ECHA, 2016b) are similar to those of tattoo complications. This is despite differences in treatment, which are reflected in the medical and indirect costs (not estimated in Table 142) or any possible aesthetic effects. For the purpose of this analysis, the valuation scenario for severe chronic dermatitis in (ECHA, 2016b), and thus, the derived willingness to pay value to avoid this experience (equal to €2 000/case (lower value) or to €12 000/case (higher value) as shown in Table 142), is considered a suitable proxy for the pain and suffering of people with tattoo complications.

Other systemic, reproductive, developmental or carcinogenic illnesses have much higher willingness to pay to avoid. See Table 141 for examples.

D.6.2. Environmental impacts

As the rationale for this restriction proposal is human health, the environmental impacts arising from substances in tattoo inks and their comparison with those of the alternatives are not discussed further.

D.6.3. Risk reduction capacity

The restriction options include in their scope substances that could contribute to tattoo adverse effects. Any new substances, meeting the criteria for inclusion in the scope of the proposed restriction options, will be progressively added, i.e., any new substances classified as CMR, skin sensitisers/irritants/corrosives, eye irritants/damaging or included in Annex II and IV (with conditions on use, purity, etc., i.e., column g-i) of the CPR (the latter for RO1 only). A few substances not included in the scope of RO1 and RO2 but suspected to lead to human health effects are highlighted for consideration in future assessment as currently there is no sufficient information to conclude on their risks to human health. (See Appendix D.1. Substances for future evaluation)

However, it is theoretically possible that the implementation of the proposed restriction options as well as future tattoo ink R&D could lead to the introduction of colourants never used before in this application, with limited information about their effects on human health, including when injected intradermally. This will necessitate continued examination of the substances found in tattoo inks and PMU. These activities could be facilitated with increased exchange of information on surveillance activities as well as via a centralised registry containing information on the chemical composition of tattoo inks and PMU manufactured and imported to the EEA31 market.

Therefore, while RO1 would lead to a decline in the number of cases of adverse tattoo effects, it is uncertain whether it will fully eliminate them due to the uncertainty associated with a number of currently used, or to be used in the future, substances that are not well-researched, and therefore, their impacts on human health are not well understood.

As RO2 proposes less strict concentration limits in comparison to RO1, it is possible that it would lead to the avoidance of fewer cases of adverse effects in comparison to RO1, leading to slightly lower risk reduction capacity than RO1. However, this conclusion is uncertain.

D.7. Practicality and monitorability

D.7.1. Practicality

Practicality in the context of an Annex XV restriction dossier under REACH is defined in terms of three criteria: implementability, enforceability and manageability.

a) Implementability

The proposed restriction options propose similar, and in the case of RO2, slightly less strict than ResAP, measures which have been used as a basis for national legislation in seven Member States and additional two EEA members. Surveillance results have shown that the majority of tattoo inks and PMU are in compliance with national legislation, which suggests industry's ability to comply with the proposed restriction options.

Earlier sections of the dossier demonstrated that the majority of the colourants for which there is information on their use in tattoo inks and PMU remain outside the scope of the proposed restriction options (see Identification of potential alternative substances and techniques fulfilling the function and Availability of alternatives) and there are a number of other colourants (also with food, cosmetic or medical applications) which could be investigated for their application in tattoo inks.

The transitional period reflects the industry capability to comply with the proposed restriction options. The proposed one year is a longer than the factored in the implementation of some national legislations (e.g., Germany's transitional period was from 13.11.2008 to 1.5.2009, taking into account the high level of knowledge of industry with the then foreseen legislation).

b) Enforceability

Enforcement of national legislation based on ResAP is already taking place in just under a third of EEA31 Member States. They have systems in place to monitor compliance and to share information on non-compliant products – RAPEX. A number of other Member States, conduct occasional checks of tattoo inks and PMU, e.g., Denmark and Italy. Member States without legislation could build on the experience to date. Stakeholders in the past have expressed concerns regarding different interpretation and therefore, enforcement in Member States with national legislation. (ECHA CfE, 2016a) This dossier and the proposed restriction options are expected to address these concerns.

To assist with the compliance check of relevant actors, the dossier provides information on the substances found in tattoo inks that present risk to human health and highlights the groups of substances that are considered most problematic: PAHs for black and dark inks, PAAs for red inks and its nuances, as well as selected problematic impurities commonly found in variety of tattoo ink and PMU colours (see section Table 3 of CoE ResAP Impurities). To achieve greater return on their enforcement efforts, Member States can focus efforts on ensuring that these substances are no longer present in tattoo inks and PMU. Occasional detailed analysis of selected tattoo inks and PMU may help prioritise other substances for more frequent screening. A EU-wide registry of tattoo inks could assist with the prioritisation.

Analytical methods exist for all groups of substances in the scope of the proposed restriction options, except for azo dyes which may decompose to PAAs with CMR properties. For the PAAs methods are available, however they would benefit from a harmonised analytical methods even for the dissolved concentration. Appendix D.2 provides information on the analytical methods that can be used to enforce the restriction. The work is an update of the earlier work by the JRC (JRC, 2015a), using contributions received via the Working Group (Denmark, Germany, Italy and Norway) the Forum for Exchange of Information on Enforcement (Forum) and the Call for Evidence ran by ECHA in 2016. (ECHA CfE, 2016a)

Information on the limit of detection of the currently used methods has been taken into account in the setting of the concentration limits for individual and groups of substances in the scope of RO1 and RO2. Although the availability of harmonised analytical methods is not a requirement for proposing a restriction, stakeholders identified the need for harmonisation of analytical methods to avoid different treatment in different Member States. (ECHA CfE, 2016a) To select an appropriate method, one of the important questions to be resolved is under what conditions metals can be considered soluble and therefore, bioavailable (i.e., in terms of the solvent, pH, temperature, time, etc.). This is an important question as some metals can be found as impurities in tattoo inks but could also be part of the complex bounded matrix of the pigment and therefore, not bioavailable. From the perspective of risk to human health, only soluble metals present an issue on the basis of current evidence. Therefore, it is important to select such methods that detect and quantify only the concentration of soluble metals in tattoo inks. (ECHA CfE, 2016a)

Another issue brought up by stakeholders is the sales of non-compliant tattoo inks and PMU via the internet, especially via online resellers. (ECHA CfE, 2016a) The collaboration of online resellers in the enforcement of restriction measure will be paramount for the success of the restriction measure.

It is expected that enforcement authorities would be able to prepare for monitoring of the proposed restriction within one year of its entry into force given the exiting experience in the EEA. This was taken into account in the determination of the transitional period (balanced against other important factors such as availability of alternatives, familiarity of industry with the restriction requirements, etc.).

c) Manageability

Given the similarity with existing measures (ResAP, the CPR, and the CLP Regulation) and the stakeholder's raised awareness of the issue, the restriction should be clear and understandable to all actors involved. Furthermore, the level of administrative burden is not expected to be higher than in the Member States with national legislation. The current compliance rate suggests that the existing regulations are manageable for industry. Therefore, the implementation of the restriction (which are similar to ResAP) is also expected to be manageable and is proportional to the risk avoided. In addition, sections D.5. Other impacts and D.8.1 Affordability showed that the impact on individual actors (tattoo ink and PMU manufacturers, tattoo artists, PMU practitioners and customers) are manageable, although selected stakeholders (e.g., those manufacturers who have not begun to develop alternatives) may experience larger impacts.

D.7.2. Monitorability

The implementation of the proposed restriction options can be monitored via surveillance programs and existing tools such as RAPEX. Of particular importance would be the monitoring of the use of tattoo inks and PMU by tattoo artists and PMU practitioners who

would have the obligation under the proposed restriction options to inject intradermally only compliant inks. This is important due to the numerous possibilities to procure tattoo inks, including to mix them in their studios.

In addition, the following could assist with the monitoring of the impact of the proposed restriction measure and the assessment of necessary further measures:

- the introduction by national health boards of a separate, EU-harmonised diagnostic codes for tattoo ink and PMU complications to enable tracking of adverse effects and to provide relevant epidemiological information for long-term studies of the association between tattooing (and PMU procedures) and cancer, reproductive and developmental issues, sarcoidosis, or other systemic illnesses for which there is currently limited information
- the introduction of an EU-wide registry of tattoo inks and PMU marketed on the EEA31-market which among other information, would gathers data on the chemical composition of the mixtures injected intradermally. This will provide information on new substances finding application in tattoo inks and PMU, which in turn will help with the assessment of the effectiveness of the proposed measure and the need for further regulatory action.

D.8. Proportionality and comparison of restriction options

D.8.1. Affordability, cost effectiveness and break-even analysis

D.8.1.1. Affordability

a) Tattoo ink manufacturers

Manufacturers with ResAP-compliant tattoo inks have reported that their margins have eroded, due to the pressure to compete with non-compliant tattoo inks and their non-discerning customer base (i.e., tattoo artists). However, it is expected that those already compliant with ResAP, would not have to incur substantial additional costs to comply with the proposed restriction options. The largest burden of the regulation would fall on those manufacturers which have not developed tattoo inks meeting ResAP's recommendations. As stated previously, EU manufacturers are reported to have higher compliance rate with ResAP requirements, therefore, the largest burden would fall on non-compliant importers. Currently, non-compliant manufacturers are reported to have a higher profit margin, as their manufacturing costs are about 50% lower than those of ResAP compliant inks, while their products have similar (0-20% lower) market prices. (stakeholder consultations)

For the purpose of this analysis, it is assumed that tattoo ink and PMU formulators would be able to pass downstream their higher costs to be incurred due to the proposed restriction options in the form of higher market prices for their products. Industry has expressed concerns that they are unable to pass on higher costs. With the entry of the proposed restriction options all formulators would need to comply with the regulation and therefore, the pressure from lower-cost, non-ResAP compliant inks would abate.

b) Tattoo artists

Tattoos can be very diverse and their price, amount of time and ink used varies greatly, depending on the skill of the tattoo artist, design (custom or pre-designed, realistic or abstract), black or multi-colour, outline or shaded, etc. The starting price for customers of smaller tattoos in many Western and Northern European Member States is on average €80-100, which is also similar to the average rate per hour of tattoo service. In Eastern Europe, prices have been reported somewhat lower: €30-40 euro for a very small tattoo. However, everywhere the prices of sought-after tattoo artists can be significantly higher. (ECHA CfE, 2016a) (stakeholder consultation)

Tattoo artists incur total costs per tattoo between €20-40 for supplies, rent, labour, and other overhead. Costs can be lower for sought-after tattoo artists as they are often sponsored and receive complimentary tattoo ink, needles, equipment and other supplies from manufacturers. Costs could also be expected to be lower in some Eastern European Member States.

The cost for tattoo ink is estimated to account for up to 14% (in Western Europe) to 31% (in Eastern European Member States) of the total cost per tattoo for tattoo artists. Therefore, if as a result of the proposed restriction options, the share of the tattoo ink of total costs per tattoo would increases to 16% (in Western Europe) to 35% (in Eastern European Member States). In other words, the marginal costs of the proposed restriction would be less than €1 per tattoo. It is expected that this increase would have a minor impact on the profit margin of a tattoo.

c) PMU practitioners

Prices of PMU procedures such as eyeliner, lipliner, or eyebrow enhancement also vary substantially in different Member States. They also depend on the reputation of the studio (which could also be a tattoo studio) or beauty (spa) centre and whether the centres offer packages (bundles) of various procedures. Stakeholder consultations have reported an average price of a procedure of about €350 but prices in Eastern and Southern European Member may be lower. Therefore, if as a result of the proposed restriction options, the cost of PMU increases by 20%, the share of the PMU of total costs per procedure would increases from 14% to 16% or the marginal cost of a restriction would be about €4/procedure. It is expected that this increase would have a minor impact on the profit margin of a PMU procedure.

d) Customers

It is likely that any tattoo and PMU cost increases caused by the proposed restriction options will be passed on to consumers, as according to market research in the US demand for tattoo and PMU services is inelastic. It is driven primarily by demographics and cultural (including fashion) trends rather than other economic forces. Despite having the hallmark of a luxury service, the industry revenue hardly declined during the most recent recession. The price of a tattoo was also not seen as a priority among those deciding on a tattoo: only 8% of respondents to a survey stated that price is an important factor in their decision to get a tattoo. Demand in the future is expected to continue to be unaffected by changes in disposable income. (IBISWorld, 2016) (SB, 2015)

In conclusion, even though it is likely that the introduction of one of the restriction options would lead to higher costs for industry, those would likely be affordable for downstream users: tattoo artists, PMU professionals and consumers.

D.8.1.2. Cost-effectiveness

As shown, the proposed restriction options would likely lead to costs and other negative impacts to industry. Table 143 shows that these are expected to be relatively small and manageable for industry and other actors. The cost-effectiveness of RO1 is estimated at about €60/litre non-compliant tattoo ink replaced in EEA31. The cost-effectiveness of RO2 is likely to be higher as substitution costs are expected to be somewhat lower than those estimated for RO1.

D.8.1.3. Break-even analysis

For RO1 to break even, between 320 (calculated using cost of illness (COI) plus higher WTP values) and 1 060 (COI plus lower WTP values) cases of chronic allergic reactions (i.e., requiring surgical removal) need to be avoided on an annual basis. This is between 0.02-0.06% of the estimated number of people getting tattoos for the first time each year (19-63 avoided removals for every 100 000 tattooed people) in EEA22 – the Member States currently without national legislation.

It is reasonable to expect that these cases would be avoided as a result of the proposed restriction measure as the estimated average prevalence rate of tattoo complications is 1.7% (see point d) in section D.6.1. Human health impacts and not all costs are taken into account (see point c).

In addition, the removal of tattoos due to an allergic or papulo-nodular reaction is just one group of the health outcomes. As stated in section D.6.1. Human health impacts, a number of people experience complications that require topical or systemic corticosteroids as well as

experience mild ongoing complaints from their tattoos and PMU.[65] This is in addition to the potential contribution of tattoo ink and PMU exposure to carcinogenic, reproductive, developmental and other systemic adverse effects.

Therefore, although full cost-benefit comparison it is not possible, it is reasonable to assume that the benefits would outweigh the costs, as very few cases of only one type of adverse effects (non-infectious, inflammatory) are necessary for the restriction to break even. Quantification and monetisation of other adverse effects (systemic, carcinogenic, reproductive or developmental) would lead to higher overall value of benefits from RO1.

As the concentration limits of RO2 are higher than RO1, it can be hypothesised that RO2 offers a lower level of protection and therefore, fewer benefits. However, as costs for RO2 are also lower than RO1, it is difficult to determine the overall proportionality of RO2 in comparison to RO1.

D.8.2. Comparison of Restriction Options

As shown in the preceding sections and summarised in Table 143. both proposed restriction options (RO1 and RO2) would likely lead to costs and other negative impacts to industry that are of similar nature and magnitude. The main difference between the two restriction options are the concentration limits. As the concentration limits of RO2 are higher than RO1, it can be hypothesised that RO2 offers a lower level of protection and therefore, lower risk reduction capacity and fewer benefits. At the same time, as more tattoo inks currently on the market likely already comply with RO2 requirements, the substitution costs would likely be lower than RO1. Testing costs for RO2 would also be possibly lower than RO1 as the information on classified substances is required to be included in the label and the substance data sheet if they are present in concentrations exceeding their CLP limits in mixtures.

Therefore, as the costs of RO2 are anticipated to be slightly lower, this option would be slightly more cost-effective (in terms of euro per volume non-compliant tattoo ink substituted), slightly more affordable for stakeholders and would require fewer avoided cases to break even. At the same time, it is expected that the risk reduction capacity, and therefore, the benefits, of RO2 would also be slightly lower. It is uncertain whether they are sufficiently different than RO1 to conclude that RO2 is more proportionate than RO1 on a cost-benefit basis.

Table 143 compares the two options qualitatively. An overall conclusion on which option is more proportionate is difficult to reach.

[65] Various studies have reported mild complaints after sun exposure as shown in Annex D: 14% reported itch and 23% swelling after sun exposure (Kluger, 2016b); 52% of complaints (42% of total respondents reported a complaint) were sun induced (Hutton Carlsten & Serup, 2014); 15.6% of respondents expressed complaints after sun exposure (Høgsberg, et al., 2013).

Table 142 Total compliance costs and cost-effectiveness of the proposed restriction options

2016 values, euro, annual	Restriction Option 1 (RO1)	Restriction Option 2 (RO2)
Total restriction costs	€4.6 million	lower
Substitution	€4.4 million	lower
Enforcement	€0.2 million	similar
Social impacts	moderate	similar
Wider economic impacts	minimal	similar
Distributional impacts	minimal	similar
Cost-effectiveness	€60/litre non-compliant tattoo inks removed from the market	higher
Risk reduction capacity	it would reduce risks but not fully eliminate them	possibly lower
Benefits	equivalent to the avoided cases of tattoo adverse effects (cutaneous, systemic, reproductive, developmental, malignant)	possibly lower
Break-even	Lower than 320 – 1 060 avoided cases of tattoo removal due to non-infectious inflammatory complications	possibly fewer cases required for break-even

In summary, it can be concluded that the proposed restriction options are proportionate, as they are cost-effective, affordable and would lead to benefits in terms of avoided complications of tattoo inks and PMU associated with exposure to chemicals and other health effects.

Appendix D.1 Substances for future assessment

During the preparation of the dossier, the working group gathered information on a number of substances for which there is an indication that they may present risk due to exposure via intradermally injected tattoo inks and PMU. Primarily due to information gaps about the hazards and risks of the substances, their risk assessment was difficult due to time constraints. Therefore, these substances are documented in this Appendix for the purpose of identifying other risk management options to address the risks arising from exposure to these substances (e.g., via harmonised classification in accordance with the CLP Regulation) or to ensure these substances are considered in future evaluations of the risks from tattoo and PMU inks.

Substances on Table 1 of CoE ResAP(2008)1

Given the limited information available for the following two substances risk cannot be demonstrated for substances with Table entry number: #1: 6-amino-2-ethoxynaphthaline and #27: 2,4-xylidine. These substances are not classified and are not very good candidates for any of the harmonised classification groups included in the scope of RO1 and RO2.

2,4-xylidine is registered under REACH and found in tattoo inks during surveillance. (JRC, 2015b) It has 102 notifications but only 31.1% of notifiers considered it a candidate for eye irritant classification, category 2. Fewer notifiers considered it in any of the other harmonised classification groups included in the scope of RO1 and RO2.

6-amino-2-ethoxynaphthaline is not registered under REACH.

Table 143 List of substances on CoE ResAP Table 1 not included in the scope of the

Substance	EC	CAS	Priority for C&L
6-amino-2-ethoxynaphthaline		293733-21-8	
2,4-xylidine	202-440-0	95-68-1	Acute Tox. 3 (97.1%), STOT RE 2 (96.1%), Aquatic Chronic 2 (95.1%), Acute Tox. 2 (32.4%), Eye Irrit. 2 (31.4%), Not Classified (2.9%), STOT RE 1 (2.0%), Acute Tox. 4 (1.0%), Muta. 2 (1.0%), Skin Irrit. 2 (1.0%)

See Appendix B.2: PAAs and azo colourants for further information supporting the exclusion of these substances.

Colourants used in tattoo inks

Of the close to 100 colourants historically used in tattoo inks (JRC, 2015b) which will not be affected by the proposed restriction options, there are five which can be considered candidates for possible harmonised classification under the CLP Regulation for categories in the scope of the proposed restriction options. As good candidates for classification are considered substances for which more than 50% of their notifiers have listed the classification category. These are listed below (with the percentage notifiers for each classification category) for further investigation in subsequent review of the tattoo ink regulation:

- Pigment violet 12 (CAS 81-64-1): Eye Irrit. 2 (96.1%), STOT SE 3 (95.9%), Skin Irrit. 2 (95.9%), Aquatic Acute 1 (3.3%), Aquatic Chronic 1 (3.3%), Not Classified

(0.9%), Skin Sens. 1 (0.2%), Muta. 2 (0.1%) – the substance is registered under REACH

- Lawsone (2-hydroxyl-1,4-naphtoquinone; HNQ) (CAS 83-72-7): Eye Irrit. 2 (89.3%), Skin Irrit. 2 (89.3%), STOT SE 3 (82.1%), Acute Tox. 4 (7.1%), Not Classified (7.1%), Aquatic Chronic 2 (3.6%)
- Pigment yellow 36 (CAS 37300-23-5): Acute Tox. 4 (100.0%), Aquatic Acute 1 (100.0%), Aquatic Chronic 1 (100.0%), Carc. 1A (100.0%), Skin Sens. 1 (100.0%)
- Pigment yellow 154 (CAS 68134-22-5): Eye Irrit. 2 (56.0%), Not Classified (43.2%) – the substance is registered under REACH
- Pigment yellow 36 (CAS 37300-23-5): Acute Tox. 4 (92.0%), Aquatic Acute 1 (92.0%), Aquatic Chronic 1 (92.0%), Carc. 1A (92.0%), Skin Sens. 1 (92.0%), Not Classified (8.0%)

Other colourants for which stakeholders expressed concerns related to their hazard profile and risk related to intradermal exposure to tattoo inks and permanent make-up:

- Swedish surveillance has found in tattoo inks Pigment Violet 1 (Xanthylium, 9-(2-carboxyphenyl)-3,6-bis(diethylamino)-, molybdatetungstatephosphate, EC 215-413-3, CAS 1326-03-0, CI 45170:2) which is similar to Solvent Red 49 or 49:1 (3',6'-bis(diethylamino)spiro[isobenzofuran-1(3H),9'-[9H]xanthene]-3-one, EC 208-096-8, CAS 509-34-2, CI 45170:1) and Basic Violet 10 (9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthylium chloride, EC 201-383-9, CAS 81-88-9, CI 45170) which are prohibited for use in cosmetic products in Annex II of the CPR (entry #398) and fall within the scope of the proposed restriction options. (ECHA CfE, 2016a) The pigment is pre-registered under REACH. It has no harmonised classification and no relevant self-classifications for human health have been submitted to ECHA.
- Concerns related to the risk profile of Pigment Green 36 (in comparison to Pigment Green 7) were expressed on several occasions during consultations with stakeholders for the preparation of this dossier. Pigment Green 36 ([1,3,8,16,18,24-hexabromo-2,4,9,10,11,15,17,22,23,25-decachloro-29H,31H-phthalocyaninato(2-)-N29,N30,N31,N32]copper, EC 238-238-4, CAS 14302-13-7, CI 74265) is registered under REACH, it has no harmonised classification and no relevant self-classifications have been submitted to ECHA.
- Pigment Red 202 (2,9-dichloro-5,12-dihydroquino[2,3-b]acridine-7,14-dione, EC 221-424-4, CAS 3089-17-6, CI 73907) is reportedly similar to Pigment Red 122 (5,12-dihydro-2,9-dimethylquino[2,3-b]acridine-7,14-dione, EC 213-561-3, CAS 980-26-7, CI 73915), which is proposed to be restricted under RO1 and RO2 on the grounds that its use is restricted to rinse-off products only under Annex IV of the CPR. Pigment Red 202 is registered, has no harmonised classification and no relevant classification notifications have been submitted to ECHA to date.

Substances on Annex III of the CPR

Article 14 of the CPR states that cosmetic products shall not contain restricted substances listed on Annex III which are not used in accordance with the restrictions laid down in the annex. These include e.g. restrictions on the body parts where the cosmetic products can be applied or the product type that may or may not contain the substances listed. For some of these substances, restrictions on their use are imposed on the basis of SCCS opinions that conclude that these substances may pose a human health risk in applications leading to prolonged contact with human skin, mucous membranes or in the vicinity of the eye, i.e., similar conditions that justified the inclusion of a number of substances on Annex II and Annex IV in RO1 and RO2. As the substances on Annex III have been reviewed for specific applications only by the SCCS, a detailed review of individual substances listed on the annex would be required to justify their inclusion in the scope of the proposed restriction options. The high workload for this assessment required a deprioritisation for this restriction dossier. The main reason for this deprioritisation was that of the substances on Annex III which are not captured in the scope of RO1 and RO2 on the basis of their harmonised classification, there was information that only methanol and two additional substances have been found in tattoo inks. (JRC, 2015b) An individual quantitative assessment was prepared for methanol but as Table 147 shows, the limited information for the remaining two, non-classified substances did not allow such assessment. With that said, future examination of the risks from tattoo inks may benefit from examining the substances in Table 147 and the remaining substances on Annex III of the CPR outside the scope of RO1 and RO2, as they may become more relevant once the substances restricted with the current proposal are substituted.

Table 144 Substances on Annex III of the CPR used in tattoo inks outside the scope of RO1 and RO2

Substance name	EC #	CAS #	CI #	Registered	Annex III entry #	Percent notifications in hazard categories
Benzoic acid, 2-hydroxy- (10), Salicylic acid (1) and its salts, Salicylic acid, Salicylic Acid	200-712-3	69-72-7		Yes	98	Acute Tox. 4 (97.8%), Eye Dam. 1 (48.7%), Eye Irrit. 2 (48.4%), Skin Irrit. 2 (6.5%), STOT SE 3 (6.1%), Repr. 2 (3.6%), Not Classified (0.4%), Aquatic Chronic 3 (0.2%), STOT RE 1 (0.2%), STOT SE 1 (0.0%), STOT SE 2 (0.0%), Skin Sens. 1 (0.0%)
Pyridinium, 1- methyl-4-[(methyl phenylhydrazono)methyl]-, methyl sulfate, Pigment yellow 87	269-503-2	68259-00-7, 14110-84-6	21107:1	No	275	Acute Tox. 4 (95.3%), Aquatic Chronic 2 (57.8%), Eye Irrit. 2 (39.1%), Skin Irrit. 2 (31.2%), Aquatic Acute 1 (28.9%), Not Classified (3.1%), STOT SE 3 (3.1%), Aquatic Chronic 4 (0.8%)

Appendix D.2 Analytical method

With regard to the enforceability of the proposed restriction, the applicability of the analytical methods listed in JRC report (http://publications.jrc.ec.europa.eu/repository/bitstream/JRC94760/wp1_tr_pubsy.pdf) have been assessed.

Data provided from Member States authorities and information gathered in the Call for evidences have been also considered.

Table 145 PAAs: International standard based methods

Substance (with ref. to JRC report)	**Matrix**	**Method in JRC report**	**Applicability to tattoo inks and PMU**
PAAs listed at p. 140[1]	Tattoo inks	Based on standard EN 14362-1:2012 adapted to the matrix "ink" – Slovenian expert (p 141 and 149) (now superseded by EN ISO 14362-1:2017)	Validation data of the method adapted to tattoo inks are not reported. No clear indication of the substances is given. Given the absence of data on repeatability and reproducibility it is not possible to assess the applicability of the method to tattoo inks and PMU. Nevertheless, Swedish authorities enforcing tattoo inks confirmed the analysis of PAAs is done by using a modified version of EN 14362.
PAAs listed at p. 143[1]	Tattoo inks and PMU	"*Determination of aromatic amines in tattoos and PMU with LC/MS*" based on EN 71-7 adapted to the matrix "ink" and recommended by CoE ResAp (2008)1 (table 4.c) – Swiss experts	Quantification of free amines: validation study published by Hauri (2005). Only some performance characteristics have been determined (LOD, repeatability and recovery) and only two amines are reported (repeatability for aniline and o-toluidine, recovery for aniline). It should be demonstrated that the composition of "inks for pens" is similar to tattoo inks and PMU one. The applicability is potentially guaranteed, but a validation study should be implemented. Reductive cleavage procedure: validation data are absent. The assessment of applicability to "tattoo inks and PMU" is not possible.
PAAs listed at p. 145[1]	Tattoo inks	Standard operational procedure used by Italian experts is based on EN ISO 17234-1:2010 (p. 145) (now superseded by EN ISO 17234-1:2015)	Validation data refer to LOQ, repeatability and reproducibility. Validation data reported in JRC report have been compared with available data from an Italian official laboratory, which are related to UNI EN ISO 17234-1:2015 used in Italy. 4-metossi-m-fenilendiamine (CAS 615-05-4) is not present in the validation data from Italian laboratory, instead 4-amminoazobenzene (CAS 60-09-3) is absent in JRC report (pg 145). LOQ reported in the JRC report is 1.5

			mg/Kg instead LOQ obtained with UNI EN ISO 17234-1:2015 is 1.0 mg/Kg. Repeatability and reproducibility are comparable to the ones in Appendix B of EN ISO 17234-1:2015, even if they are determined for a different matrix. The method is potentially applicable to tattoo inks and PMU.
PAAs (no substance details)	Tattoo inks	"*Determination of carcinogenic aromatic amines in tatto inks by HPLC/MS/MS after reductive cleavage according EN 14362*" developed on the basis of EN 14362-1- Swiss experts (p. 149)	Not applicable. Quantification of amines after reductive cleavage has low repeatability and reproducibility due to low solubility of pigments. Repeatability data obtained from a validation study with inks spiked samples is not reliable as reported in JRC report.
PAAs (no substance details)	Tattoo inks and PMU	"*Determination of aromatic amines in tattoo ink, permanent makeup and texile using GC-MS*" (CHE01-WV494) – Dutch experts (p. 150). It is not clear if it represents an update of the method SIG01-ND428 recommended in the CoE ResAp (2008)1 based on EN 14362-1 (tables 4.a & 4.b)	Recovery and repeatability, LOD and LOQ of the method are reported. Potentially applicable but the validation study is not complete. Intra-laboratory reproducibility data have to be included. Repeatability data reported in JRC report are not coherent with Table 4.b of CoE ResAp (2008)1 concerning the recommended performance characteristics of the method.
PAAs listed at p. 151[1]	Tattoo inks	"*GC/MS analysis for primary aromatic amines (PAA) liberated from azo colorants and free PAA*" developed on the basis of the method reported in the ResAP, method modified by EN 14362 – Danish experts (p. 151)	In the JRC report validation data of the method are not reported. In the document "*Chemical substances in tattoo ink - survey of chemical substances in consumer products*" (p. 154) results from analysis of tattoo inks samples in duplicate, with indication of repeatability and LOD. The method is potentially applicable to tattoo inks, but the validation study is not complete and has to be integrated with reproducibility data[2].

Note:

[1]Wrong CAS numbers

[2]Given the variability of the reductive cleavage (due the low solubility of pigments) and the differences of results obtained from Member States when using adaptation of the same method (e.g. EN ISO 14362-1, low repeatability/reproducibility) validation studies on reproducibility inter-laboratory have to be carried out in order to establish the reliability of methods for "tattoo inks/PMU".

Table 146 Other in-house methods

Substance (with ref. to JRC report)	**Matrix**	**Method in JRC report**	**Applicability to tattoo inks and PMU**
PAAs listed at p. 147[1]	Air samples/studies on surface contamination	"*Aromatic amines in air and on surfaces*" (MDHS 75/2) – English experts (p. 147)	The method is not applicable to tattoo inks and PMU because the extraction procedure (sample preparation) is designed for air. Other extraction methods specifically for tattoo inks are available and reported at p. 148 of the JRC report).
PAAs (no substance details)	Food contact materials (migration test)	"*Determination of primary aromatic amines in acidic migration solutions by LC-MS/MS*" – Austrian experts (p. 147)	In the JRC report the analytical procedure is missing. Available information do not allow to assess the applicability to tattoo inks.
PAAs (no substance details)	Tattoo inks	*"Determination of free carcinogenic aromatic amines in tattoo inks by HPLC/MS/MS"* – Swiss experts (p. 148)	In the JRC report data on LOQ and repeatability obtained from a validation study with tattoo inks spiked samples. The method is potentially applicable to tattoo inks, but the validation study is not complete and has to be integrated with reproducibility data (intra-laboratory). In general, taking into account also information provided by Dutch experts on the best technique to analyze PAAs, methods based on LC-MS-MS seems to be the best option to analyze tattoo inks for presence of free aromatic ammines and for aromatic amines which could be formed. Such technique allows to overcome the disadvantages of GC-MS, in particular the possible false positive results due to the injector high temperature.
PAAs (no substance details)	Colorants, cosmetics, finger paints, inks for pens and tattoos	*"In-house HPLC/MS method based on: "Determination of carcinogenic aromatic amines in dyes, cosmetics, finger paints and inks for pens and tattoo with LC/MS"* bases on	In the JRC report data on LOQ and repeatability obtained from a validation study with tattoo inks spiked samples. However, validation data are not complete: reproducibility data, matrix and analytes are not reported in the report. The sample preparation is also missing. Available information do not allow to assess the applicability to tattoo inks

		Hauri – French experts (p.148)	
PAAs listed at p. 152[1] (p-fenildietilenammine is not reported)	Tattoo inks	"*GC/MS analysis for p-phenylendiamine (PDD) and free PAA*" – Danish experts (p.151)	In the JRC report validation data are absent. In the document "*Chemical substances in tattoo ink - survey of chemical substances in consumer products*" (p. 154) data in duplicate, with indication of repeatability and LOD are available. The method is potentially applicable to tattoo inks, but the validation study is not complete and has to be integrated with reproducibility data (intra-laboratory)

Note:

[1]Wrong CAS numbers

[2]Given the variability of the reductive cleavage (due the low solubility of pigments) and the differences of results obtained from Member States when using adaptation of the same method (e.g. EN ISO 14362-1, low repeatability/reproducibility) validation studies on reproducibility inter-laboratory have to be carried out in order to establish the reliability of methods for "tattoo inks/PMU".

Table 147 International standard methods

Substance (with reference to JRC report)	**Matrix**	**Method in JRC report**	**Applicability to tattoo ink and PMU**
4-amminoazobenzene	Textile materials	EN 14362-3 (p.141) (the old version included in the report has been superseded by EN ISO 14362-3:2017)	In the JRC report 2015 (p. 141) it is mentioned that Swedish experts use a modified version of EN 14362-3 but validation data are not there. Swedish authorities enforcing tattoo inks confirmed the analysis of PAAs is done by using a modified version of EN 14362.
PAAs on p. 144[1]	Toys (constituent materials such as textiles, wood, leather, paper, others)	EN 71-11:2005 (p. 144)	In absence of methods based on this standard for which validation data are available for tattoo ink matrix, the assessment of the applicability is not possible. This standard considers a procedure similar to the one set in EN 71-7 on the basis of which methods for tattoo inks have been developed (recommendation in ResAP (2008)1, table 4.c).
4-amminoazobenzene	Cuoi tinti	EN ISO 17234-2:2011 (p. 146) (in the report and old version of the standard is reported. It has been now replaced by EN ISO 17234-2:2015)	In absence of methods based on this standard for which validation data are available for tattoo ink matrix, the assessment of the applicability is not possible.

PAAs (n.20 listed in the JRC report)	Laminate samples or nylon cooking utensils (migration test for food contact material)	"*Specific determination of 20 primary aromatic amines in aqueous food simulants by liquid chromatography-electrospray ionization-tandem mass spectrometry*" (J. Chromatography A, 1091: 40-50) (p. 153)	CoE ResAP foresees a sample treatment with acidic solution applied to tattoo inks based on EN 71-7:2002 (sample treatment with HCl 0.07 M solution and sonication). It should be demonstrated that a solution with 3%acetic acid allows recoveries higher than the solution of HCl 0.07 M or than methanol. In absence of validation for tattoo ink matrix, the assessment of the applicability is not possible
aniline, 2-anisidine, 3-chloro-4methoxyanline, 2,4-dimethylaniline, o-toluidine	Printed sheets	"*Determination and Quantification of Primary Aromatic Amine in Printer Ink"* (p. 153)	The technique is considered applicable. The sample preparation with water has to be demonstrated more effective solution with 3% acetic acid, solution of HCl 0.07 M or methanol alone. In absence of validation for tattoo ink matrix, the assessment of the applicability is not possible.

Note:

[1]Wrong CAS numbers

Table 148 Methods described in literature: Methods for metals, International standard methods

Substance (with reference to JRC report)	**Matrix**	**Method in JRC report**	**Applicability to tattoo ink and PMU**
Aluminum, Antimony, Arsenic, Barium, Boron, Cadmium, Chromium, Cobalt, Copper, Lead, Manganese, Mercury, Nickel, Selenium, Strontium, Tin, Zinc	Parts of toys	EN 71-3:2013+A1: 2014	Not applicable. Method describes an extraction procedure and analysis of soluble elements from a solid surface. For tattoo ink, a sample digestion procedure is required in order to dissolve the matrix. Furthermore, the final composition of the solution must be taken into account when dealing with ICP-MS techniques.
Mercury	Foodstuff/modified for tattoo inks	EN 13806:2002	Applicable in the modified version. Cold-vapour atomic absorption spectrometry (CV-AAS) is a suitable technique for the quantification of a critical and volatile element as Mercury. Repeatability may be improved for ink.

Lead, Cadmium, Chromium, Molybdenum	Foodstuff/modified for tattoo inks	EN 14083:2003	Applicable in the modified version. Graphite furnace atomic absorption spectrometry (GF-AAS) following pressure digestion is a suitable method for the determination of these elements, however this technique does not allow simultaneous determination of all elements of interest. LoDs are not so satisfactory, since they rather high although the techniques used is sensitive especially for Cd. Repeatability may be improved for ink.
Arsenic, Cadmium, Lead, Mercury	Foodstuff/modified for tattoo inks	EN 15763:2009	Applicable in the modified version. Determination of Hg by ICP-MS is not so easy to perform, in some cases it leads to misleading results.
Aluminum, Antimony, Arsenic, Barium, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Mercury, Molybdenum, Nickel, Potassium, Selenium, Tin, Titanium, Zinc, Zirconium	Leather products/ modified for tattoo inks	EN ISO 17072-1:2011	Not applicable. Method describes an extraction procedure and analysis of soluble elements from a solid surface. For tattoo ink, a sample digestion procedure is required in order to dissolve the matrix.
Aluminum, Antimony, Arsenic, Barium, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Mercury, Molybdenum, Nickel, Potassium, Selenium, Silicon Sodium, Tin, Titanium, Zinc, Zirconium	Leather products	EN ISO 17072-2:2011	Not applicable as it is. An acid microwave assisted digestion procedure specific for tattoo ink should be developed. The mentioned spectrometric techniques (ICP-OES, ICP-MS, AAS, SFA) can be conveniently applied according to the type of element.
Aluminum, Antimony, Arsenic, Barium, Beryllium, Boron, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Mercury, Molybdenum,	Sediments, soils and oils/ modified for tattoo inks	EPA 3051A	May be applicable after testing the suitability of the extracting/dissolving procedure in microwave oven for tattoo ink.

Nickel, Potassium, Selenium, Silver, Sodium, Strontium, Tellurium, Vanadium, Zinc			
Aluminum, Antimony, Arsenic, Barium, Beryllium, Boron, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Mercury, Molybdenum, Nickel, Potassium, Selenium, Silver, Sodium, Strontium, Tellurium, Vanadium, Zinc	Ashes, biological tissues, oils, oil contaminated soils, sediments, sludges, and soils/modified for tattoo inks	EPA 3052	May be applicable after testing the acid assisted digestion procedure in microwave oven for tattoo ink. HF and HCl may be not necessary as reagents of mixture.
Chromium (VI)	Soils, sludges, sediments, and similar waste materials/modified for tattoo inks	EPA 3060A	Applicable in the modified version.
Chromium (VI)	Drinking water/modified for tattoo inks	EPA 218.7	May be applicable, but the matrix is very different from water. Since tattoo ink is a quite inhomogeneous solution, samples are supposed to be difficult to analyse by direct injection.
Aluminum, Antimony, Arsenic, Barium, Beryllium, Bismuth, Boron, Cadmium, Cesium, Calcium, Cerium, Chromium, Cobalt, Copper, Dysprosium, Erbium, Europium, Gadolinium, Gallium, Germanium, Gold, Hafnium, Holmium, Indium, Iridium, Lanthanum, Lead, Lithium, Lutetium, Magnesium, Manganese, Molybdenum, Neodymium, Nickel, Palladium, Phosphorus, Platinum, Potassium, Praseodymium, Rubidium, Rhenium, Rhodium, Ruthenium, Samarium, Scandium,	Drinking water, surface water, groundwater, wastewater/modified for tattoo inks	EN ISO 17294-2	Applicable in the modified version.

Selenium, Silver, Sodium, Strontium, Terbium, Tellurium, Thorium, Thallium, Thulium, Tin, Tungsten, Uranium, Vanadium, Yttrium, Ytterbium, Zinc, Zirconium			
Aluminum, Antimony, Arsenic, Barium, Beryllium, Bismuth, Boron, Cadmium, Calcium, Chromium, Cobalt, Copper, Gallium, Indium, Iron, Lead, Lithium, Magnesium, Manganese, Molybdenum, Nickel, Phosphorus, Potassium, Selenium, Silicon, Silver, Sodium, Strontium, Sulphur, Tin, Titanium, Tungsten, Vanadium, Zinc, Zirconium	Water quality/modified for tattoo inks	EN ISO 11885	Not appropriate. The entire procedure is time consuming and ICP-OES technique is not so sensitive for complex matrices.
Mercury	Drinking, surface, ground, rain and waste water	EN ISO 12846:2012 *	No comments.

Notes: * Supersedes DIN EN 1483

Table 149 In-house validated methods*

Substance **(with reference to JRC report)**	**Matrix**	**Method in JRC report**	**Applicability to tattoo ink and PMU**
Cadmium, Lead, Nickel	Cosmetics, food contact materials	Determination of heavy metals (Cd, Pb, Ni) in cosmetics and food contact materials (Slovakia)	Applicable if tested on tattoo inks.
Mercury	Cosmetics, food contact materials	Determination of mercury in cosmetics and food contact materials (Slovakia)	Applicable and recommended in the quantification of mercury.
Antimony, Chromium (VI), Cobalt, Copper, Mercury, Zinc	Cosmetics, food contact materials	Determination of heavy metals (Hg, Zn, Cu, Cr (VI), Co, Sb) in cosmetics and food contact materials (Slovakia)	Data lacking, no evaluation is carried out.

Antimony, Arsenic, Barium, Cadmium, Chromium, Cobalt, Copper, Gold, Iridium, Lead, Lithium, Mercury, Molybdenum, Nickel, Osmium, Palladium, Platinum, Rhodium, Ruthenium, Selenium, Silver, Sodium, Thallium, Tin, Uranium, Vanadium, Zinc	Unknown	ICP-MS (in-house method) (France)	As the laboratory stated on this method: "The test method was never applied to tattoo inks.". The matrix is not reported. May be applicable if tested on tattoo inks.
Cadmium, Chromium, Cobalt, Lead, Nickel	Cosmetics	MI-08 Determinazione degli elementi in cosmetici - Accredia Rev. 5, 2014 (Italy) (Determination of elements in cosmetics)	Applicable if tested on tattoo inks. LoDs are reported for three elements out of five only. LoD for Cr (1.26 mg/kg) seems to be too high to be used for control purposes being this above the maximum allowed concentration of impurities in products for tattoo and PMU recommended in the Resolution ResAP(2008)1 on requirements and criteria for the safety of tattoos and permanent make-up (Table 3). Cr is also indicated in the list of substances prohibited in cosmetic products of Annex II (Regulation N.123/2009).
Unknown, not specified.	Tattoo inks	Metals and other elements in tattoo inks (Slovenia)	Applicable. Since ICP-MS measurements could be strongly interfered, the applicability depends on the ICP-MS instrument to be used.
Antimony, Arsenic, Barium, Cadmium, Chromium, Cobalt, Copper, Lead, Mercury, Nickel, Selenium, Tin, Zinc	Tattoo inks	CHE01-WV495: Determination of certain elements in tattoo inks using ICP-MS (The Netherlands)	Applicable. This digestion procedure is advantageous, since HF, used in other methods, might be corrosive for some optical parts of the ICP-MS.
Metals and other elements (not specified)	Tattoo inks	ICP/MS screening analysis for metals and other elements (Denmark)	Not really recommended. The TotalQuant analysis gives a large range of element concentration, this is inappropriate to the aims of an official control for a REACH restriction.

Notes: *For this kind of methods, more details on method validation parameters are needed to evaluate the application to tattoo inks. In some cases there is a lack of information.

The six methods described in literature are not evaluated for their applicability, since these kind of methods cannot be recommended for checking compliance with restrictions of Annex

XVII (REACH). A clear and harmonized validation procedure with all performance characteristics is always required when dealing with official controls.

Table 150 Methods described in literature: Methods for colorants, International standard methods*

Substance (s) **(with reference to JRC report)**	**Matrix**	**Method in JRC report**	**Applicability to tattoo ink and PMU**
Carcinogenic dyestuff listed at p 156 of JRC report	Textiles	EN ISO 16373-2:2014	The coloured test specimen is extracted from textiles with pyridine/water mixture at 100 °C and analysed by LC/MS or LC/DAD. Validation data (LOQ, LOD, Repeatability and Reproducibility) are reported. No reference to the applicability to tattoo inks is present.
Allergenic dyestuff listed at p 156 of JRC report	Textiles		
Other dyestuff	Textiles		
Carcinogenic dyestuff listed at p 159 of JRC report	Textiles	EN ISO 16373-3:2014	This standard specifies a method for the detection and quantification of carcinogenic dyestuff in dyed, printed and coated samples (textiles). Repeatability and Reproducibility are reported with reference to different textile matrices. No reference to the applicability to tattoo inks is present.
Colorants listed at p 160 of JRC report	Toys	EN 71-11:2005	Validation data (LOQ, LOD, Repeatability and Reproducibility) are reported. No reference to the applicability to tattoo inks is present.

Table 151 Methods described in literature: Methods for colorants, In-house validated methods

Substance (s) (with reference to JRC report)	Matrix	Method in JRC report	Applicability to tattoo ink and PMU
Acid Red 1	Unknown	Slovakia	No data.
Colorants	Hair colours	SOP 1201: Intern metod för analys av färgämnen i hårfärgsprodukter med LC-MS (Sweden) (Internal method for analysis of colorants in hair colours by LC-MS)	The method is reported as screening method for hair colour products. No reference to the applicability to tattoo inks is present.
Colorants	Tattoo inks	Identification of colorants in tattoo inks with MALDI/TOF (Switzerland)	Only qualitative repeatability and reproducibility are available. It could be used as screening method.
Colorants	Tattoo inks	Identification of colorants in tattoo inks with colorimetry (Switzerland)	Only qualitative repeatability and reproducibility are available. It could be used as screening method.

Table 152 Methods for the polycyclic aromatic hydrocarbons (PAHs), International standard methods

Substance (s) (with reference to JRC report)	Matrix	Method in JRC report	Applicability to tattoo ink and PMU
benzo[α]pyrene	Finger paints	EN 71-7:2014	Validation data (LOQ, LOD, Repeatability and Reproducibility) are available for the matrix finger paint.No reference to the applicability to tattoo inks is present. May be applicable if tested on tattoo inks
PAHs listed at p 183 of JRC report	Food matrices	CEN/TS 16621:2014	Validation data are lacking and strongly dependent from the matrix. It cannot be assumed that tattoo inks are similar to food matrices.

Table 153 Methods for the polycyclic aromatic hydrocarbons (PAHs), In-house validated methods

Substance (s) (with reference to JRC report)	Matrix	Method in JRC report	Applicability to tattoo ink and PMU
PAHs listed at p. 186 of the JRC report	Tattoo inks	Determination of PAHs in tattoo inks with GC/MS (Italy)	Validation data (LOQ, Repeatability and Reproducibility) are available for tattoo inks. The method is applicable to tattoo inks.
PAHs listed at p. 187 of the JRC report	Tattoo inks	PAHs in tattoo inks by HPLC/UV/FLD after microwave assisted extraction with toluene (Switzerland)	Validation data are lacking and strongly dependent from the matrix. It may be applicable to tattoo inks but a validation study is needed.
PAHs listed at p. 188 of the JRC report		CHE01-WV405 Determination of polycyclic aromatic hydrocarbons (PAH's) in tattoo ink and rubber using a GC-MS system (The Netherlands)	Validation data are lacking. It may be applicable to tattoo inks but a validation study is needed.

Table 154 Methods for the polycyclic aromatic hydrocarbons (PAHs), Methods described in literature

Substance (s) (with reference to JRC report)	Matrix	Method in JRC report	Applicability to tattoo ink and PMU
PAHs. No reference to the substances is given.	Tattoo inks	Chemical Substances in Tattoo Ink. Survey of chemical substances in consumer products, 116 Danish Environmental Protection Agency, 2012, ISBN 978-87-92779-87-8.	LOD is reported in a range, depending on the substance. Validation data are lacking. It may be applicable to tattoo inks but a validation study is needed.
PAHs listed at p. 190 of the JRC report	Tattoo inks	Tattoo inks contain polycyclic aromatic hydrocarbons that additionally generate deleterious singlet oxygen Regensburger et al., Experimental Dermatology, 2010, 8 (19), 275-281.	LOD is reported in a range, depending on the substance. Validation data are lacking. It may be applicable to tattoo inks but a validation study is needed.

Table 155 Methods for phthalates, International standard methods

Substance (s) (with reference to JRC report)	Matrix	Method in JRC report	Applicability to tattoo ink and PMU
Phthalates listed at p 192 paragraph 1 of the JRC report	Cosmetic products ready to inject	EN 16521:2014	The LOQ is reported. Other validation data are lacking. No reference to the applicability to tattoo inks is present.
Phthalates listed at p 192 paragraph 2 of the JRC report	Textiles	EN ISO 14389:2014	Validation data (LOQ, LOD, Repeatability and Reproducibility) are reported. No reference to the applicability to tattoo inks is present.

Notes: The six methods described in literature are not evaluated for their applicability, since these kind of methods cannot be recommended for checking compliance with restrictions of Annex XVII (REACH). A clear and harmonized validation procedure with all performance characteristics is always required when dealing with official controls.

Table 156 Methods for phthalates, In-house validated methods

Substance (s) (with reference to JRC report)	Matrix	Method in JRC report	Applicability to tattoo ink and PMU
12 phthalates (no reference to specific substances is given)	Cosmetic products (nail polish etc.)	Determination of phthalates in cosmetics by GC-MS (Austria)	Validation data (LOQ, Repeatability and Reproducibility) are available. No reference to the applicability to tattoo inks is present.
Phthalates listed at pg 192 paragraph 2 of the JRC report	Cosmetics, food contact materials	Determination of phthalate esters in cosmetics (Slovakia)	Validation data (LOQ, LOD, Repeatability and reproducibility) are reported. No reference to the applicability to tattoo inks is present.

Table 157 Methods for nitosamines, International standard methods

Substance (s) (with reference to JRC report)	Matrix	Method in JRC report	Applicability to tattoo ink and PMU
Nitrosamines listed at p 196 of the JRC report	Toys (elastomeric materials) and finger paints	EN 71-12:2013	Validation data (LOQ, Repeatability and Reproducibility) are available. No reference to the applicability to tattoo inks is present.

Table 158 Methods for nitosamines, in-house validated methods

Substance (s) (with reference to JRC report)	Matrix	Method in JRC report	Applicability to tattoo ink and PMU
Nitrosamines listed at p 198 of the JRC report	cosmetics, finger paints & tattoo inks	Nitrosamines in cosmetics, finger paints and tattoo inks by LC/MS/MS (Switzerland)	Validation data (LOQ, Repeatability and Reproducibility) are available but strongly dependent from the substance and matrix. It may be applicable to tattoo inks but a validation study is needed.

Appendix D.3 RAPEX notifications for tattoo inks and PMU

Table 159 RAPEX notifications related to the chemical risk

Notification	Year/ Week	MS	Brand name/Product	Substance	Legal basis	Origin	Action
0034/16	2016-03	DK	Starbrite/Tribal black	benzo(a)pyrene: 0.5 mg/kg)		US	Withdrawal of the product from the market
0035/16	2016-03	DK	Intenze / Mario Barth/Gold Label tattoo ink/Light green	aromatic amines (measured value for o-anisidine: 40 mg/kg)		US	Withdrawal of the product from the market
0036/16	2016-03	DK	Intenze/Lime Green, Lemon Yellow, Golden Yellow, Golden Rod, Persian Red, Cherry Bomb, Cherry Bomb Mario Gold	aromatic amines (o-anisidine and aniline) (measured values: up to 5 ppm for each) and/or barium (measured value: up to 54000 ppm)		US	Withdrawal of the product from the market
0097/17	2017-05	DE	Eternal Ink/ Light Red	nickel (measured value 16.1 mg/kg).	Res	US	Withdrawal of the product from the market
0166/16	2016-07	IT	Eternal Ink/ Light Red	aromatic amines o-toluidine (measured value 92 mg/kg), 2.4-diaminotoluene (measured value 2780 mg/kg) and 2-methyl-5-nitroaniline (measured value 46 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0203/16	2016-08	DE	Intenze/Mario's Dragon Green Dark	barium (measured value 11700 mg/kg)	Res	US	Withdrawal of the product from the market (By: Distributor)
0347/16	2016-12	IT	Eternal Ink/ Lightning Yellow	aromatic amines o-anisidine and o-toluidine (measured	Res	US	Ban on the marketing of the product and any

				value 24 mg/kg and 31 mg/kg)			accompanying measures
0483/16	2016-16	IT	Eternal Ink/ True Gold	aromatic amine o-toluidine (measured value 48 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0644/16	2016-21	GE	ALMA PRIMA/KOTU-Tribal black	PAHs,, including benzo(a)pyrene (measured value benzo(a)pyrene: 0.2 mg/kg; total of PAHs: 29.5 mg/kg)	Res	Unknown	Withdrawal of the product from the market (By: Distributor) Ban on the marketing of the product and any accompanying measures
0650/15	2015-21	GE	Bio Touch/Dark red	nickel (measured value 18.2 mg/kg)	Res	US	Warning consumers of the risks
0668/15	2015-22	SE	Eternal Ink/ Solid Gold	aromatic amines (measured values for o-anisidine and o-toluidine 14 mg/kg and 86 mg/kg respectively)	Res	US	Withdrawal of the product from the market
0669/15	2015-22	SE	Intenze/Grey wash dark	PAHs (measured value of PAHs: 20 mg/kg)	Res	US	Withdrawal of the product from the market
0670/15	2015-22	SE	Unknown/Tattoo Fastness High-grade Color/Brown, Green, Red, White, Yellow colours	aromatic amines (measured values for 4-methyl-m-phenylenediamine up to 6220 mg/kg), barium (measured value up to 4.5 g/kg)	Res	Unknown	Withdrawal of the product from the market

				and zinc (measured value up to 0.5 g/kg)			
0671/15	2015-22	SE	BIOTouch/Micro Pigment Cosmetic Color SUNSET	barium (measured values 62 mg/kg), zinc (measured values 102 mg/kg) and aniline (measured values 53 mg/kg)	Res	US	Withdrawal of the product from the market
0672/15	2015-22	SE	Magic Cosmetic/Micro Cream Pigment for Permanent Make-up	arsenic, barium, lead and zinc (measured values respectively 49 mg/kg, 103 mg/kg, 25 mg/kg and 107 mg/kg)	Res	Israel	Withdrawal of the product from the market
0673/15	2015-22	SE	Kuro Sumi/Kuro Sumi Colors Tattoo Ink	PAHs, including benzo(a)pyrene and naphthalene (measured value of PAHs: up to 20 mg/kg), as well as cadmium, lead and zinc (measured values 28 mg/kg, 41 mg/kg and 246 mg/kg)	Res	CN	Withdrawal of the product from the market
0674/15	2015-22	SE	Kuro Sumi/Kuro Sumi Colors Tattoo Ink	PAHs, (including benzo(a)pyrene and naphthalene) (measured value of PAHs: 3.7 mg/kg) as well as lead (measured value 17 mg/kg)	Res	JP	Withdrawal of the product from the market

0675/15	2015-22	SE	Pure Colors/Strawberries & Cream (tattoo ink & PMU)	barium (measured value:17.8 g/kg)	Res	US	Withdrawal of the product from the market
0676/15	2015-22	SE	Purebeau/Venus (PMU)	barium (measured value 2.5 g/kg)	Res	DE	Withdrawal of the product from the market
0677/15	2015-22	SE	Millennium Colors/MOMS	PAHs, (including benzo(a)pyrene) (measured value of PAHs up to 22 mg/kg)	Res	US	Withdrawal of the product from the market
0712/16	2016-23	DE	GOOCHIE/PMU colour	nickel (measured value: 22 mg/kg), arsenic (measured value: 6.8 mg/kg) and lead (measured value: 3.3 mg/kg)	Res	CN	Withdrawal of the product from the market (By: Distributor)
0731/16	2016-24	DE	Carmen Wallstein/Unknown (PMU)	nickel (measured value 24 mg/kg)	Res	DE	Withdrawal of the product from the market (By: Manufacturer)
0884/15	2015-28	DE	Dragonhawk Tattoo/Tribal Black	PAHs, (including benzo(a)pyrene) (measured value of PAHs up to 2.5 mg/kg)	Res	CN	Import rejected at border
0892/16	2016-30	DE	Golden Rose/Dark coffee; chocolate (PMU)	nickel ("dark coffee" measured value 51.8 mg/kg and "chocolate" measured value 48.2 mg/kg)	Res	US	Withdrawal of the product from the market
0985/15	2015-31	FR	DERMAGLO/Unknown	0.3% of phenol	Res	Unknown	Withdrawal of the product from the market
0986/15	2015-31	FR	Intenze/Mario's Dragon Green	aromatic amine o-anisidine	Res	Unknown	Withdrawal of the product

			Dark	(measured value: 60 mg/kg) and barium (measured value: 11140 mg/kg)			from the market
1084/16	2016-35	DE	Intenze/Gray Wash Dark	PAHs, including benzo(a)pyrene (measured value benzo(a)pyrene: 0.1 mg/kg; total of PAHs: 16.9 mg/kg)	Res	US	Destruction of the product (By: Retailer) Withdrawal of the product from the market
1166/15	2015-38	IT	Eternal Ink/Nude Bush	barium (measured value 550 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
1168/15	2015-38	IT	FUSION INK/True Blood	aromatic amine o-anisidine (measured value: 21 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
1193/15	2015-39	IT	Intenze/Dark Chocolate	antimony (measured value up to 3.2 mg/kg), arsenic (measured value up to 14.9 mg/kg), nickel (measured value up to 106 mg/kg) and lead (measured value up to 5.76 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
1199/15	2015-39	IT	Eternal Ink/Light red	nickel (measured value 11 mg/kg)	Res	US	Withdrawal of the product from the market
1228/15	2015-40	IT	Eternal Ink/Nuclear green	barium (measured value 6200	Res	US	Recall of the product from end users

				mg/kg)			
1262/15	2015-41	IT	Eternal Ink/Lightning Yellow	aromatic amines o-anisidine and o-toluidine (measured value 19 mg/kg and 68 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying Measures
1333/17	2017 - 40	IT	BIOTEK/STRONG BLACK	(PAH)s, including benzo(a)pyrene (measured values: total of PAHs: 1201 mg/kg)	Res	IT	Ban on the marketing of the product and any accompanying measures
1279/17	2017 - 38	IT	Eternal Ink/Dark Red	aromatic amine o-anisidine (measured value 27 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
1088/17	2017 - 33	IT	Eternal Ink/Deep red	aromatic amine anisidine (measured value: 39 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
1087/17	2017 - 33	IT	Eternal Ink/Deep Red	aromatic amine anisidine (measured value: 20 mg/kg).	Res	US	Ban on the marketing of the product and any accompanying measures
1086/17	2017 - 33	IT	Intenze/Dark Tone	(PAH)s, including benzo(a)pyrene (measured values: benzo(a)pyrene: 0.02 mg/kg; total of PAHs: 145 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0665/17	2017 - 21	NL	Arcane Pigments, Alla Prima / Lining Black	(PAH)s, including naphthalene (measured values: naphthalene: 1.41 mg/kg; total of PAHs:	Res	US	Ban on the marketing of the product and any accompanying measures

				1.56 mg/kg)			
0660/17	2017 - 20	NL	Eternal Ink/Triple black	(PAH)s, including naphthalene (measured value: naphthalene: 9.73 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0659/17	2017 - 20	NL	Intenze/Lining Black	(PAH)s, including naphthalene (measured values: naphthalene: 2.41 mg/kg; total of PAHs: 2.53 mg/kg)	Res	UK	Ban on the marketing of the product and any accompanying measures
0658/17	2017 - 20	NL	Kuro Sumi/Tattoo Outlining Ink	(PAH) benzo(a)pyrene (measured value: 0.105 mg/kg)	Res	UK	Ban on the marketing of the product and any accompanying measures
0657/17	2017 - 20	NL	Unknown/Premium Tattoo Ink - Tomato is Red	cadmium (measured value: 0.54 mg/kg), lead (measured value: 19.76 mg/kg) and polycyclic aromatic hydrocarbons (PAH)s, including benzo(a)pyrene (measured values: 153 ug/kg for benzo(a)pyrene; total of PAHs: 1.45 mg/kg)	Res	CN	Ban on the marketing of the product and any accompanying measures
0656/17	2017 - 20	NL	Eternal Ink/Triple black	(PAH)s, including naphthalene (measured values: naphthalene: 3.07 mg/kg; total of PAHs: 3.08 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures

0655/17	2017 - 20	NL	Fusion/Power Black	(PAH) naphthalene (measured value: 7.23 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0654/17	2017 - 20	NL	Intenze/Lining black	(PAH)s, including naphthalene (measured values: naphthalene: 1.67 mg/kg; total of PAHs: 1.75 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0653/17	2017 - 20	NL	Intenze/True black	(PAH)s, including naphthalene (measured values: naphthalene: 2.25 mg/kg; total of PAHs: 2.38 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0652/17	2017 - 20	NL	Intenze /Dimension Black	(PAH)s, including naphthalene (measured values: naphthalene: 3.01 mg/kg; total of PAHs: 3.09 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0651/17	2017 - 20	NL	Eternal Ink/Triple Black	(PAH) naphthalene (measured value: 13.87 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0650/17	2017 - 20	NL	Derma International/#B B Best Black	arsenic (measured value: 3.2 mg/kg), lead (measured value: 3.1 mg/kg), cobalt (measured value: 40.6 mg/kg) and nickel (measured value: 53	Res	US	Ban on the marketing of the product and any accompanying measures

				mg/kg)			
0649/17	2017 - 20	NL	Derma International/#9 Black	arsenic (measured value: 5.47 mg/kg), lead (measured value: 4.73 mg/kg), cobalt (measured value: 72.3 mg/kg), nickel (measured value: 91.1 mg/kg) and zinc (measured value: 90.07 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0648/17	2017 - 20	NL	Alla Prima /Unknown	(PAH)s, including naphthalene (measured values: naphthalene: 1.29 mg/kg; total of PAHs: 1.47 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0647/17	2017 - 20	NL	Unknown/Tattoo Specific Color - Black	(PAH)s, including naphthalene and benzo(a)pyrene (measured values: naphthalene: 1.18 mg/kg; benzo(a)pyrene: 0.97 mg/kg; total of PAHs: 2.07 mg/kg)	Res	Unknown	Ban on the marketing of the product and any accompanying measures
0646/17	2017 - 20	NL	Dynamic/BLK	(PAH)s, including naphthalene (measured values: naphthalene: 1.36 mg/kg; total of PAHs: 1.53 mg/kg)	Res	US	Ban on the marketing of the product and any accompanying measures
0645/17	2017 - 20	NL	Intenze/black sumi	(PAH)s, including naphthalene	Res	US	Ban on the marketing of the product

				(measured values: naphthalene: 1.78 mg/kg; total of PAHs: 1.9 mg/kg)			and any accompanying measures
0644/17	2017 - 20	NL	Kokkai Sumi Ink /Lining - Tribal - Tattoo ink	cadmium (measured value: 0.53 mg/kg) and lead (measured value: 19.19 mg/kg)	Res	CN	Ban on the marketing of the product and any accompanying measures

Source: (JRC, 2015b), RAPEX

Table 160 RAPEX notifications related to microbiological risk

Notification	Year/ Week	MS	Brand name/Product	Microbiological agent	Legal basis	Origin	Action
0049/06	2006	FR	Intenze/Lemon yellow (1), Hard orange (2)	(1) Moraxella spp, (2) Staphylococcus warneri		US	Voluntary stop of distribution
0890/07	2007-34	NL	Euro Sumi Outlining ink	Pseudomonas spp:1500 cfu /ml		UK	Sales ban ordered by the authorities
1050/07	2007-41	DE	Eternal Tattoo/Colour Plum No 29	Aerobic mesophile bacteria count: 7.7 x 10^5 CFU/g		US	Voluntary withdrawal from the market by the importer
1071/09	2009-31	NL	Eternal Ink	Pseudomonas spp		US	Sales ban and withdrawal from the market ordered by the authorities.
1609/10	2010-44	IT	Starbrite 2 /Golden Yellow, Baby Blue	Multiple-use containers (15 ml and 30 ml), absence of a non-return valve= no guarantee of preserving the sterility of the pigment		US	Imports rejected by the customs authorities
0133/15	2015-5	DE	Vibes/Energy Ink/Brigth Green	Aerobic mesophilic bacteria: 1.6 x		DE	Withdrawal of the product from the

				10^6 cfu/ml			market
0023/16	2016-10	DE	Intenze/Bob Tyrell Light Tone	Aerobic mesophilic bacteria: up to 13000 cfu/g	NL	US	Ban on the marketing of the product and any accompanying measures
0358/08	2008-11	DE	Fantasia Colour Pigment, Med. Blue	Lot No 002: aerobic mesophiles total bacteria count: 8.1 x 10⁷ CFU/g (identified as pseudomonas aeruginosa) - Lot No 1006: aerobic mesophiles total bacteria count: 3.6 x 10⁶ CFU/g; pseudononas aeruginosa: 1.1 x 10⁶ CFU/g; yeasts: 900 000 CFU/g		US	Recall from consumers ordered by the by the authorities

Source: (JRC, 2015b), RAPEX

Annex E. Assumptions, uncertainties and sensitivities

E.1. Related to risk assessment

Uncertainties and Sensitivity Analysis

One of the main sources of uncertainty regarding the exposure assessment is the estimate for the amount of ink used in tattooing. As stated above only very limited data exist as this issue was investigated in only two published studies. The value used for this proposal (14.36 mg/cm^2 of ink) is based on a study by Engel et al. (Engel, et al., 2008). In the original publication the authors give a mean value of 2.53 mg/cm^2 for used pigment over all experiments. However, the experiments were performed under different settings (different needles, tattooing was performed by artist or by scientists, human or pig skin was used, concentration of ink (Pigment Red 22, PR 22) 10% or 25 %, commercial pigment (purity ~80 %) or self-synthesized (>98 %)). This mean value was used for further work (Lehner, et al., 2011) and also cited by the JRC (JRC, 2015b) the BfR (BfR, 2012) and the Danish EPA (DEPA, 2012) (DEPA, 2014).

For the purpose of this proposal a concentration of 25 % PR 22 is considered to be a more realistic composition for tattoo inks. As a result all experiments for 25 % were pooled (N=9) for this proposal. The individual values are given in the table below.

Table 161: Experimental data from Engel et al., 2008.

#	PR 22 (25 %) mg/cm^2	Remarks
1	0.6	Tattoo artists, pig skin
2	0.95	Human skin
3	1.42	Pig skin
4	1.69	Human skin
5	2.6	Pig skin
6	3.44	Pig skin
7	3.59	Pig skin
8	5.19	Pig skin
9	9.42	Pig skin
	3.21	Mean
	2.60	Median
	3.59	75th percentile[66]
	6.04	90th percentile
	7.73	95th percentile

The Dossier Submitter decided to use the 75th percentile (3.59 mg pigment/cm^2) for further calculation resulting in 14.36 mg/cm^2 of tattoo ink, when 25% pigment is assumed. If a

[66] Approximation performed with MS Excel, "QUARTILE" or "QUANTILE" function respectively.

single tattoo session results in a 300 cm^2 tattoo, this equals 300cm^2 x 14.36mg/cm^2 = 4 308 mg ink/tattoo session. The following issues were taken into account:

- The highest value 9.42 mg/cm^2 may be used as a worst case assumption. However this value is significantly higher than the other results which could not be attributed to the experimental setting alone.
- The pigment estimate is multiplied by 4 to obtain the estimate for total amount of ink (containing 25 % of pigment). Multiplying the highest value 9.42 mg/cm^2 may result in an unreasonable overestimation.
- The data set is very limited: There were only 9 reported individual results for the procedures carried out using 25% Pigment Red 22. In addition, an unknown number of additional procedures were also performed but the results were not reported.
- Results from experiments performed by tattoo artists yielded the lowest amount of pigment used (0.6 mg/cm^2)
- In the only other study - by Prior - an amount of 0.4 mg/cm^2 was reported as the average amount of ink used (with 67 % of black pigment).

In addition, Engel et. al. (Engel, et al., 2010) found that the amount of pigment used was influenced by the equipment and that the amount was higher with commercial pigment despite the lower concentration of PR22. This was attributed to other agglomeration and sedimentation properties, which may influence the amount of pigment required. These issues add to the uncertainty because the exposure estimate is based on the study results. On the other hand a variability of equipment and ink composition and quality must also be assumed for tattooing in general. As stated above the exact composition of tattoo inks and the resulting amount of ink used may depend on the supplier, the colour used, the purpose of the ink and presumably also on the personal style and proficiency of the tattoo artist.

Naturally, the size of the individual tattoos is very variable and the skin area actually covered depends on the motif. The applied 300 cm^2 of full covered area is an estimate for the highest area that can be tattooed in one session.

Applying the exposure scenarios developed in this document, Table 164 summarizes the issues to be considered during the uncertainty and sensitivity analyses with respect to the exposure assessment.

Table 7. Overview of the main sources of uncertainty and how they will drive the RCR, concentration limits and the sensitivity of the final result.

Source of uncertainty	Description	Effect on RCR	Effect on concentration limit	Sensitivity for the result
The amount of pigment/ink deposited in a tattoo (mg/cm^2)	The estimate for used ink may be an overestimation because the 75th percentile from experimental data was used and the calculation includes multiplication of the estimate by 4 (due to 25% pigment in the ink). The data set applied is very limited (9 reported numbers + unknown total number of experiments). Comparison with other literature data also suggests that the typical value of deposited ink	↑	↓	High

	may be smaller. If the professional tattoo artist does apply less ink per cm^2 than 14.36 mg ink/cm^2,which have been indicated in expert judgements, then the risk assessed in this assessment would overestimate the risk and set the concentration limits too low (where based on the exposure assessment).			
The application of different tattoo equipment	In the study by Engel et al. (2008) the variability in the amount of pigment in the skin may also be due to the use of different tattoo application equipment.	Both ways	Both ways	Medium
The amount of pigment in the ink	In the calculation the content of pigment in the ink is assumed to be 25 %. As in some cases 25% will be too low (presumably leading to the use of less ink in total) and in some cases too high (presumably leading to the use of more ink in total) this may influence the result in both ways.	Both ways	Both ways	Low
Uptake of pigment	In the scenario a 100% distribution of pigment in the system is assumed. This is most likely not the case. In the study by (Engel et al., 2008) a reduction of only 32% was observed during 6 weeks. If there is not a 100% distribution of pigment in the system the estimated RCR values will be too high and the concentration limits too low (where based on the exposure assessment).			Low
Uptake of soluble substances	In the scenario a 100% uptake of soluble substances such as impurities are assumed. This is likely to be the case. However, in case a 100% uptake does not take place the estimated RCR values will be too high and the concentration limits too low (where based on the exposure assessment).			Low
Continuous release of impurities from pigments	A continuous release of impurities from pigments may possibly give rise to additional exposure. However, since the solubility of pigments generally is very low this is unlikely to occur to a greater extent. Further, the release should supply a higher amount than was originally supplied with the liquid in the tattoo ink when excretion takes place. If impurities are released in such high amounts the risk estimated would be too low and the concentration limits too high (where based on the exposure assessment).			Low
Excretion of pigments	In the scenario it is assumed that the absorbed pigments are excreted after having had their effect within the body system. It is possible that this may occur due to observations of coloured lymph nodes. If the pigment is not excreted the RCR values will be too low and the concentration limits too high (where based on the exposure			Medium

	assessment).			
Excretion of impurities	In the scenario it is assumed that the absorbed impurities are excreted after having had their effect within the body system. This is likely to be the case. If the known impurities were e.g. known as being hydrophobic the excretion may be less likely to occur. However, the known impurities are not known to be hydrophobic. However, if the impurities are not excreted the RCR values will be too low and the concentration limits too high (where based on the exposure assessment).			Medium
Lack of excretion of continuously released impurities	In case that a continuous release of impurities from pigments takes place and that these impurities are not excreted the system will experience a higher concentration than what is present in the tattoo ink. However the assumption that impurities are not excreted may not be likely.			High

- There still remains uncertainties regarding the appropriate methodology for assessing risks due to intradermal exposure and risks arising from mixtures. The challenges for risk assessment of pigments in tattoo inks has been raised by Serup (Serup, 2017a). The Dossier Submitter recognizes these challenges. However, as no other alternative and appropriate method has been found, the Dossier Submitter has applied the approach for risk assessment in REACH.

- The Dossier Submitter assumes that the risks associated with exposure to a substance at an equivalent dose are expected to be at least as high, if not higher, for intradermal exposure via tattooing compared to exposure to substances applied on the skin. However, it is acknowledged that in some cases this conclusion may not hold true considering that a tattoo may only be applied once, or a limited numbers of times, and while it leads to long-term exposure, this exposure may be different than the exposure associated with for example a cosmetic product applied and removed multiple times (up to daily application over most of a lifetime).

- The number of substances included in the scope that have actually been used in tattoo inks is unknown. A restriction would therefore likely cover various substances that would never find use in tattoo inks.
- The rationale for inclusion of some of the CPR Annex II substances is clear, particularly in relation to recent amendments to the CPR/CPD where there is an associated opinion of the SCCS. However, for many of the substances there are no such associated opinions. For example, some of the inclusions relate specifically to certain uses in cosmetic products (e.g., hair dyes or substances used as a fragrance ingredient) and not others. It is uncertain to what extent other uses have been examined in the decision to place the substance on Annex II and what the implications are for risks associated with potential use in tattoo inks.
- While Annex II of the CPR does not include any concentration threshold for substances prohibited from use and for only a few of the substances in the 'restricted

field of application' product types in Annex IV, adapting this for a restriction on tattoo inks might require consideration of such a low concentration limit. In particular, some substances might be present in detectable but toxicologically negligible concentrations, with their removal being impractical or would require substantial resources, exceeding any benefits of their elimination. Examples of such situations have not been collected on the basis of the experience of the Member States with national legislation based on the two resolutions. However, enforcement of Annex II and IV under the CPR allows for the non-intended presence of traces of some substances, stemming from impurities of natural or synthetic ingredients, the manufacturing process, storage, migration from packaging, which is technically unavoidable in good manufacturing practice, unless a purity requirement is stated. The concentration thresholds for cosmetic products would also presumably require updating to make them relevant for tattoo inks.

- This restriction carries only forward concerns about the conditions related to colourants used in cosmetic products and regulated under the CPR. Historical information shows that pigments other than those on Annex IV have also been used in tattoo inks. There are currently no conditions on their use, other than those related to the groups of substances included in the scope of this restriction proposal.
- Column h lists maximum concentrations for colourants allowed in Annex IV CPR which are intended to come into contact with the skin. The inclusion of the provisions of column h into the restriction is based on the argument that concentrations which are not allowed on the skin should also not be allowed under the skin. This would be a minimum requirement because the skin barrier, which is a factor in the absorption of substances applied on the skin, is circumvented in the case of injection of tattoo inks. A degree of uncertainty lies in the fact that no risk assessment of the respective substances has been performed for the application "injection under the skin". It is possible that for tattooing, a lower maximum concentration needs to be allocated to certain substances.
- The justification for the maximum allowed concentrations of impurities in products for tattoos and PMU included in CoE ResAp(2008)1 Table 3 is not available to the Dossier Submitter.
- The content ranges for selected substances reported by JRC and used in the calculation of RCRs are based on a large variety of national surveys and market surveillance activities and are difficult to compare. Statistical details, such as mean, median and percentile values are to a large degree lacking.
- The detection limit for PAAs vary across different laboratories who apply different standards.
- The detection limits the Dossier Submitter used for setting the concentration limits for PAHs may be under estimated (set based on detection limit as the risk based concentration was below this) and therefore a lower concentration limit for PAHs could be achievable.
- Most/all analytical methods cannot differentiate between soluble and insoluble barium and measure only the total content of elements.
- The solubility of different compounds varies, so the conclusions on the risk will depend on which substances/pigments/compounds are present in any given tattoo ink. The extent to which the risk will vary depending on solubility is unknown.

- Dose response relationships for substances included in the restriction are investigated by the Dossier Submitter only for a small number of the substances included in the scope.
- As highlighted by the RAC (ECHA, 2013), dose response relationships for arsenic were derived by linear extrapolation. Extrapolating outside the range of observation inevitably introduces uncertainties. As set out by the RAC, the mechanistic evidence is suggestive of non-linearity; it is therefore acknowledged that the excess risks in the low exposure range might be an overestimate.
- The different entries in the legislative text of CPR Annex IV are mainly identified by a Colour index number (CI number). Since several of the relevant CI numbers can be associated with more than one substance, the European Commission's database for information on cosmetic substances (Cosmetic ingredient database, CosIng) has been used as a source file to identify the correct CAS and EC numbers for the entries in Annex IV. There are uncertainties related to the use of CosIng to match the CI numbers with their corresponding CAS and EC numbers for some of the entries in CPR Annex IV, i.e. how the following legal text in Annex IV should be interpreted: "substance name….. and its insoluble barium, strontium and zirconium lakes, salts and pigments". The legal text indicates that at least 4 individual CAS/EC numbers should be associated with these entries in Annex IV, but this cannot be confirmed by the information in CosIng. The Dossier Submitter can therefore not be certain that all relevant substances on CPR Annex IV are captured by the scope of the restriction.
- There is a strong indication that photo-decomposition of azo-colorants that contain 3,3'-dichlorobenzidine and azo-colorants that may decompose via amide hydrolysis are 99,5% responsible for the PAAs (with h We have inserted a short text in Appendix B5 (highlighted in yellow).Please have a look and amend if you think it is necessary. armonised classification) observed in tattoo inks. However, this could also be verified by other investigations, which have not been performed.
- Since azo-colorants not described by the stakeholder as being used could be used in the future, all possible relevant PAAs have been identified and included in the scope of the restriction proposal. Thus PAAs that may not be relevant is also included in the restriction proposal.
- The critical aspect concerning laser treatment is the decomposition and the substances formed during laser treatment. The hazard and risk from laser treatment of tattoos implies uncertainties in the hazard and risk assessment which the Dossier Submitter has not addressed in detail.

There are several sources of uncertainties in the risk assessment of substances to reproduction in the present restriction proposal. Hereby, uncertainties related to identification/derivation of NOAEL/LOAEL and DNEL values have been discussed individually for each substance in Appendix B.3. Hazard assessment for reprotoxic substances

- The applied general approach of the DNEL setup following the REACH Guidance does not consider higher risks of sensitive population groups. The estimated RCRs may underestimate risks for young adults, children or adults with weakened immune defense (To the knowledge of the Dossier Submitter an EU-wide ban of tattooing for under the age of 18 is not existing).
- Further uncertainties arise from the chosen risk assessment strategy based on one overall DNEL for reprotoxic effects and setting of group concentration limits for

substances toxic to reproduction as described in RO 1 & 2 that should cover the relevant range of risk levels. The risk of individual substances based on their estimated concentration limits were not considered in this proposal. This may lead to under- or overestimation of the risk level for the individual substances. Underestimation may have occurred for potent reprotoxic substances with DNELs lower than 0.001 mg/kg bw/d (as for example for tributyltin chloride). Overestimation is obviously given for other substances with DNELs higher than 0.001 mg/kg bw/d which may be true for the majority of known reprotoxic substances.

- There is general uncertainty for the Category 2 reprotoxicants. The Dossier Submitter proposes to include those in RO 1 & 2 with a group concentration limit 10 fold higher than the group concentration limit proposed for Category 1A/B reprotoxicants. Category 2 reprotoxicants were not subject of an individual hazard assessment and were not quantitatively assessed with regards to their risk level.
- The group concentration limits proposed for Category 1 and 2 reprotoxicants do not differentiate between effects on fertility (on male and female adults) and development effects (e.g. on the progeny that may be affected by tattooing pregnant females). There is uncertainty in this approach as fertility and developmental effect are not necessarily comparable. Separate DNELs for fertility and developmental toxicity may exist. However the difference will not come into effect for most of the substances (those with DNEL above 0.001 mg/kg bw/d).
- The proposal suggests concentration limits on individual reprotoxic substances which do not reflect exposure to several compounds from one or multiple tattoo inks that may act on the reproduction system via similar or different modes of action.
- The exposure to reprotoxic substances may also be expected from other sources which have (in this proposal) not been considered.
- If present, risk estimates should be compared with biomonitoring data. Within this proposal concentration levels in urine or blood could be present for some of the assessed substances (e.g. for the reprotoxic phthalates), but have not been considered as this verification would have required to estimate the exposure from several sources. This was not feasible for the high amount of substances assessed.
- With regards to RO2 and the option to apply an individual concentration limit, there are uncertainties due to the imbalance of considering the individual concentrations only for those substances that have already been found in tattoo inks in comparison to other substances which would need an individual concentration limit to ensure RCR <1. This would result in a higher protection level for those already identified in tattoo inks than those not yet examined.

E.2. Related to impact assessment

This section discusses the impact of the main SEA assumptions on total restriction costs, cost-effectiveness, break-even and overall proportionality of Restriction option 1 (RO1) and Restriction option 2 (RO2).

1. Tattoo ink and PMU on the EEA31 market

Annex C already noted that the future volumes of tattoo ink and PMU on the EEA31 market are uncertain. There is no historical information regarding the volumes of ink placed on the EEA31 market to extrapolate short- and long-term growth. Therefore, information about future volumes can be inferred only on the basis of information available on the demand for tattoos and PMU in the future. The long-terms demand for tattoo inks and PMU would depend not only on how many new people get tattoos but also how many tattoos a person tends to have, their size, style and colour. How these trends change creates an uncertainty. As stated in part A. of Annex C, it is assumed that the demand will grow at similar rates as the demand in recent years. Therefore, for the purpose of the analysis of the impacts of the proposed restriction options, it is assumed that the amount of tattoo ink and PMU on EEA31 market is expected to remain at current levels – 166 000 litres annually on average. For sensitivity purposes, the effects of two additional scenarios presented in Table 131 are tested. The Low tonnage baseline scenario assumes that future generations would not have the same desire to have a tattoo as their parents, while the High tonnage scenario assumes that preferences for tattoos will grow faster in the short term and continue at the same rate as during 2003-2014 after that.

As shown in Table 165, the cost-effectiveness for RO1 would deteriorate by 25.5% in the Low tonnage baseline scenario but would not change significantly in the High tonnage baselines scenario. The impacts for RO2 are expected to be similar.

Table 162 Tattoo ink and PMU on EEA31 market - projections

Scenario	Low Tonnage	Main Baseline	High Tonnage
Total restriction costs (yr)	3 042 190	4 589 609	5 174 969
Replaced tattoo ink & PMU (litres/yr)	38 859	78 693	87 911
Cost-effectiveness (€/litre non-compliant tattoo inks replaced)	78	58	59
Break-even - low (only effects on skin) (# cases avoided)	700	1 050	1 190
Break-even - high (only effects on skin) (# cases avoided)	210	320	360

2. Share of compliant inks currently on the EEA31 market

As stated in section D.4.1. Substitution costs, the assumptions on the share of compliant tattoo inks and PMU with the restriction options currently on the market will impact the conclusions with respect to substitution costs. The main analysis presented in Annex D is developed on the basis of the assumption that about 50% of the tattoo inks and about 90% of the PMU on the market are compliant with RO1 and RO2 requirements and therefore,

would not need to be reformulated substantially and their prices would not increase as a result of the proposed restriction options.

Therefore, for sensitivity purposes, it is tested if the impact of the lower and higher share of alternatives (compliant tattoo inks and PMU) currently on the market, i.e., in the High share of alternatives scenario assumes that only 30% of tattoo inks and no PMU currently on the market would not be compliant with RO1, while in the Low share of alternatives scenario – 70% of tattoo inks and 20% of PMU would not be compliant with the proposed restriction options.

Table 166 shows that these assumptions have an impact on the proportionality of the restriction: i.e., the cost-effectiveness for RO1 will improve by 16.5% and deteriorate by 5.6% respectively in the High share and in the Low share of alternatives scenario.

Table 163 Impact of the assumption related to the share of tattoo inks and PMU currently on the market that would have to incur cost as a result of RO1

Indicator	High share of alternatives	Main Scenario	Low share of alternatives
Total restriction costs - annual	2 331 456	4 589 609	6 847 762
Replaced tattoo ink & PMU (litres/yr)	46 567	78 693	110 820
Cost-effectiveness (€/litre non-compliant tattoo inks replaced)	50	58	62
Break-even - low (only effects on skin) (# cases avoided)	540	1 050	1 570
Break-even - high (only effects on skin) (# cases avoided)	160	320	480

3. Share of compliant inks currently on the EEA31 market

As stated in section D.4.1. Substitution costs, the price difference between compliant and non-compliant tattoo inks and PMU on the market is assumed 15% and 20% respectively. This is on the basis of the average response by stakeholders. The price difference was reported to range from "none" to close to 40% for tattoo inks and 70% for PMU. (stakeholder consultations) To test the impacts of these assumptions, two additional scenarios are prepared: no price difference and high price difference. The latter assumes that the price difference between compliant and non-compliant tattoo inks and PMU would be double those in the main scenario: respectively, 30% and 40%.

Table 167 shows that these assumptions have a substantial impact on the proportionality of the restriction: in the event the prices of tattoo inks and PMU increase by 30% or 40% respectively, the proportionality of RO1 can be demonstrated. The situation for RO2 is expected to be similar.

Table 164 Impact of price difference assumption on RO1

Indicator	No price difference	Main Scenario	High price difference
Total restriction costs - annual	235 762	4 589 609	8 943 456
Replaced tattoo ink & PMU (litres/yr)	78 693	78 693	78 693
Cost-effectiveness (€/litre non-compliant tattoo inks replaced)	3	58	114
Break-even - low (only effects on skin) (# cases avoided)	50	1 050	2 050
Break-even - high (only effects on skin) (# cases avoided)	20	320	620

4. Combined impact on proportionality

Table 168 shows that the combined impact of these three assumptions would lead to the highest deterioration in the cost-effectiveness of RO1: The combination of Low tonnage & Low share of alternatives & High price difference leads to the highest deterioration of the cost-effectiveness of RO1 by close to 65%. The impact of the polar opposite combination of assumptions on the costs effectiveness is substantial; however, the largest improvement of the cost-effectiveness is due to the price difference assumption (while all other assumptions remain as in the main scenario). The situation is expected to be similar for RO2.

Table 165 Combined impact of assumptions on RO1

Indicator	Low tonnage/Low share of alternatives/High price difference	High tonnage/ High share of alternatives/No price difference	No price difference
Total restriction costs - annual	8 943 456	235 762	235 762
Replaced tattoo ink & PMU (litres/yr)	55 032	52 078	78 693
Cost-effectiveness (€/litre non-compliant tattoo inks replaced)	163	5	3
Break-even - low (only effects on skin) (# cases avoided)	2 050	50	50
Break-even - high (only effects on skin) (# cases avoided)	620	20	20
% change	-64.1%	1188%	1847%

Therefore, the proposed restriction options to break even in the worst case scenario 2 050 surgical removals due to complication of tattoo inks would need to be avoided (calculated using cost of illness (COI) plus low WTP values) or 620 (COI plus high WTP values). This is respectively about 0.12% or 0.04% of the estimated number of people getting tattoos for the first time each year in EEA22.

It is reasonable to expect that these cases would be avoided as a result of the proposed restriction options as the estimated average prevalence rate of tattoo complications is 1.7% (see point e) in section D.6.1. Human health impacts and not all costs are taken into account (see point c).

In addition, the removal of tattoos due to an allergic or papulo-nodular reaction is just one group of the health outcomes. As stated in section D.6.1. Human health impacts, a number of people experience complications that require topical or systemic corticosteroids as well as experience mild ongoing complaints from their tattoos and PMU. This is in addition to the potential contribution of tattoo ink and PMU exposure to carcinogenic, reproductive, developmental and other systemic complications.

In summary, it can be concluded that the proposed restriction options are proportionate, as they are cost-effective, affordable and would lead to benefits in terms of avoided complications of tattoo inks and PMU associated with exposure to chemicals and other health effects (systemic, carcinogenic, reproductive or developmental) even when main assumptions are relaxed.

Annex F. Stakeholder information

F.1. Call for evidence

In order to gather information from relevant stakeholders on substances used in tattoo inks and PMU, a call for evidence was launched on ECHA's website. Specifically, information was sought relating to the substances used, tonnages, emissions and exposure, costs of tattoo inks, PMU and tattooing services, issues related to enforceability and alternatives (their risk, technical characteristics and costs).

Consultation started on 31 August 2016 and ended on 23 November 2016. In total 12 comments were received. Respondents included Member States, companies, industry or trade associations, NGOs and individuals. Received comments were taken into account in the development of the report.

The responses received included information on test methods and limits of quantification; information on substances used in tattoo inks and permanent make-up; survey results on how many people have tattoos and how many of them have declared any skin reactions, as well how many people are informed about risks; information on currently existing regulations on tattoo inks and PMU and experience in complying with them; and information of number of tattoo sessions and the average price per tattoo/PMU session.

More information is available in the background note for the call for evidence: https://echa.europa.eu/previous-calls-for-comments-and-evidence/-/substance-rev/14502/term

F.2. Survey on decorative tattoo sizes and costs

In addition to the call for evidence, ECHA conducted a survey/ interviews with several tattoo artists in Finland about the decorative tattoos.

The questions included information about the sizes and costs of different tattoos (simple and complex design), quantity of used ink in different sizes of tattoos, information how often different size of tattoos are made, information on expenses of used materials (ink, needles, aftercare, gloves, other supplies) and information on the market trends. See the survey in the Appendix.

In total, five responses were received. The gathered information was used in the analysis of impact assessment.

F.3. Questionnaire on the tattoo process by the Danish EPA

To refine the exposure scenario for tattoo inks, the Danish EPA in cooperation with the German Authorities developed the questionnaire. The questionnaire was open for replies from the 24th of January to the 7th of February 2017.

Seventy-three tattoo artists started to fill in the questionnaire. Forty-four tattoo artists completed the full questionnaire and 29 filled in parts of the questionnaire giving a completion rate of 60.3%.

On the 31 January 2017 a meeting was held with the chairs and board members of the three major tattoo artist associations in Denmark in order to validate the replies. Eight participants (Danish tattoo artists) validated the replies both as experienced tattoo artist as well as representatives of their associations.

The tattoo artists were asked to describe:

- the size of a "normal" or "typical" tattoo;
- percentage of customers that returns for another tattoo;
- the most frequent time interval between tattoos for a normal customer;
- how many sessions they would normally need to complete a large tattoo;
- the most important factor influencing how much it would be possible to tattoo in one session (such as pain, tattoo artist capacity, how much the skin can take etc.);
- how long time it takes for a tattoo to heal;
- what is the minimum time needed before a tattoo can be covered up;
- any circumstances that should be taking into account when trying to establish an exposure scenario for tattoo inks.

Please see the full summary of the results of this questionnaire in Appendix F.1.

F.4. Participation in the 3rd European Congress on Tattoo and Pigment Research (ECTP)

On 28-30 March 2017 ECHA participated in the 3rd European Congress on Tattoo and Pigment Research (ECTP) organised by the European Society of Tattoo and Pigment Research (ESTP). ECHA gave a presentation entitled: Potential legislation on tattoo inks and personal makeup (PMU) in the EU under REACH. The presentation gave a brief outline about the scope of the early draft of restriction dossier. Three questions were posed to the audience: need for labelling requirements, suitable transitional period, substances (pigments) that are difficult to substitute and other substances not mentioned in the current scope that need investigation.

The conference was attended by about 150 representatives of academia, dermatologists, tattoo ink and PMU manufacturers, tattoo artists, and government authorities.

Comments were received which were taken into account. In addition to this, ECHA participants followed up with selected researchers and representatives from industry in support of the development of the restriction dossier.

F.5. Consultation with Forum

To update the information gathered by the JRC on available analytical methods (JRC, 2015a) for selected substances in tattoo inks, the dossier submitter consulted the Forum on exchange of information on enforcement (the Forum). On 9 November 2016, the Dossier Submitter presented the restriction proposal in the Forum meeting. The Dossier Submitter also requested feedback from the Forum on whether the methods described in the JRC report are still relevant or whether there are new methods that are being used/developed that are not described in the report.

One response was received from the Swedish Medical Products Agency (the authority enforcing tattoo inks in Sweden), which was taken into account in drafting the restriction proposal.

A summary of available analytical methods is presented in Appendix D.3 based on information received from the Forum consultation, ECHA Call for Evidence and the JRC report (JRC, 2015a).

F.6. Stakeholders consulted by JRC

During the preparation of the Joint Research Centre (JRC) reports ((JRC, 2015a), (JRC, 2015b), (JRC, 2016a), (JRC, 2016b)), various stakeholders were consulted.

In April 2014 an international webinar on tattoos was organised to exchange information on safety issues related to tattoos, where representatives from the European Commission, OECD, twelve Member States, one EFTA country and other jurisdictions took part.

Five Consumer Safety Network Subgroup on Tattoos and Permanent Make-up (CSN-STPM) meetings were organised to share the knowledge, discuss the data collected and propose recommendations. The members of the CSN-STPM included both experts from Competent Authorities and stakeholders, including tattoo artists, ink manufacturers, dermatologists, consumers associations and the Council of Europe.

A number of questionnaires were prepared on different topics: regulatory framework, analytical methods, statistics, ink ingredients, health effects, CoE ResAP(2008)1, risk communication and data gaps identification. The questionnaires can be found in the JRC reports.

Targeted questionnaires were prepared and sent to relevant recipients. Those included: 28 EU Member States' plus 4 EFTA countries' Authorities; CSN- STPM members; other jurisdictions via the OECD secretariat; tattoo and PMU professional associations; ink manufacturers/distributors/private labels; and dermatologist associations. For more information please see the JRC reports.

F.7. Survey of tattoo ink and PMU manufacturers

ECHA conducted a web survey of tattoo ink and PMU manufacturers to supplement information gathered by the JRC to assist with the estimation of the potential impacts of the restriction and to confirm some concerns expressed by stakeholders. The survey was advertised via ECHA E-news and social media channels. It was also circulated by the ESTP and other stakeholders with linkages to the industry. The web survey ran between August 7 and September 10, 2017. Two responses were received. To complement the information received, five additional interviews with European and international tattoo and PMU manufacturers were conducted. The information was used in the quantification of socio-economic impacts in Annex D and E.

F.8. Interviews of dermatologists

To estimate the costs of treatment of non-infectious inflammatory tattoo complications, interviews with dermatologist prominent in the field of tattoo research were conducted. ECHA interviewed four dermatologists to obtain cost information in four Member States (Belgium, Denmark, Finland, and the Netherlands). To complement this information, four additional inquiries were made to medical institutions. Two responded. The information received is presented in Annex D, in the section on Human health impacts.

Appendix F.1 Questionnaire on the tattoo process

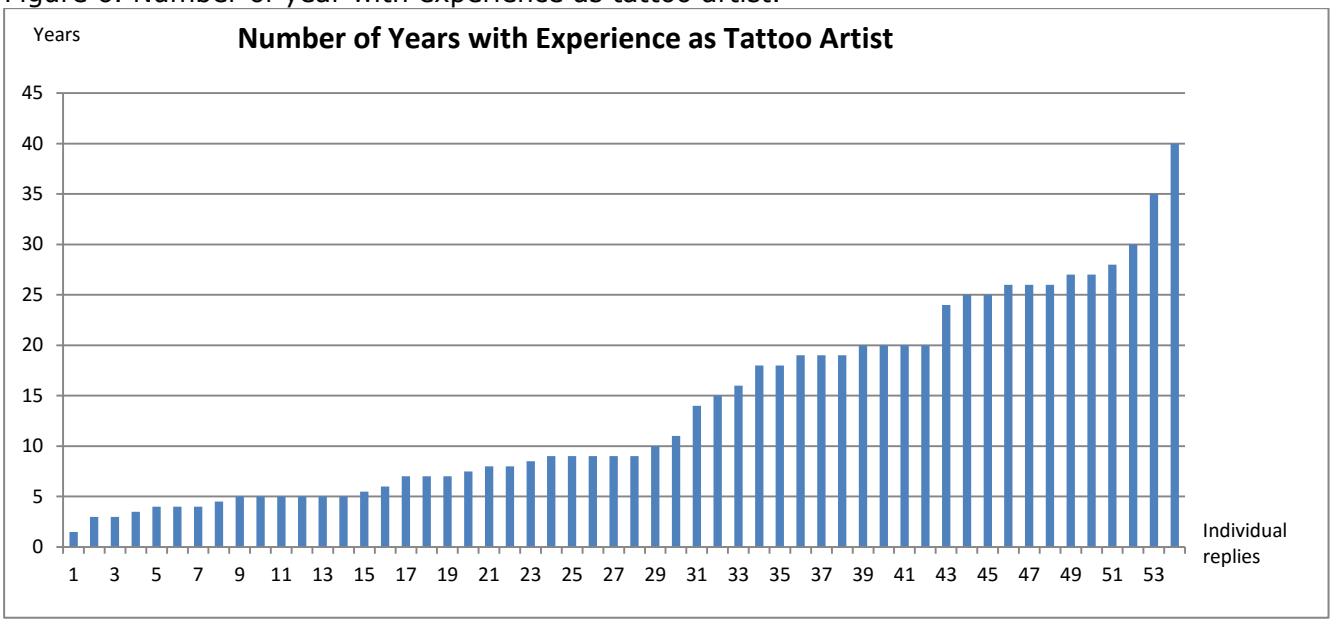
Methodology - questionnaire

The questionnaire was developed by the Danish EPA in cooperation with the German Authorities in order to refine the exposure scenario for tattoo inks. For reference, the English draft version of the questionnaire is attached to the document. The questionnaire was then translated into Danish and adopted to an on-line version open for reply from the 24th of January to the 7th of February 2017.

73 tattoo artists started to fill in the questionnaire. 44 tattoo artists completed the full questionnaire and 29 filled in parts of the questionnaire giving a completion rate of 60.3%.

54 tattoo artists filled in the number of years of experience. The experience of the tattoo artists varied from 1.5 years to 40 years. The average years of experience were 14 years with a median of 9 years. Two indicated that they were apprentice.

Figure 6. Number of year with experience as tattoo artist.



Midway 0n the 31th of January 2017 a meeting was held with the chairs and board members of the three major tattoo artist associations in Denmark in order to validate the replies. The 8 participants representing the Danish tattoo artists validated the replies both as experienced tattoo artist as well as representatives of their associations.

In the questionnaire a large tattoo scenario was developed (Question 8, 9 and 10 – see also appendix 2). This was based on the report from (JRC, 2016b) (See Text box 1 and Table 169). However, this did not resonate with the approach of the tattoo artist. In their view, a large tattoo is a tattoo that covers a body part such as for example an arm, a leg or the back. This type of tattoo is completed in a series of sessions. Thus, the replies to the questions on large tattoos did not make sense and are thus omitted from the analysis. The concept of a large tattoo is also not applied.

Text box 1. The description of the exposure scenario in (JRC, 2016b) (5.2 Exposure). (For references see (JRC, 2016b))

The level of exposure to chemicals due to the presence of tattoos depends on several factors, among which the quantity of inks injected in the derma and the number and size of the tattoo(s).

The quantity of pigment used for performing a tattoo has been experimentally evaluated and described in the literature [77]. Just after the application, on average around 2.53 mg of pigment were present in 1 cm² of skin. This would mean that for a tattoo of about 400 cm², the skin contains a total amount of 1 g of pigment.

The size of a tattoo can greatly vary and in the literature different classifications exist and various units are used (Table 5.5) [10, 13, 16, 17, 56, 70, 71, 77-79]. The Belgian "Conseil Supérieur de la Santé" (CSS) reported that a tattoo covering one arm, the back or the entire body is about 800, 4500 and 16400 cm2, respectively. Tattoos can broadly be divided into small, medium and large according to their area.

An internet survey with 3411 participants [70] showed that most tattooed German people (61%) have tattoos bigger than 300 cm² (16% even larger than 900 cm2), while in Denmark and the United States tattoos are smaller than 182 cm² in about 70% of cases according to studies with less than 350 participants [56, 80]. Regarding the difference between genders, tattoos in women tend to be smaller than in men, usually smaller than 182 cm², both in Europe and in the US.

In the general tattooed population more than 50% usually have their tattoos placed on the extremities, followed by the trunk and by the head/neck, which generally represent less than 5%. Localisation seems to depend on gender and women more often tattoo Country Age (Years) Frequency (%) In Europe Denmark their trunk compared to men who rather do it on their extremities (arms and legs) [56, 67, 70, 71, 74, 78]

Apart from few exceptions, both data from the questionnaires and from the literature showed that at least half of the tattooed people have more than one tattoo. No clear trend related to gender can be derived from the data available.

However, according to the biggest study [70] the majority of women and men have 2-3, or 4 and more tattoos respectively.

Table 166. Size of tattoos as described in JRC et al. (JRC, 2016b) (5.2 Exposure).

Ranges	Surface (cm^2)	Location	Surface (cm^2)
Small	<30	Arm	800
Medium	30 - 300	Back	4500
Large	>300	Entire body	16400

Size of a normal tattoo

The tattoo artists were asked to describe the size of a "normal" or "typical" tattoo.

In total 51 tattoo artists replied to this question. Four replies described the size qualitatively. In replies where ranges were given the average was reported here. In one reply an area of up to 10.000 cm^2 was given. This is considered an outlier, since the rest of the replies are within the range of 10 to 450 cm^2 and it is assumed that the question was not correctly understood.

The results are presented in Figure 7. The average area was 176.7 cm^2. The median is found to be 150 cm^2.

The four tattoo artist, who gave qualitative replies, stated the following:

- *"It vary so much, I can't tell"*
- *"I mostly tattoo whole arms, legs or bodies in sessions of 2-3 hours and 1-2 times a month"*
- *"I basically only tattoo the whole arm or back in stages"*
- *"A normal tattoo is probably something in-between something that takes a whole day (6-8 hours and something that is relatively quickly done (around 45 minutes), so I would say a normal tattoo is 3 hours"*

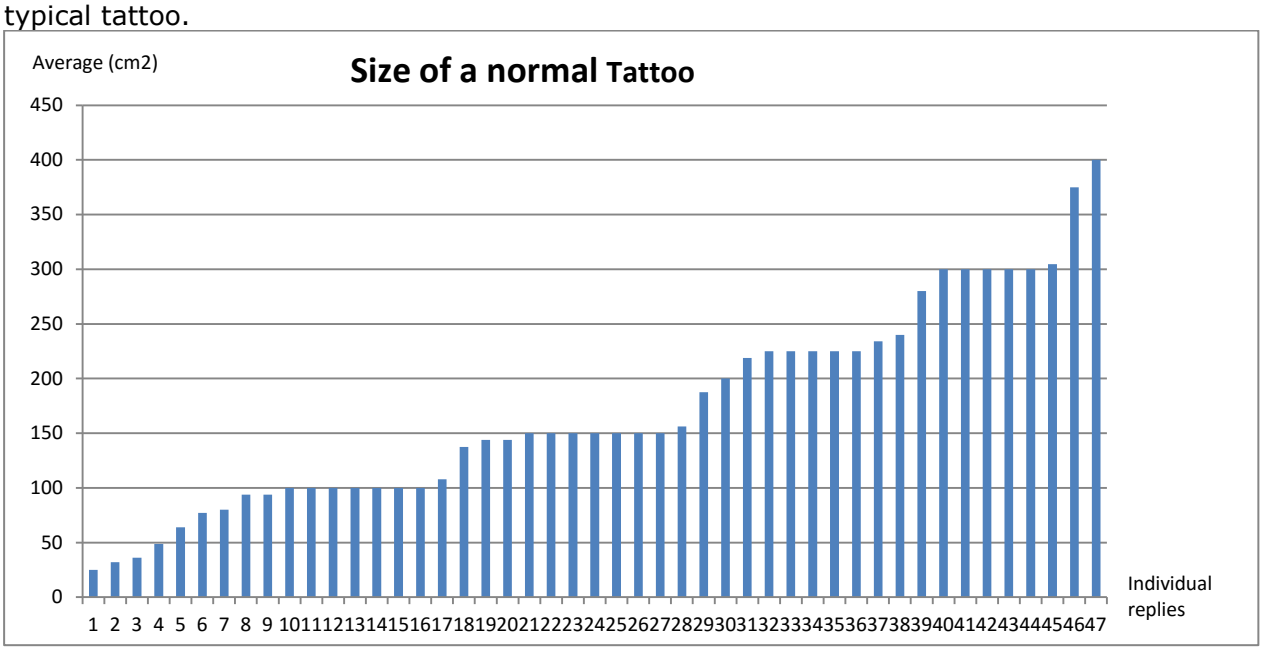
Further information was provided in some replies:

- *"Ca. the size of the forearm H = 30 cm and B = 15 cm"*
- *"It depends on what you are specialized in. If you take the average size then perhaps the ones produced the most are H = 6 and B = 6"*

When this result were presented to the tattoo artists they explained that many clients have small tattoos (10 x 15 cm) made, but that it is also very popular to have larger body parts such as an arm or lower leg tattooed. The larger tattoos are normally performed in more than one session over a longer time period. Further, they explained that while the experienced tattoo artists often make the larger tattoos apprentice make several small ones that the experienced artist supervise. Thus, when asked the size of a normal tattoo the experienced artist and the apprentice would give different replies. However, the artist confirmed that when looking at the number of tattoos made, the small tattoos would outnumber the tattooing of large body parts.

The replies to the question on the size of a normal tattoo (see Figure 7) did not distinguish between a small tattoos and the process of having a larger tattoo, since both are considered as normal. However, the intention of the question was not to catch the situation where a larger body part is tattooed, since this is addressed in other questions in the questionnaire. Rather, the information intended to obtain was the size of small tattoos frequently made.

Figure 7. The individual replies on the question concerning the size in cm^2 of a normal or typical tattoo.



In order to get an average number for a small tattoo, tattoos larger than the arm of an adult woman were excluded. Typically, an arm of an adult male 21+ years (see table x) is in average 314 cm^2 and a leg is 682 cm^2. For an adult female 21+ years an arm is in average 237 cm^2 and a leg is 598 cm^2. Thus all replies above 237 cm^2 were excluded (thus 11 replies were excluded). This results in an average size of 138 cm^2 for a small tattoo. Due to the uncertainties this number is rounded to 140 cm^2.

This assumption about the higher frequency of the small tattoos correspond with the findings of (JRC, 2016b), who found that in Denmark and the USA 70% of the tattooed population has a tattoo smaller than 182 cm^2. However, in Germany the same report states that 61% of the tattooed population had a tattoo bigger than 300 cm^2, which illustrate how popular tattooing larger body parts are becoming.

Several tattoos – repeated tattooing

The tattoo artists were asked about the percentage of customers that returns for another tattoo. In total 51 tattoo artist replied. The results are given in Table 170.

The majority of tattoo artist (74.5%) experience that around 75% or more than 75% of their clients come back to have more tattoos.

Thus, it can be assumed that it is normal that the clients come back for more tattoos. However, in the questionnaire it was not specified if the client comes back to have more small tattoos, to have a larger area tattooed in several sessions or to have a minor correction of the tattoo, which the tattoo artist explained, is normal praxis.

However, based on the results repeated tattooing is assumed to be normal.

Table 167. The percentage of customers that returns for another tattoo.

How many of your customers – approximately - return for another tattoo?	Responses	Percent
Ca. 25%	3	5.9%
Ca. 50%	9	17.6%
Ca. 75%	21	41.2%
Over 75%	17	33.3%
Don't know	1	2.0%

Time interval between tattoos for a "normal" costumer

The tattoo artists were asked about the most frequent time interval between tattoos for a normal customer. In total 51 tattoo artist replied. Only one reply was qualitative. In replies where ranges were given the average was applied. One tattoo artist replies 3-5 years whereas the rest of the replies lie within 1 to 15 months. The results are given in Figure 8.

The qualitative reply was:

- *"Today, it is quite normal to have a very big tattoo"*

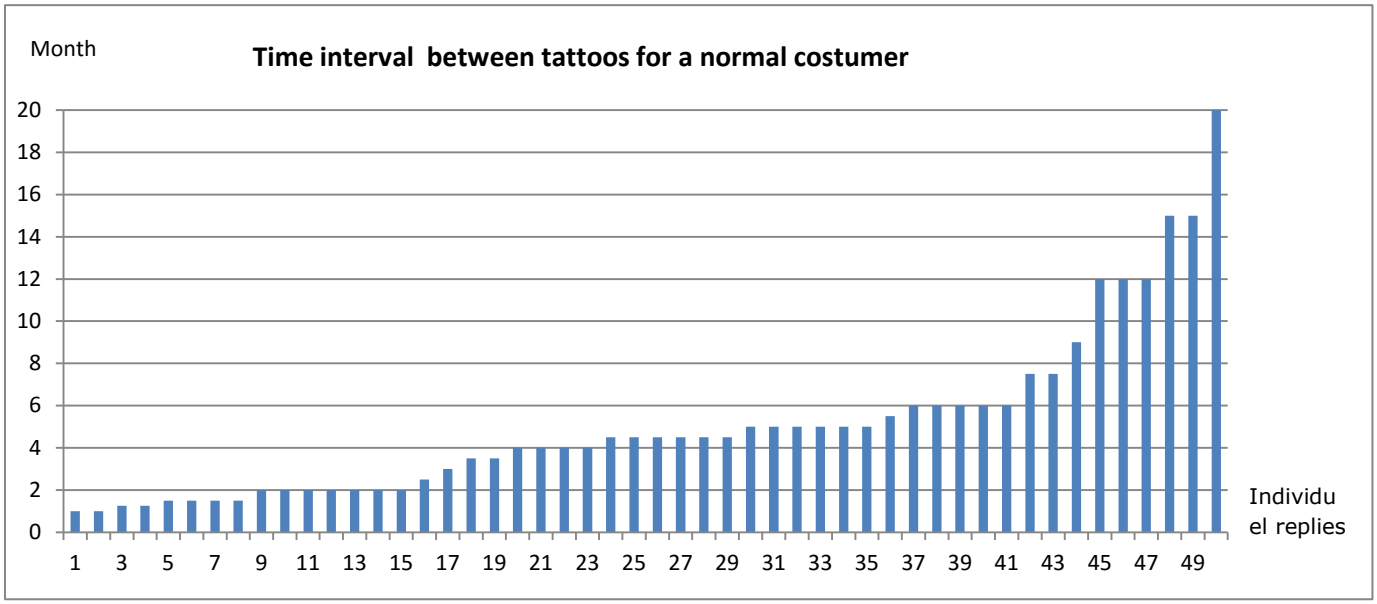
Further information was provided:

- *"1.5 month since I do not want to tattoo my customers too often. I am personally convinced that the body will think it is an attack from the outside"*
- *With larger projects 1 month, with single pieces in average 1 year.*

When this result was discussed with the tattoo artist, the tattoo artist explained that in the case where the costumer is having a large tattoo they will typically come back e.g. once a month for a year to complete the whole tattoo, whereas for smaller tattoos that are finished within one session there is typically a longer period in-between the costumer comes back to have a new tattoo. Further, a third possibility is when the costumer comes back to have a minor correction of the tattoo. This is normally done within 6 month after the tattoo is made. These values will be applied in the development of the scenario.

However, in the questionnaire these possibilities were not specified. However, the time intervals given in the questionnaire fits into the various possibilities as explained by the tattoo artists.

Figure 8. The time interval in moths between typical tattoos.



Redrawing and corrections of tattoos

The tattoo artists were asked if there typically would be a need to redraw a normal or typical tattoo. In total there were 51 replies.

Table 168. Tattoo artists that redraw tattoos

Is there a need to refresh a normal tattoo?	**replies**	**percent**
Yes	13	25.5%
No	36	70.6%
Don’t know	2	3.9%

The result shows a disagreement among the tattoo artist since ca. 25% says yes and ca. 70% says no. When the tattoo artists were asked to validate the result they explained that after a tattoo is made they normally offer the costumer the possibility to come back and have their tattoo checked. This normally takes place 2-6 month after the tattoo is made. During the check the tattoo is corrected in case minor errors have occurred. Normally, for a

skilled tattoo artist the corrections are tiny. It is thus not to be seen as a redrawing. The artist also informed us that an actual redrawing of the tattoo due to the tattoo being faded normally takes place 20 to 25 years after it is made.

However, in the questionnaire this was not specified.

This validation also explains the replies given when the tattoo artist were asked about the period for having a tattoo redrawn or refreshed. In total 47 tattoo artist replied to this question. Four replied that their tattoos should never be redrawn. 3 replied qualitatively.

The qualitative replies clearly referred to the situation of small correction following shortly after the tattoo is made:

- *"Minus"*
- *"It is not possible to answer it depends on the client, the tattoo artist, the skin and the caretaking of the tattoo"*
- *"Prefer to see the tattoo when it has healed to see if there is a need for redrawing, since some inks tends to disappear in the healing process"*

Besides, some added additional information:

- *"1 month if there is an error. Otherwise newer"*
- *"2 month to tighten everything, not a redrawing as such"*
- *"As a minimum 3 weeks and within 3 month. It is always included in the price by me. It is important to see the work after 3 weeks, so that both the costumer and I are satisfied. It takes one year before the tattoo has settled. And no sun in 1 year otherwise it will fade 50%. I have tested it. Sunscreens don't work. Dark textiles are needed."*
- *"If it must, then not until 8 weeks after! AT THE EARLIGST"*
- *"It depends on how much the costumer has healed and if it needs. No-one is interested in more ink in the skin than necessary. From 3 weeks to 3 months if it has to be "free" and has a mistake or if it has lost the colours. A normal tattoo only very seldom needs to be redrawn - if ever – if the artist know what he/she is doing. And if we talk about many years, and normal wear and renewal of the skin – again different from person to person – but if the work is done properly there is no need for redrawing."*
- *"If there are mistakes, 6-8 weeks after it is made"*

Using 6 month as a limit, it is estimated that beside the 4, who replied that their tattoos should never be redrawn, 7 replies related to an actual redrawing after several years and 33 replies related to the small corrections of newly made tattoos. For the correction of minor mistakes an average period of 2.3 months was found, with a median of 2 months.

Figure 9. The time interval in months between having the tattoo made and having it checked.

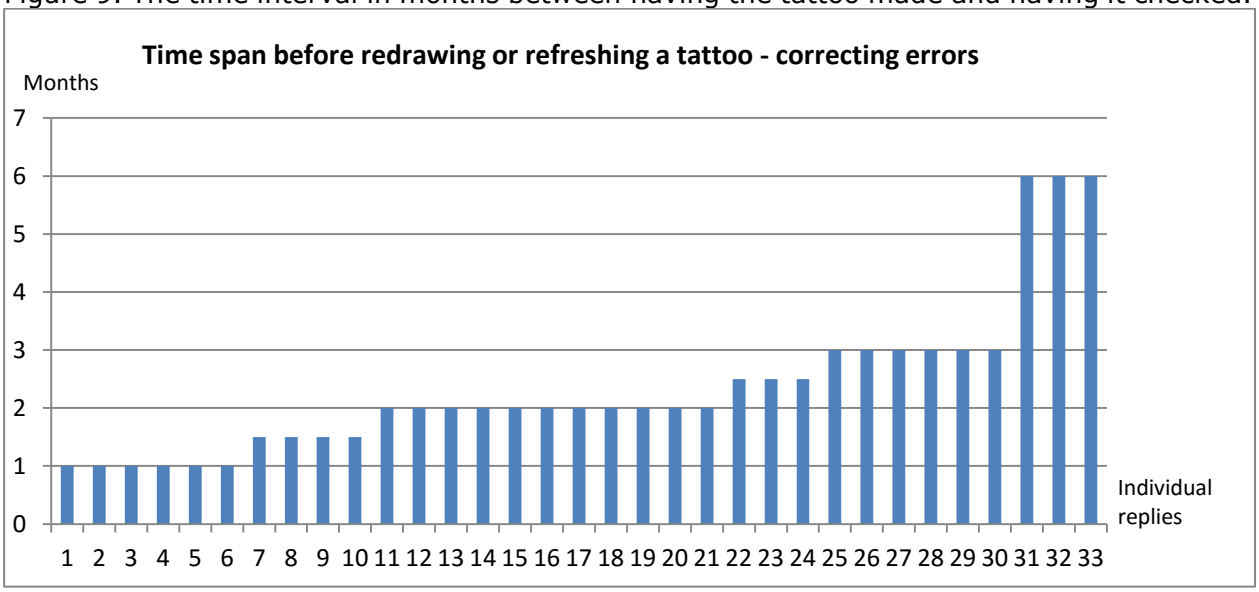
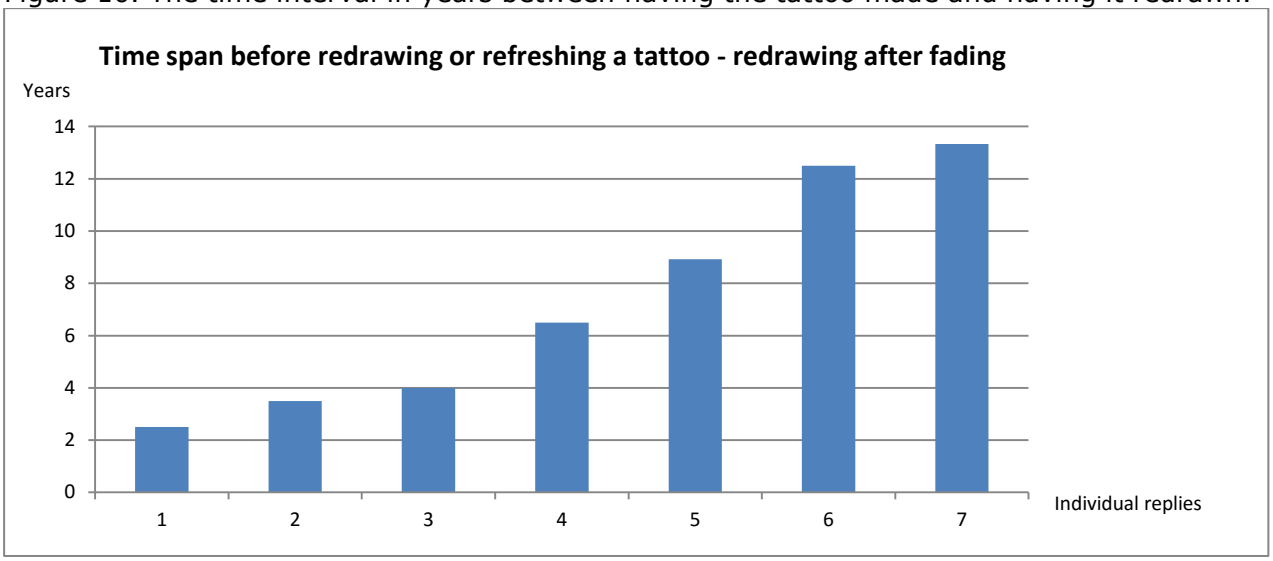


Figure 10. The time interval in years between having the tattoo made and having it redrawn.



Maximum area in one session

The tattoo artists were asked how large an area they could tattoo in one session. There were 44 replies. 5 replied qualitatively. One only indicated the height. In replies where ranges were given the average was applied.

The qualitative replies given:

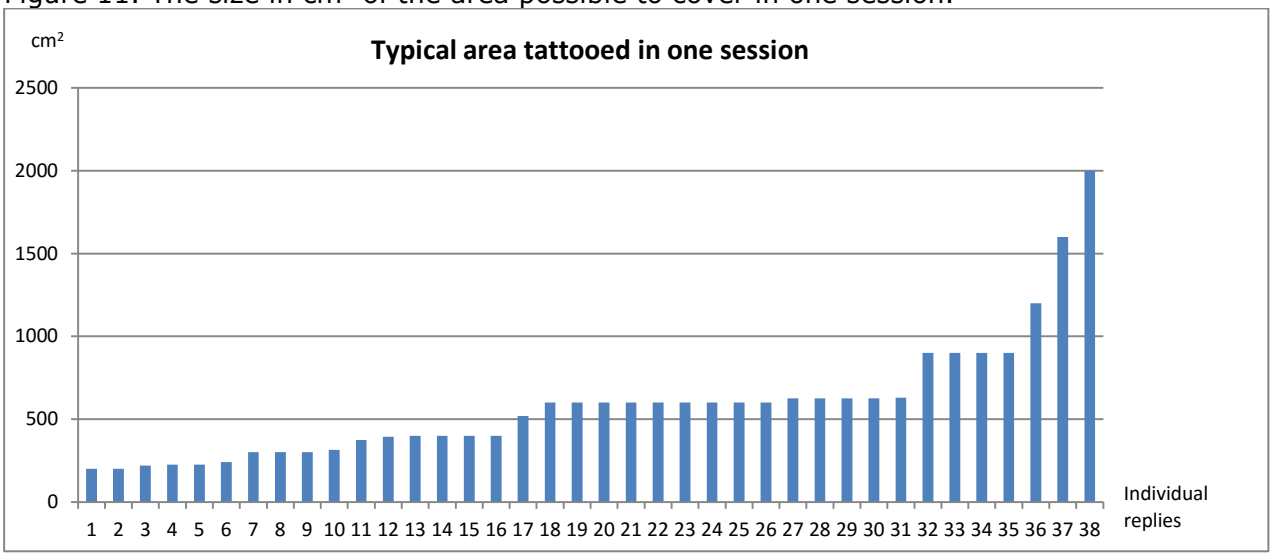
- *"It depends on the motive, details, the client and many other factors"*
- *"?"*
- *"Depends on the tattoo sometimes the full tattoo sometime only half a tattoo."*
- *"ca. 8 hours in the chair"*
- *"4-5 hours"*

Further information was provided:

- *"25x25 cm depending on the details"*
- *"30x30 and maybe more depending on what the skin can stand"*
- *"6 hours, it depends again on the costumer/the skin/the pain and not the least the style of the tattoo – can make a whole arm with a tribal tattoo in one session or 10x15 cm in photorealistic.. but around 6 hours – ca. 20 x 30, but I am fast"*
- *"Depends on the details but around 20x10 cm*
- *A coloured and detailed tattoo (one session = 4 hours: 15x15 cm). A black and simple tattoo (one session = 4 hours: 25x25 cm)*
- *A size of 30x20 cm can be made in one session if it is black and grey, is it full colours it will take two sessions.*

The average height was found to 23.4 cm and the average with to 24.6 cm. The average area was 588.0 cm^2, with a median of 600 cm^2.

Figure 11. The size in cm^2 of the area possible to cover in one session.



When this result was validated by the tattoo artists they said that the reply would be very depended on how much the area within the tattoo that is coloured. If the area is fully covered with colour the tattoo would be much more time consuming than if it was more simple tattoo such as for example the text of a poem. Thus for a full colour tattoo it is assumed that the area that can be tattooed is only ca. 300 cm^2.

Completion of a large tattoo in a sequence of sessions

The tattoo artists were asked how many sessions they would normally need to complete a large tattoo. In total 16 tattoo artist replied to this question.

Table 169. The number of sessions needed to complete a large tattoo

Number of sessions and hours	**number**
1 session of 4 hours	3
1 session of 6-8 hours	1
1-2 sessions	1
1-2 sessions of 8 hours	1
1-2 sessions of 2-3 hours	1
2 sessions of 4 hours	1
3 sessions	1
3 sessions of 6 hours	1
3-6 sessions of 6 hours	1
8 sessions of 2,5 hours	1
8 sessions of 4 hours or 4 sessions of 8 hours	1
10 sessions of 3-5 hours	1
Don’t know	1
Depends on the client	1
Total	**16**

When the replies were validated the tattoo artist explained that - as for the size of the tattoo to be completed in one session - the reply depends on the tattoo. Thus it can be assumed that if the area is to be filled with ink the scenario of several sessions should be applied. Thus several sessions for a large tattoo can be assumed. According to the questionnaire, up to 10 sessions can be the case.

Further, the tattoo artist were asked how many days they would need in-between the sessions. In total 19 tattoo artist replied to this question. Two replied qualitatively.

Table 170. The number of days in-between two sessions

Number of days in-between sessions	**Number of replies**
14	3
14 - 21	1
14 - 30	1
21	2
21 - 28	1
30	5
35	1
42	1
2 sessions	1
Minimum 21 days – never less and preferently longer so that the skin can heal and just so that it is possible to penetrate the skin	1
It depend on the size and the details in the motive, which can vary	1
If it is a tattoo of ca. 30x20cm in full colour it has to be done in two session of 6-7 hours and there has to be 25-30 days in-between for the skin to stabilize	1
Total	**19**

The replies varied from 2 to 6 weeks. In average the tattoo artist think there should be 25 days between two tattoo sessions.

However, when this result was presented to the tattoo artist, the artist explained that it depends on in how close you tattoo to the tattooed area of the first session.

One artist also explained that if a customer flies in from another country, he will e.g. make 4 or 5 session in the following days in order to complete an arm or a leg. The customer would then naturally rest for a longer period before being tattooed again.

Another explained that after having a large tattoo it is easy to feel if the body can handle more tattooing. If the body is not ready the customers distinctly reject continuing the tattooing.

<u>The length of a session</u>

The tattoo artists were asked according to their experience what would be the most important factor influencing how much it would be possible to tattoo in one session, such as pain, tattoo artist capacity, how much the skin can take etc. In total 43 tattoo artist replied. Some indicated more than one factor. The skin capacity and the pain for the consumers appear to be the most important factors for the lengths of a tattoo session.

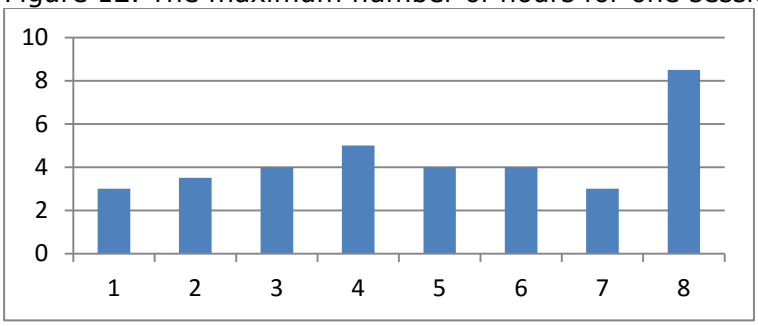
Table 171. Important factors for how much a tattoo artist can tattoo in one session

Skin capacity	21
Pain for the customer	17
Physical capacity of the tattoo artist/ability to concentrate of the tattoo artist	8
The well-being of the customer	6
Costumer is satiated (not hungry)	4
CustomersCustomers health	2
The colour of the ink	2
The location of the tattoo and the skin type	2
The economy of the tattoo artist	1
The motivation of the tattoo artist	1
The colour density	1

Some also wrote how many hours it normally would take (see Figure 12). The indicated hours are in general a little lower than the number of hours indicated under the former questions concerning the completion of a larger tattoo in a sequence of sessions.

When this result was validated the tattoo artists indicated that the normal time for a session would be around 4.5 hours.

Figure 12. The maximum number of hours for one session.



Time for healing

The tattoo artists were asked how long time it takes for a tattoo to heal. In total 44 tattoo artists replied. One answered qualitatively. When an interval was given the average is applied.

When the result from the question was validated by the tattoo artist it became clear that there were three answers to the question. This was confirmed by the additional information given in the replies. The artists explained that after a tattoo a scab/crust is created. This falls of after ca. 1 week. The skin continues the healing process and looks fine and healed after 4 to 6 weeks. However, the skin has not fully recovered after the tattooing until 6-12 month.

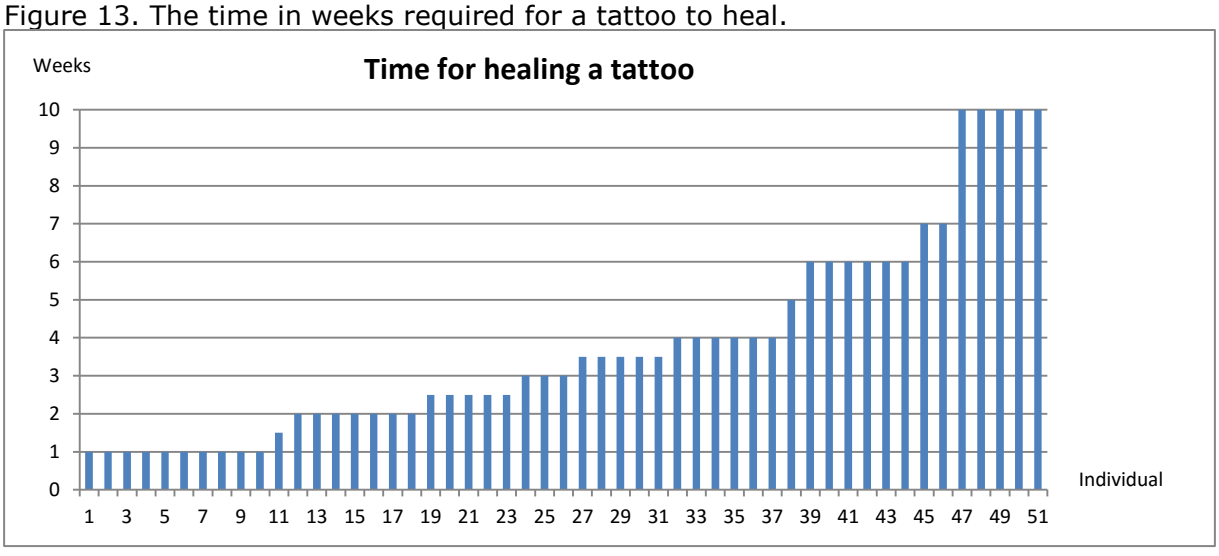
The qualitative reply:

- *"It depends on the client"*

Additional information was given in the replies:

- *"1 year to fully heal. The wound heals in 5 to 8 days using Pantalon ointment."*
- *"3 month to fully heal"*
- *"7 days for the scab/crust to fall off and ca. 1 month before the skin is normal"*
- *"The skin heals form the outside and in. The surface is healed after ca. 14 days but not until after 6 weeks is it complete"*
- *"In total 6 weeks. Scalps fall of after 4 to 5 days."*
- *"The surface is healed in 10 t0 20 days and fully healing after 30-50 days.*
- *"Surface heals in 3-4 weeks. The skin is first 100% healed after 1 year."*
- *"The scab/crust falls off after max. 1 week. Fully healed in 1 month."*
- *"The surface or fully healed? There is a big difference depending on the aftercare chosen; it can look fully healed after 2-3 weeks. However, the wound is only healed superficially and the skin still works beneath and can still be damaged. The skin is actually not fully recovered until years after the tattooing. The tattooed skin is more sensitive to sun, heat and cold the first 2 years after tattooing.. in general one would say the tattoo is healed after 3 month."*
- *"Surface healing 7-10 days. Total healing 5-8 weeks."*
- *"Surface healing 10-12 days, before I have to tattoo the same area I have to wait minimum 6 weeks"*
- *"Surface healing 5-8 days, fully 14-18 days."*

Figure 13. The time in weeks required for a tattoo to heal.



Thus the answers are understood to fall into three different groups one relating to the immediate wound and scab/crust, one group relating to the first healing and one to the fully healed skin. As can also be seen from the replies with additional information some replies gave information about more than one stage of the healing.

Time span in-between tattoos

The tattoo artists were asked what time span would be required as a minimum between a customer having two tatto0s. In total 43 tattoo artists replied to the question. 5 gave a qualitative reply.

Qualitative replies:

"Depends on several things, same place on the body or different place"

"It is up to the costumer as long as you do not have to tattoo on top of a brand new tattoo"

"It depends on the placing and size etc. Not possible to answer"

"Depends on the area"

"It is up to the costumer"

Also addition information was provided:

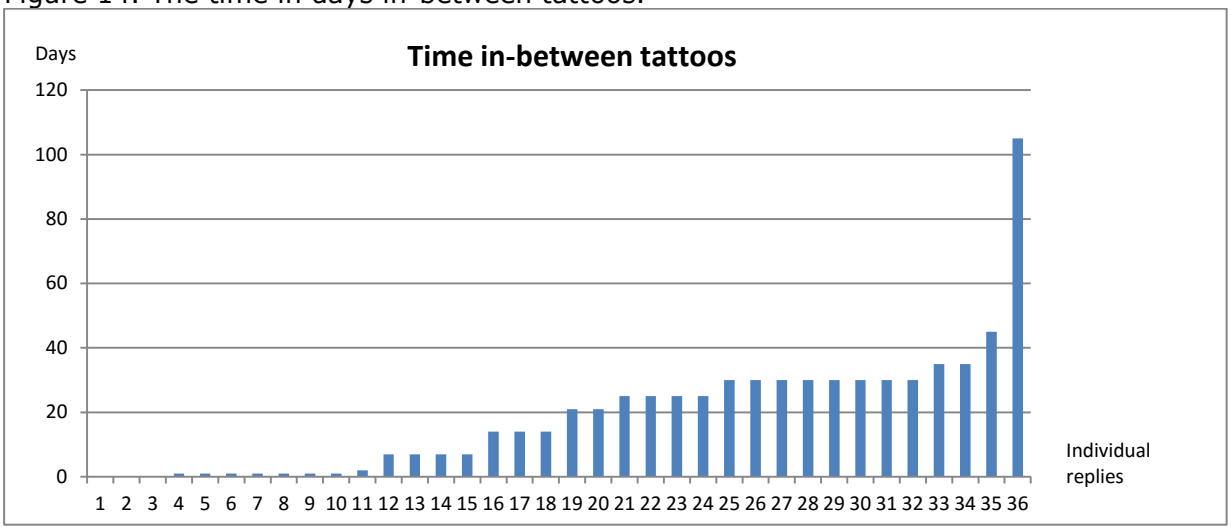
- *"3 month between two individual tattoos and 3 weeks between sessions when doing a larger tattoo"*
- *"The next day is fine as long as it is another place on the body"*
- *"Depends on the size of the tattoo and the general health of the costumer. Is it a small tattoo then 2 days are fine. For a normal size 3-4 weeks and large tattoos 2 moths."*
- *"When the surface is healed, you can make a tattoo another place. If you have to tattoo the same place there should at least be 6 weeks and sometimes more."*
- *"Different places no lower limit. Same place minimum 14 days"*
- *Same place 14 days. Different places 7 days"*
- *I always recommend AT LEAST 3 weeks so the body can recover and of course if possible even longer. It depends on the general health and healing process of the costumer"*
- *It depends on if it is the same area. If it is two different places it can be done right away. If it is the same area there must be 3-4 weeks in-between.*

When the result from the question was validated by the tattoo artist it became clear that the question had been somewhat unclear. If the question is, what is the timespan required as a minimum between two tattoos on different parts of the body? Then the reply is none. However, if the question relate to the same place then the reply is 4-6 weeks.

In the replies it is possible to see that some artists have clearly understood the questions as related to different parts of the body and given the reply zero or one day. Some artists still considering the questions as related to different parts of the body still recommend 2-3 weeks in order for the body to recover. Considering the replies to the next question on cover up, those who did not relate the question to different parts of the body understood the question as relating to minor corrections or continued work on a large tattoo, since they gave replies that were 3 weeks or more.

When validated by the tattoo artist they again said that a normal scenario is that the customer comes once a month during a year in order to complete a larger tattoo such as the leg or the back. Further they explained that it is also related to people get their salary so that they can afford the next piece of the tattoo.

Figure 14. The time in days in-between tattoos.



Cover up tattoos

To cover up a tattoo means to make a new tattoo on top of the old one. The tattoo artists were asked how much time is required as a minimum from having a tattoo to having it covered up. The results are shown in Figure 15. In total 44 tattoo artists replied. 10 replies were qualitative. The average is 6 month.

Qualitative responses:

- *"?"*
- *"?"*
- *"The tattoo must as a minimum be fully healed before you can go into the skin and change the pigments again."*
- *"A cover-up tattoo is typically made on top of an old tattoo that is damaged from the sun and thus most of the colour would have disappeared. New tattoos can be difficult and sometimes impossible to cover-up. If a new tattoo has to be covered, sometimes they have to be treated with laser first."*
- *"I do not have experience with cover-up tattooing"*
- *"It depends on how deep the ink lies, how new or old the tattoo is and other factors. There is a huge preparation in cover ups (making the right drawing etc.) .."*
- *"It is hard to say. It is individual"*
- *"As long as possible"*
- *"The tattoo should as a minimum be fully healed before it can be tattooed again"*
- *"Preferably several years but it can vary according to the motive and if the skin is damaged."*

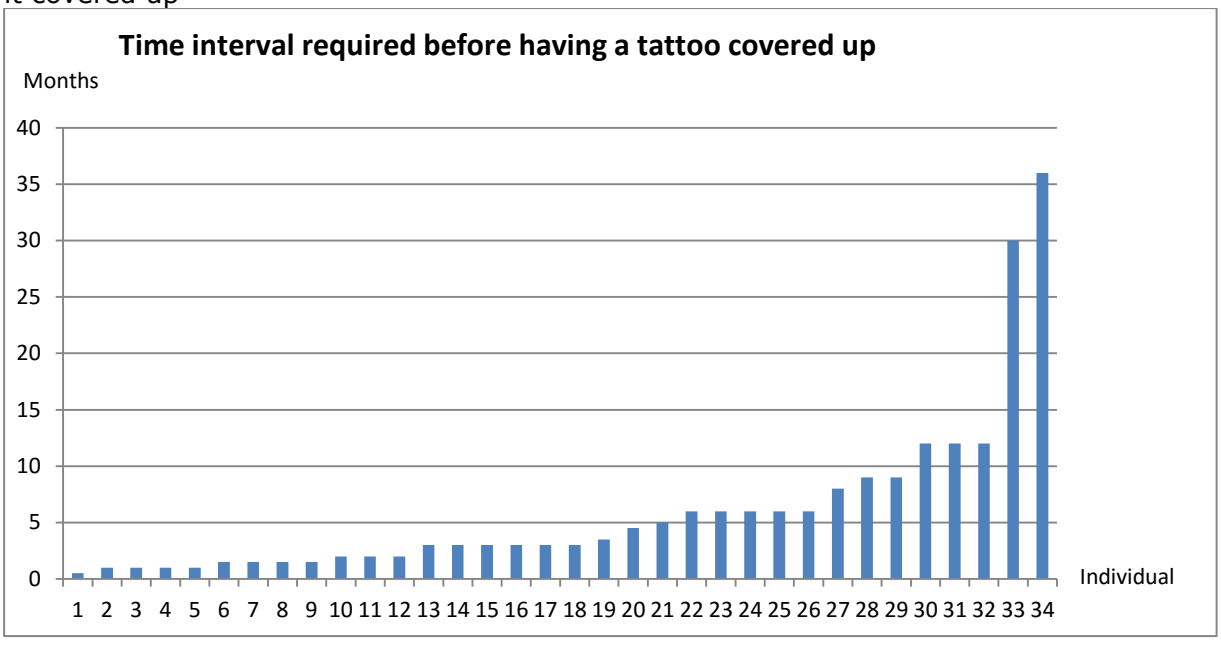
Further, additional information was given:

- *"2 month, but is has to be estimated based on the individual tattoos"*
- *"3-4 months depending on the skin type and healing process"*
- *"It depends on the motive that has to be covered, but preferable 2-3 years so the tattoo is more faded out. However, it depends just as much on the tattoo artist that*

performed the tattoo. If it is a good tattoo artist who has made some good and regular lines then it is no-go no-matter age of the tattoo – unless we are talking 20 – 30 years."

- *"It depends on the skin. As a minimum 3 months otherwise laser treatment would be preferred before making a new tattoo."*
- *"The older the tattoo is the easier it is to cover. Minimum 6 month. If it is just a line maybe 2-3 month is possible."*
- *"It depends on how it is made and it has to be healed. Somewhere between 6 month and one year."*
- *"It depends on what you want to cover up, how the tattoo is placed in the skin, if there are any scars, if it is a small or large tattoo. It can be anything from 4 month to one year."*
- *"It depends on the size of the tattoo etc. and if there are scars due to tattooing errors. If it is only lines maybe 3-4 month is enough. If the tattoo has shadows 6-7 month is required and for a full coloured tattoo 8- 10 months is required. However, it also depends on the brand of the ink (the producer). Sometimes the ink of different producers can't be used in the same tattoo including cover ups.. this may cause an allergic reaction."*
- *"I would say minimum 3 month in order for the skin to recover otherwise you make scars on scars. The skin needs time and peace to recover and depending on how tuff (ugly, scar, bad making, bad healing) the tattoo is it may require more time."*

Figure 15. The minimum required time interval in months between having a tattoo and having it covered up



At the meeting the tattoo artist validated this number and said that hopefully no-one need a cover up before the tattoo is fainting i.e. ca. 20 years. However, in principle a cover up can be performed when the skin has fully recovered from the tattoo i.e. from around 6 months. However, a fully coloured tattoo would require a longer period than a tattoo consisting of a

few lines. This is consistent with the qualitative replies and the additional information provided in the questionnaire.

The artist also explained that is quite normal that people have had a tattoo on their holyday, which they regret and then they like to have a cover up of that tattoo. However, cover ups may be complicated since the tattoo artist does not know what ink has been applied and the depth of the tattoo.

Other issue

The tattoo artists were asked if there were any circumstances that should be taking into account when trying to establish an exposure scenario for tattoo inks. In total 36 tattoo artist replied. Some replies did not provide much information, such as, ”I do not understand the question”, ”not” or “I think you are doing a good job”, these have been omitted here. However, many of the replies do point in the same direction as has been concluded from the other questions and the validations by the tattoo artist.

- *“It is almost impossible to define a normal size tattoo. Many tattoo artists only makes whole days sessions on one customer, whereas apprentice often makes many small tattoos and may thus have 5 to 7 customers a day. So if normal means the most, then the small ones becomes the normal”*
- *“There is a large difference in how fast tattoo artists work. A large tattoo can take from 10 to 40 hours - even with the same motive - depending on who makes it. Thus there may be different perception on what would qualify as a large tattoo”*
- *“There is a large difference between tattoo artists that work professionally and those who work as amateurs. Also customers are different.”*
- *“There can be operations before and after a tattoo, that has to be taken into account. E.g. for how long should you wait after an operation and how shortly before an operation can you have a tattoo”*
- *“Half of the tattoo artists operating today has not had any apprenticeship and thus have bad habits. They do not care about hygiene; buy cheap equipment on the internet as well as inks and needles. Everyone can buy tattoo equipment on the internet and everyone can open a shop without any security for the customers. Something has to be done.”*
- *“NO-ONE gets tattooed every day. Your way of addressing a “large tattoo” is ridiculous.”*
- *“Many of the questions you ask are impossible to answer, in particular the questions on large tattoos. Most large tattoos are adapted to the costumer and the tattoo artist, thus the colour density, the size, the time etc. is individual. You have to use 6 hours for a portrait the size of a hand as well as for lines covering the whole body. It all depends on the motive.”*
- *“There is a large difference on the styles applied and the time it takes. That makes it hard to answer your questions.”*
- *“It is very hard to estimate the time consumption - for example how long can a session be, how long does it takes for a tattoo to heal etc. because it vary from person to person.”*

- *"It is different from person to person how they react on a tattoo and how the tattoos heal up. In most cases it is hard to generalize and have uniform guidelines."*
- *"The healing process is important because itching and irritation is often misinterpreted as a negative health effect but actually it is just the normal healing process just like for any other wound."*
- *"Yes, please be aware of cover up tattoos. Everything depends on the size and the colour density – and the motive and if there are scars in the tattoo.*
- *"Yes, the experience of the tattoo artist counts. Concerning healing and what the customers can handle please go to the tattoo shop and have a talk with the tattoo artist, and then it is much easier to explain. Also you can follow the web page ut.aiden.."*
- *"It is not possible to put the process into a table since all the questions need detailed explanations depending on various factors and the customers. Hygiene, ink, performance and environment. Is the tattoo done by an experienced professional educated by another tattoo artist or is it an entrepreneur doing it at home. Copies of inks and equipment. Distributors that sells to private."*
- *"Yes, for example it is typical for my style that for the large projects I would make all the lines in the first section. In that session the areas that are coloured are much smaller than the size of the tattoo. I am aware that the immune system should not be burdened unnecessarily thus I tell the client to be rested, having eaten and drinking enough liquid. It all influence for how long you can tattoo a person but also the healing time."*
- *"One tattoo scenario cannot be done, since the scenarios are just as different as the tattoos."*
- *"I have observed that many tattoo artists tattoo to deep. Some customers talk about the artist being absentminded and that the service stops as soon as the tattoo is done"*
- *"The chemistry of the various types of ink. Itching can occur if inks from different producers are mixed. It does not happen very often, but there is a risk."*
- *"You can't say how long it takes to make a large tattoo since it varies depending on the skin of the customer and an artistic tattoo artist like me makes new art each time. Thus the question cannot be answered."*
- *"You need to use your common sense. We are individuals and manage differently during the tattoo process. It all depends on your blood sugar. The higher your blood sugar the better you can cope the tattoo process."*
- *"We are working with humans and skin and it is difficult to generalize."*
- *"Size is broad concept. I do not think that it can be measured like you are trying to do. A tattoo of 10x10 cm can be densely packed with ink or it can be open with many free areas"*
- *"Tattoos are very popular in periods. The customers gets tattoo done in periods and then often several years passes (5-10 years), where they do not get any tattoo, and then they may start again. As a tattoo artist I think that the normal costumer will not*

be exposed to tattoo inks on a regular basis through-out a lifespan. Many just have 2 or 3 tattoos and many just did it when they were young."

- *"Be aware about tattoos on scars, such as when it should be allowed to tattoo on scars from operations, self-induced scars, wounds and scars from accidents."*
- *"Tattoo artist that has not had the proper teaching in an apprenticeship from another experience tattoo artist they do not know where the inks comes from and this can result in some of the scenarios seen some years ago with car paint etc. being applied."*
- *"There is such a big difference in how different artist work. And a lot of these scenarios will be answered different from artist to artist and not only one way is the right one. But the problem is that there are far worse and untalented artists in the business then there are good ones. Artists that haven't had the proper training or experience. This means that how a perfect tattoo scenario is supposed to go with minimum damage to the skin, good healing and thereby a good long-lasting tattoo is rare. Only some can live up to standardised scenarios like these. And if these scenarios you are developing are made out of the idea of how not to do it properly, just to fit as many artists as possible and that's the standard, it's worthless."*
- *"There is a big difference on the experience of the tattoo artist and there is also a difference between tattoo artists depending on whether they are owner of the shop or whether they are hired on a permanent basis or paid by the hour. Those paid by the hour may be more slow and may also not take so much care of the costumer and the skin and may thus harm the skin with infections, since the tattoo process will be to violent. Unfortunately, we experience customers that tell that a tattoo artist paid by the hour has made a tattoo where there are serious infections and where we can judge from the size that too much time has been spend on the same skin area. This also counts for guest tattoo artists.*
- *"Be aware that the scenarios will be strongly dependent on the experience of the tattoo artist and how the costumer takes care of the tattoo afterwards. The questions in the questionnaire are not well suited for describing the tattoo process."*

<u>Conclusion</u>

Despite the critique of the questionnaire by the tattoo artists – (one could say that the questionnaire gave information to do a better questionnaire) some conclusions may be extracted from the replies and the validations by the tattoo artist of the replies.

It can thus be concluded that:

- Frequently made small tattoos are estimated to a size of 140 cm^2.
- Larger tattoos consist of full body parts e.g. an arm or a leg.
- Simple line or text doesn't take as much ink as full colour tattoo and is completed faster.
- Repeated tattooing appears to be the normal. Thus the customers visit the tattoo artist on a regular basis.
- Larger tattoos are often made during several sessions - once a month throughout a year appears to be normal, but this can vary - whereas small tattoos have longer intervals typically around a year or more.

- The tattoo artist frequently offer the customer to come back to have their tattoo checked where minor mistakes can be corrected. The customers normally come for corrections within a period of ca. 2 months.
- On average an area of 600 cm^2 can be made in one session. However, for a full colour tattoo (high colour density) it is estimated that the area that can be tattooed in one session is smaller. The area for a full coloured tattoo is estimated to ca. 300 cm^2.
- Depending on the image and the colour density the number of sessions needed to complete a larger tattoo varies. For a difficult motive/high colour density tattoo up to 10 sessions may be needed whereas for low colour density tattoos a larger tattoo can be completed in one session. This also depends on the experience of the tattoo artist.
- In general in average the tattoo artist recommend at least 25 days between two tattoo sessions. It is possible to continue tattooing several days in a row - though only for a short period - on different parts of the body. The timespan required between two tattoos in the same area is 4-6 weeks e.g. in one session lines can be drawn and in the next session 4-6 weeks later the areas can be coloured.
- The skin capacity and the pain for the consumers appear to be the most important factors for the lengths of a tattoo session.
- After a tattoo has been made a scab/crust is created. This falls off after ca. 1 week. The skin continues the healing process and looks fine and healed after 4 to 6 weeks. However, the skin has not fully recovered after the tattooing process until 6-12 month later.
- A cover up is a new tattoo on top of an old tattoo. There should normally as a minimum be a period of 6 month between the tattoo is made and a cover up of the tattoo. For full colour tattoos a longer period is necessary – probably approximately one year.
- After 20-30 years a tattoo may fade and a cover up may be required in order to maintain the tattoo.

Questionnaire

Questionnaire on exposure scenario for tattoo inks

Currently the European Chemical Agency (ECHA) together with Germany, Denmark, Italy and Norway are developing an EU regulation for chemical substances in tattoo ink. Many information concerning tattoos has already been collected, however knowledge on the process of tattooing is still limited.

By filling in the questionnaire you help the authorities in Europe to design a more appropriate regulation of chemical substances in tattoo inks.

The questionnaire contains three exposure scenarios, A Normal Tattoo, A Large Tattoo and Max Tattooing.

Before answering the questions on tattooing please fill in information about yourself and your experience as a tattoo artist.

Personal info

Name and contact details:

Years of experience as tattoo artist:

Do you represent other tattoo artists e.g. as owner of a shop or as representative of an organisation (Please indicate with X and if yes please describe):

No = ___

Yes = ___

Scenario "A Normal Tattoo"

These questions refer to a normal or typical situation.

1. Please estimate the normal or typical (median) size of a tattoo. Describe it as A cm^2 = H cm x W cm, where A is the area, H is the height and w is the width.

   A =

   H =

   W =

2. How often does your customer come back for having another tattoo (please indicate in %):
3. How many tattoo do your customers normally/typically have before they stop getting more tattoos:
4. Please estimate the timespan for a normal costumer between two tattoos:
5. Does a normal tattoo need to be refreshed/tattooed over (If yes, please skip question no. 3):

   No = ___ (If no, please skip question no. 6)

   Yes = ___

6. How much time after the tattooing does a tattoo need to be refreshed?
7. How long time does it take for a tattoo to heal?

Scenario "A Large Tattoo"

These questions refer to the situation where a customer gets a large tattoo. Take a moment to think what you consider a large tattoo. Perhaps it is a tattoo that covers the whole back or the shoulder and upper arm.

1. Please describe how big a tattoo, which you would consider as a large tattoo is. Please describe as A cm^2 = H cm x W cm, where A is the area, H is the height and w is the width.

   A =

   H =

   W =

2. Is it possible to complete a large tattoo in one session/on the same day? (indicate with X)

   Yes = ___

No = ___ (If no, please skip question no. 3)

3. How often would a costumer need to come back to complete the tattoo? Please indicate the typical number of sessions/days needed:

   and the typical timespan between the session/days:

4. How large an area is normally tattooed in one session/on the same day: Please describe as A cm^2 = H cm x W cm, where A is the area, H is the height and w is the width.

   A =

   H =

   W =

Scenario Max Tattooing

This scenario addresses how much it would be possible to tattoo one person. Thus we make a theoretical example where a costumer comes to the tattoo shop as often as possible and each time get as large a tattoo as possible.

1. How large a tattoo would a person be able to get in one session/on the same day? Please describe as A cm^2 = H cm x W cm, where A is the area, H is the height and w is the width.

   A =

   H =

   W =

2. Please indicate the most important factor that set the limits for the size of the tattoo in one session/on the same day. Is it relevant to consider issues as how much pain the person can cope with, how long time the tattoo artist is capable of working, how much the skin can take?
3. How short could the timespan between the sessions possibly be:
4. If a tattoo is being "tattooed over" (a tattoo on an already existing tattoo), how long time should there as a minimum be between the tattoos?

Is there something you would like the authorities to pay attention to/be aware of when describing tattooing?

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