The Danish EPA’s Risk Assessment of Hazardous Substances in Tattoo Inks based on the project, "Chemical Substances in Tattoo Ink"

Considerations concerning the methodology

In the project "chemical substances in tattoo ink"' a quantitative risk assessment has not been performed, since knowledge on uptake of the various chemical substances in tattoo ink in the body is considered to be insufficient.

In the project the risk from chemical substances in tattoo ink is treated qualitatively as real cases of local reactions in the tissue as a result of tattoos are described.

A number of the substances identified in the project, such as lead, nickel, cadmium, PAH and Anilines have however very low DN(M)EL values for systemic effects. Thus based on the currently available data and assumed conditions for uptake, the probability that exposure may cause the DN(M)EL values to be exceeded and the degree of absorption it would require are assessed.

The following describes considerations related to the uptake and other aspects of the assessment of whether there is an unacceptable increased risk using the investigated tattoo inks, ie. whether DN(M)EL is exceeded.

When performing a calculation based on the available information, and there appears to be a risk (ie. if the DN(M)EL-value is exceeded), a precautionary approach is applied in the interest of consumer safety, and despite of the uncertainties associated with the calculation, it is assumed that the ink may pose an unacceptable increased risk.

An average as well as a realistic worse case scenario is considered.. The applied realistic worst-case scenario in this assessment is based on a large tattoo of approximately 30 x 30 cm. This correspond to a tattoo on the whole back. It may also be possible that a person gets a full body tattoo, but this is normally done in a stepwise manner in order for the body to heal after getting a tattoo, the time span in which the body tattoo is being made is probably so long, that the calculation of risk in connection with this scenario would correspond to the risk of getting a large tattoo of approximately 30 x 30 cm. If it shows that an ink poses a risk in connection with a tattoo of 30 x 30 cm there might not be a risk in getting a very small tattoo, but the premise is that an ink must be usable for all sizes of tattoos.

In the project report the authors point out that due to lack of knowledge on the kinetics of the pigments in the skin it would be inadequate to say anything about the systemic uptake, i.e. absorption of the substances in the bloodstream. In other words, how much of the substance which is placed under the skin, can be distributed to body organs after absorption in the blood. One way to tackle this issue is to calculate the percentage that has to be absorbed systemically, before the critical dose is exceeded and thereby causes an unacceptable increased risk, and evaluate whether such an absorption rate would be realistic. For example if only an absorption of 5% is required to exceed the D(N)MEL there is a very high probability that the ink pose an unacceptable increased risk. If the calculations indicate that the absorption should be more than 100% (i.e. that more ink has to be used than was actually placed in the skin) the ink is considered not to pose an unacceptable increased risk, due to that substance.

If a substance has a a D(N)MEL and a calculated tolerable daily dose based on long term exposure, it could be questioned whether an actual exposure, that exceeds the D(N)MEL value e.g. during one week poses a risk. In the assessment as a basis - and for the sake of precaution – it was chosen to assume that if it is possible that a D(N)MEL on an average may be exceeded during several weeks it cannot be excluded that the ink poses a risk. Especially for the carcinogenic substances it is considered relevant to look at the average dose during several weeks and not just consider a few days of exceedance, since it is the total dose rather than a single day of exceeded value, which is most crucial for the increased cancer risk.
Concerning lead and its neurotoxic effects it is also appropriate to relate to a dose during a certain period of time e.g. a few weeks, since lead accumulates in the organism, which can build up a critical level in the body by repeated exposure.

Kommentar [doble1]: Er det ikke sådan at bly ikke nødvendigvis skal spredes ud og optages over en længere periode for at kunne påvirke kroppen?

As the basis for a tolerable level for non-threshold carcinogenic substances a DMEL value representing a 10^{-6} lifetime risk is chosen, i.e. a theoretical increased risk of one in a million during a lifetime.

In an uncomplicated process, the skin will heal from a tattoo in approximately 3-4 weeks. During this time the skin will try to reject the pigment. The only quantitative indication of absorption and degradation of tattoo ink in the report comes from mouse studies. The kinetics of pigment in the skin is here studied using Pigment Red 22 in mice. After 6 weeks, the pigment in the skin was reduced to 32%, i.e. 68% of the pigment can be considered to be transported and distributed elsewhere in the organism either as pigment or degradation products. In time most tattoos will fade. This also support the former conclusion that the pigment either is transported away from the skin or is degraded. When the pigment degrades, it is assumed that the degradation products are also transported away from the skin. When the pigment or degradation products are transported away, then, based on the precautionary approach, it is assumed that the substance is absorbed in the body and thus is systemic available. From the mouse experiment it has been observed that about 1/4 of the pigment can be found in the lymph nodes. Deposition of pigment in the lymph nodes may subsequently lead to other forms of systemic absorption/ distribution.

The experiment using Pigment Red 22 is used as the basis for the calculations. Thus in the following risk assessment the absorption is considered over a 6 week period. This also assures that when applying a D(N)MEL based on long term exposure (as mentioned earlier) the exposure is levelled out over several weeks

Since pigment is in particle form - to be visible - it may not be absorbed and transported in the same degree as the liquid matrix in which the pigment is suspended. It is therefore assumed that a large percentage of the pigment remains in the skin - otherwise you would not be able to see the tattoo - while the matrix of potential impurities and degradation products to a much higher degree will be available for absorption via the lymphatic system and bloodstream.

A total absorption of 68% of the pigment is as a worst case considered realistic, as this was the rate of elimination from the skin found in the mice study. For other more soluble substances i.e. impurities or possible degradation products a worst case absorption rate of up to 100% is considered.

With the exception of phthalocyanines and carbon black all the chemical substances measured in the tattoo inks were either impurities or degradation products.

Method of Calculation

In Table 1 the deposited amount of tattoo ink in mg/kg body weight for an average tattoo and a large tattoo are calculated based on the data given in the report, 'Chemicals substances in tattoo inks'.

Table 1

Scenario	Area for tattoo	Amount of tattoo ink deposited in the skin (mg/cm^2)	Deposited amount of tattoo ink – in total
Average tattoo	430 cm^2	2.53 mg/cm^2	**1.09 g**
Worst case tattoo	1090 cm^2	9.42 mg/cm^2	**10.3 g**

The following sections describe the hazardous substances found by analysis of tattoo ink, its critical effects, and the DN(M)EL-value as described in the report. Based on DN(M)EL-value and the above mentioned considerations, the risk from the use of the inks is evaluated.

In each section there is a calculation scheme where the calculation can be followed.

Calculation Scheme

1	2	3	4	5	6	7	8
Sub-stance	**Critical effect**	**NOAEL/ LOAEL** **mg/kg/d**	**DNEL/ DMEL** **mg/kg/d**	**Tolerable daily intake** **(x 70 kg)** **mg/d**	**Tolerable dose over 42 days** **(x 42 d)** **mg**	**Absorption required to exceed the DN(M)EL value** **(%)** **A) Average tattoo (1.09 g ink)** **B) Worst case tattoo (10.3 g ink)** **.. if the ink contained 100% of the substance**	**(Absorption required to exceed the DN(M)EL value) .. for the measured max concentration of the substance in the ink** **(%)**

In the calculations the daily N(L)OAEL or DN(M)EL dose is converted into a total tolerable dose during a period of 42 days for an adult person weighing 70kg (sixth column in the calculation scheme). At this stage in the calculation it is assumed that 100% of tattoo ink consists of the critical substance and based on a average tattoo and large tattoo (worst case) the absorption required to exceed the N(L)OAEL or DN(M)EL value is calculated. Finally, a correction is made for the actual content of the crititcal substance in the analyzed ink; the calculation is here done for the highest level found in the report (last column in the calculation scheme). If the calculated absorption rates is above 100%, this means that it will not be possible to exceed the D(N)MEL value, because it is not possible to absorb more substance than is actually applied to the skin.

Black and dark inks and content of PAH

Black is the most used ink. 11 black inks have been purchased. Black pigment is often used in dark inks. 19 inks were tested for PAH in relation to the inks containing black dye.

One ink (No. 11) contains BaP, which is classified as carcinogenic (carc. 1B). In the resolution of the European Council a limit of 0.0005 ppm is set. It is not known how the Council of Europe has derived this limit.

One ink contains relatively high levels of Naphthalene and 12 inks have relatively low contents. Five inks contain pyrene. A total of 14 inks contain PAHs. All 14 inks exceed the limit of 0.5 ppm for PAH as recommended in the resolution of the European Council. It is not known how the European Council has come to this limit. The limit for oils used in tires is 1 ppm for BaP and 10 ppm for total PAH content. The US EPA also has a list of 16 PAHs, which they judge as being harmful to the environment and thus monitor in the environment.

5 inks do not contain PAHs (2 black, 2 red and 1 purple).

BaP

In Table 2 calculations have been made for ink No. 11 (black) which contains 5.3 μg/g of BaP (5.3 ppm).

Table 2

Sub-stance	Critical effect	DNEL/ DMEL mg/kg/d	Tolerable daily intake (x 70 kg) mg/d	Tolerable dose over 42 days (x 42 d) mg	Absorption required to exceed the DN(M)EL value, (%) A) Average tattoo (1.09g ink) B) Worst case tattoo (10.3g ink)	(Absorption required to exceed the DN(M)EL value) .. for the measured max concentration of the substance in the ink (%)

					.. if the ink contained 100% of the substance	
BaP (impurity)	**Cancer**	**DMEL: 0.0000006 – 0.000005 mg/kg/d**	**0.000042 – 0.00035 mg/d**	**0.0018 – 0.0147 mg**	**A(0.0018 mg /1.09 g = 0.000002) 0.0002%** **B: (0.0018 mg /10.3 g = 0.0000002) 0.00002%**	**(5.3 ppm = 0.00053%)** **A: 34%** **B: 3.4%**

Considering the lower value from the DMEL-range the calculation results in an unacceptable increase in risk for the two tattoos only requiring an absorption of 34% and 3.4%, respectively during a 42 day period.

Naphthalene
Ink No. 3 (black) contains 81μg/g naphthalene (81 ppm). 12 inks have from 0.5 to 5.0 μg/g naphthalene (0.5 to 5.0 ppm). The report has not specifically addressed any DN(M)EL level for naphthalene. According to Annex 17 of REACH naphthalene is not prohibited in tires. Naphthalene is on the list of the 16 US EPA PAHs. The European Council generally has a limit for PAHs of 0.5 ppm. Based solely on naphthalene 13 out of 19 inks exceed the limit value in resolution of the European Council.

Pyrene
Ink No. 11 (black) contains 28 μg/g pyrene (28 ppm), ink No. 3 (black) contains 27 μg/g pyrene (27 ppm), ink No. 12 (black) contains 23 μg/g Pyrene (23 ppm), ink No. 23 (black) contains 15 μg/g Pyrene (15 ppm) and ink No. 10 (gray) contains 0.5 μg/g pyrene (0.5 ppm). The report has not specifically addressed any DN(M)EL level for pyrene. According to Annex 17 of REACH pyrene is not prohibited in tires. Pyrene is on the list of the 16 US EPA PAHs. The European Council generally has a limit for PAHs of 0.5 ppm. Based solely on pyrene 4 out of 19 inks exceed the resolution of the European Council.

Total PAH
14 inks contain PAH including BaP. The total concentration varies between 118 μg/g of PAH (118 ppm) to 0.8 μg/g PAH (0.8 ppm). A specific D(N)MEL value for PAH in total has not been set. The five highest values are at 118, 52.9, 32.8, 21.3 and 5.0 μg/g PAH. The European Council generally has a limit for PAHs of 0.5 ppm. All 14 inks containing PAHs (ie. 14 out of 19) exceed the limit of the resolution of the European Council.

Reddish inks and contents of primary aromatic amines

Red ink is the second most used ink and the ink that most often results in visible skin reactions. 12 red inks have been purchased. All red inks are analysed for relevant chemical elements and 7 inks have been analysed for primary aromatic amines (PAAs). Five of the reddish inks have given rise to reactions in the skin.

8 green inks have been purchased. 4 green inks have been analysed for relevant chemical elements and PAA.

3 purple inks have been purchased. All 3 purple inks have been analysed for relevant chemical elements and PAA. All 3 have given rise to reactions in the skin.

3 orange inks have been purchased. All 3 inks have been analysed for relevant chemical elements and 2 have been analysed for PAA.

1 brown ink has been purchased. The brown ink has been analysed for relevant chemical elements and PAA. The ink has given rise to the reaction in the skin.

A total of 19 inks have been analysed for PAA and all 19 analysed inks contain PAA. All the inks that have given reaction in the skin were reddish, i.e. red, purple or brown.

The EU-limit (in REACH) for each PAA in textiles **-** after potential present azo-dye has been degraded **-** is 30 ppm. This limit does not apply to aniline, 2-naphthylamine and 2.4-xylidine/2.6-xylidine. In the resolution of the European Council Declaration a limit-value for PAA has not been stated. The European Council Declaration only address the same PAAs as REACH, The detection limit for the PAAs is typically around 1-2 ppm.

Aniline

Ink No. 53 (red) has the highest content of aniline and contains 300 µg/g of aniline (300 ppm) (measured after possible degradation of the ink). The content of aniline was also measured before possible degradation of the azo-dyes present in the ink. The content here was 28 µg/g. Concerning this ink reaction in the skin has been observed. Aniline is classified as carc. 2. In Table 3, a calculation has been made **-** based on the DMEL value of 0.00002 mg/kg d from the report **-** on how much of the substance that has to be absorbed for it to constitute an unacceptable increased risk. It is seen that aniline generated from decomposition of the ink in a large tattoo may cause an unacceptable increased risk even when only 2% is absorbed. From the calculation it is estimated that the ink therefore may constitute an unacceptable increased risk.

Table 3.

Sub-stance	Critical effect	DNEL/ DMEL mg/kg/d	Tolerable daily intake (x 70 kg) mg/d	Tolerable dose over 42 days (x 42 d) mg	Absorption required to exceed the DN(M)EL value (%) A) Average tattoo	(Absorption required to exceed the DN(M)EL value) .. for the measured max concentration of the

					(1.09 g ink) **B) Worst case tattoo (10.3 g ink)** **.. if the ink contained 100% of the substance**	**substance in the ink** **(%)**
o-anilin (impurity, degr.)	**Cancer**	**DMEL:** **0.00002 mg/kg/d**	**0.0014 mg/d**	**0.059 mg**	**A: (0.059 mg/1.09 g = 0.00005) 0.005 %** **B: (0.059 mg/10.3 g = 0.000006) 0.0006 %**	**(300 ppm = 0.03 %)** **A: 17 %** **B: 2 %**

Ink No. 57 (brown) contains 230 µg/g of aniline (230 ppm) after possible degradation of the ink. The content of Aniline was before possible degradation of the azo-dye 79 µg/g. Reaction in the skin has been observed for this ink. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 3% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Ink No. 65 (orange) contains 110 µg/g of aniline (110 ppm) after possible degradation of the ink. The content of aniline before possible degradation of the azo-dye was not measured. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 5% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Ink No. 20 (orange) contains 56 µg/g of aniline (56 ppm) after possible degradation of the ink. The content of aniline before possible degradation of the azo-dye was not measured. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 10% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk. For an average tattoo the TDI is not exceeded at 100% absorption.

Ink No 24 (Red) contains 24 µg/g of aniline (24 ppm) after possible degradation of the ink. The content of aniline before possible degradation of the azo-dye was 3.7 µg/g. Reaction in the skin has been observed with this ink. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 25% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Colour No 48 (red) contains 11 µg/g of aniline (11 ppm) after possible degradation of the ink. The content of aniline before possible degradation of the azo-dye was below the detection limit. Reaction in the skin has been observed for this ink. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 53% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Ink No 37 (purple) contains 10 μg/g of aniline (10 ppm) after possible degradation of the ink. The content of Aniline before possible degradation of the azo-dye was not measured. Reaction in the skin has been observed for this ink. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 59% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Ink No. 35 (purple) contains 4.2 μg/g of aniline (4.2 ppm) after possible degradation of the ink. The content of Aniline before possible degradation of the azo-dye was 2 μg/g. Reaction in the skin has been observed with this ink. Even for a large tattoo when the ink is degraded, the DMEL value is not exceeded at 100% absorption. Thus the ink is considered not to pose an unacceptable increased risk of cancer due to aniline.

The inks No. 1, 5, 18, 24, 36, 60 also contains aniline, but at concentrations below 4 ppm and they are expected not pose an unacceptable increased risk of cancer due to aniline.

o-Anisidine
Ink No 26 (green) has the highest content of o-anisidine and contains 1775 μg/g of o-anisidine (1775 ppm) after possible degradation of the azo-dye. O-anisidine is calssified as carc. 1B. In Table 4, a calculation has been made - based on a DMEL value of 0.0004 mg/kg/d - of how much of the substance that has to be absorbed for it to constitute a risk. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 0.56% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Table 4.

Sub-stance	Critical effect	DNEL/ DMEL mg/kg/d	Tolerable daily intake (x 70 kg) mg/d	Tolerable dose over 42 days (x 42 d) mg	Absorption required to exceed the DN(M)EL value % A) Average tattoo (1.09g ink) B) Worst case tattoo (10.3g ink) .. if the ink contained 100% of the substance	(Absorption required to exceed the DN(M)EL value) .. for the measured max concentration of the substance in the ink (%)
o-anisidine (impurity, degr.)	**Cancer**	**DMEL:** **0.00004 mg/kg/d**	**0.0028 mg/d**	**0.12 mg**	**A: (0.12 mg/1.09 g=**	**(1775 ppm = 0,1775 %)** **5.6 %**

					0.0001) 0,01% **B: (0.12mg/10.3 g = 0.00001) 0.001%**	**0.56 %**

Ink No. 27 (yellow) contains 1150 μg/g of o-anisidine (1150 ppm) after possible degradation of the azo-dye. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 0.9 % absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Ink No 49 (red) contains 425 μg/g of o-anisidine (425 ppm) after possible degradation of the azo-dye. The content of o-anisidine before possible degradation of the azo-dye was 15 μg/g. Reaction in the skin has been observed for this ink. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 3% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Ink No 18 (red) contains 95 μg/g of o-anisidine (95 ppm) after possible degradation of the azo-dye. The content of o-anisidine before possible degradation of the azo-dye was 4,9 μg/g. Reaction in the skin has been observed for this ink. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 12.3% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Ink No. 48 (red) contains 55 μg/g of o-anisidine (55 ppm) after possible degradation of the azo-dye. The content of o-anisidine before possible degradation of the azo-dye was 9.0 μg/g. Reaction in the skin has been observed for this ink. Concerning a large tattoo when the ink is degraded, the DMEL value is exceeded at 21% absorption. Thus based on the calculations the ink is estimated to constitute an unacceptable increased cancer risk.

Ink No. 34 (red) contains 34 μg/g of o-anisidine (34 ppm). The content is measured before possible degradation of the azo-dye. Concerning a large tattoo, the DMEL value is exceeded at 35% absorption. Thus based on the calculations it is estimated that the ink could exceed the tolerable cancer risk.

Ink No. 36 (yellow) contains 5.6 μg/g of o-anisidine (5.6 ppm) after possible degradation of the azo-dye. The content of o-anisidine before possible degradation the azo-dye was 4.6 μg/g. Reaction in the skin has been observed for this ink. Even for a large tattoo when the ink is degraded, the DMEL value is not exceeded at 100% absorption. Thus in the calculated scenario the ink is considered to not pose an unacceptable increased risk of cancer due to o-Anisidin.

The Inks No. 1, 5, 24, 25, 35, 37, 44, 45, 53, 57 also contain o-anisidine, but at concentrations below 5.5 ppm.

3,3'-Dichlorobenzidine
3,3'-dichlorobenzidine is carcinogenic (cars. 1B). The DMEL value (as in the report is said to be very uncertain) is given in Table 5. Ink No. 24 (red) has the highest content of 3.3 '-

Dichlorobenzidine and contains 6.2 µg/g after possible degradation of the ink. Before possible degradation of the azo-dye 3.3 '-dichlorobenzidine could not be detected in the ink. The risk is calculated in Table 5. Due to the uncertainties of the DMEL and the high calculated aborption rate needed for the DMEL value to be exceeded no firm conclusion can be drawn whether an unacceptable risk level is reached.

Formateret: Engelsk (Storbritannien)

Table 5

Substance	**Critical effect**	**DNEL/ DMEL** **mg/kg/d**	**Tolerable daily intake** **(x 70 kg)** **mg/d**	**Tolerable dose over 42 days** **(x 42 d)** **mg**	**Absorption required to exceed the DN(M)EL value** **%** **A) Average tattoo (1.09g ink)** **B) Worst case tattoo (10.3g ink)** **.. if the ink contained 100% of the substance**	**(Absorption required to exceed the DN(M)EL value) .. for the measured max concentration of the substance in the ink** **(%)**
3,3-Dichlor-benzidine (impurity/ degradation)	**Cancer**	**DMEL:** **0.000015-0.00015 mg/kg d**	**0.001 – 0.01 mg/d**	**0.042 – 0.42 mg**	**A: (0.042 – 0.42 mg/1.09 g = 0.000039 – 0.00039) 0.0039 % - 0.039%** **B: (0.042 – 0.42 mg/10.3 g = 0.0000039 – 0.000039) 0.00039 % - 0.0039%**	**(6.2 ppm= 0.00062%)** **A: 620% - 6200%** **B: 62 % - 620 %**

Ink No. 53 (red) contains 5.8 µg/g of 3.3'-dichlorobenzidine after possible degradation of the azo-dye. The content of 3.3'-Dichlorbenzidine before possible degredation of the azo-dye was 3.7 µg/g. Again no firm conclusion can be drawn for this scenario. Ink No. 57 (brown) containing 4.0 µg/g

3.3 '-dichlorobenzidine, it is estimated that the required absorption (approximately 100% in the degradation of a large tattoo) is so high that the ink most likely does not cause an increased unacceptable risk. The content of 3.3'-dichlorobenzidine before possible degradation of the azo-dye could not be detected in the ink No. 57.

Other PAAs
The content of other PAAs is given in the table below

Table 6

Substance	Content (ug/g) of 2-naphthylamine before possible degradation of azo-dye	Content (ug/ g) of 2-naphthylamine after possible degradation of azo-dye	DMEL can not be set due to lack of data. Classified as carc. 1A.
Ink No.			
53 (red)	2.6	Not measured	
Substance	Content (ug/g) of p-chloroaniline before possible degradation of azo-dye	Content (ug/ g) of p-chloroaniline after possible degradation of azo-dye	DMEL can not be set due to lack of data. Classified as carc. 1B.
Ink No.			
53 (red)	6.3	100	
57 (red)	2.1	72	
48 (red)	1.1	12.4	
Substance	Content (ug/g) of 4-chloro-o-toluidine before possible degradation of azo-dye	Content (ug/ g) of 4-chloro-o-toluidine after possible degradation of azo-dye	DMEL can not be set due to lack of data. Classified as carc. 1B
Ink No.			
45 (blue)	5.9	15	
1 (red)	Not measured	1.2	
Substance	Content (ug/g) of 5-nitro-o-toluidine before possible degradation of azo-dye	Content (ug/ g) of 5-nitro-o-toluidine after possible degradation of azo-dye	DMEL can not be set due to lack of data. Classified as carc. 2
Ink No.			
57 (brown)	58	>400	
53 (red)	190 OBS	150	
24 (red)	58 OBS	14	
34 (red)	Not measured	6.3	
Substance	Content (ug/g) of 4-methoxy-m-	Content (ug/g) of 4-methoxy-m-	DMEL can not be set due to lack of

	phenylenediamine before possible degradation of azo-dye	phenylenediamine after possible degradation of azo-dye	data. Classified as carc. 1B
Ink No.			
49 (red)	Could not be detected	40	
Substance	Content (ug/g) of 4-methoxy-m-phenylenediamine before possible degradation of azo-dye	Content (ug/g) of 4-methoxy-m-phenylenediamine after possible degradation of azo-dye	DMEL can not be set due to lack of data. Classified as carc. 1B
Ink No.			
49 (red)	Could not be detected	40	
Substance	Content (ug/g) of o-Toluidine before possible degradation of azo-dye	Content (ug/g) of o-Toluidine after possible degradation of azo-dye	DMEL can not be set due to lack of data. Classified as carc. 1B
Ink No.			
44 (green)	Not measured	133	
60 (green)	Not measured	42	
53 (red)	1.4	20	
57 (red)	1.0	13	
1 (red)	Not measured	10	
24 (red)	2.9	6.5	
48 (red)	1.2	4.3	
7 (green)	1.5	2.6	
35 (purple)	0.85	1.5	
65 (orange)	Not measured	1.3	
5 (red)	Not measured	1.1	
27 (yellow)	Not measured	0.68	
Substance	Content (ug/g) of 4-Methyl-m-phenylenediamine before possible degradation of azo-dye	Content (ug/g) of 4-Methyl-m-phenylenediamine after possible degradation of azo-dye	DMEL can not be set due to lack of data. Classified as carc. 1B and sensitizing
Ink No.			
53 (red)	2.6	>400	
57 (brown)	1.8	>200	
65 (orange)	Not measured	16	
24 (red)	Not detected	2.2	
1 (red)	Not measured	1.2	
Substance	Content (ug/g) of . 2.4-xylidine / 2.6-xylidine before possible	Content (ug/g) of 2.4-xylidine / 2.6-xylidine after possible degradation	The health impact has not been evaluated for this

	degradation of azo-dye	of azo-dye	substance
Ink No.			
1 (red)	<0.75	Not measured	
5 (red)	<0.75	Not measured	
Substance	Content (ug/g) of 4-aminobiphenyl before possible degradation of azo-dye	Content (ug/g) of 4-aminobiphenyl after possible degradation of azo-dye	The health impact has not been evaluated for this substance
Ink No.			
1 (red)	1.1	Not measured	

It is not possible to assess whether the content of the above mentioned other PAAs pose a health risk, since DMEL values could not bet set. Ink No. 44, 49, 53, 57 and 60 contain PAA in concentrations above 30 ppm, which is the limit for PAAs in textiles. 5-nitro-o-toluidine, 4-methoxy-m-phenylenediamine, o-toluidine and 4-methyl-m-phenylenediamine are prohibited in textiles with a limit value of 30 ppm. p-chloroaniline also occurs in concentrations above 30 ppm, but is not prohibited in textiles.

Local skin reactions

The report describes that aluminium is under suspision for causing granuloms (i.e. small lumps under the skin, smallpox, sores and the like). However, all the inks that are known to have caused a local reaction in the skin have relatively low aluminium contents. The results of the analysis therefore suggest that it may not be high concentrations of aluminium, which causes local skin reactions.
Especially blue and white inks have high aluminium contents and the reddish inks usually give local reactions.

Table 7. Ink with high content of Al

Ink no.	**Ink**	**Concentration of Al**
Ink no. 46	(white)	11.000 μg/g Al
Ink no. 59	(white)	9000 μg/g Al
Ink no. 29	(peach)	9300 μg/g Al
Ink no. 64	(peach)	8400 μg/g Al
Ink no. 22	(white)	7800 μg/g Al
Ink no. 25	(light blue)	7400 μg/g Al
Ink no. 21	(peach)	7100 μg/g Al
Ink no. 4	(white)	6800 μg/g Al
Ink no. 62	(blue)	6500 μg/g Al
Ink no. 6	(light green)	6100 μg/g Al
Ink no. 14	(white)	5200 μg/g Al
Ink no. 38	(light blue)	3400 μg/g Al
Ink no. 8	(blue)	3300 μg/g Al
Ink no. 5	(red)	2600 μg/g Al

Ink no. 28	(orange)	2600 µg/g Al
Ink no. 60	(green)	2500 µg/g Al
Ink no. 45	(blue)	2400 µg/g Al
Ink no. 1	(red)	2300 µg/g Al
Ink no. 15	(blue)	1900 µg/g Al
Ink no. 7	(green)	1700 µg/g Al
Ink no. 65	(orange)	1700 µg/g Al
Ink no. 40	(yellow)	1600 µg/g Al
Ink no. 27	(yellow)	1400 µg/g Al
Ink no. 26	(light green)	1300 µg/g Al
Ink no. 20	(orange)	1100 µg/g Al
Ink no. 41	(green)	1100 µg/g Al
Ink no. 37*	(purple)	1030 µg/g Al
Ink no. 19	(yellow)	1000 µg/g Al

* Local skin reactions have been observed

Table 8. Content of Al in ink, where a local skin reaction was observed

Ink no.	**Ink**	**Concentration of Al**
Ink no. 37	(purple)	1030 µg/g Al
Ink no. 18	(red)	590 µg/g Al
Ink no. 24	(red)	710 µg/g Al
Ink no. 35	(purple)	1,8 µg/g Al
Ink no. 36	(yellow)	960 µg/g Al
Ink no. 48	(red)	61 µg/g Al
Ink no. 49	(red)	52 µg/g Al
Ink no. 53	(red)	23 µg/g Al
Ink no. 57	(brown)	14 µg/g Al

Of 8 inks that showed local skin reactions 6 contains high concentrations of PAAs. This suggests, that it may be the PAAs or the azo-dyes itself that are responsible or contribute to local reactions.

In addition, those who are tattooed with red and yellow inks claim that "it itches when the sun shines on the tattoo." This might suggest that light degrades the azo-dye to PAAs, which subsequently react with the skin. It is possible to envisage that depending on how much azo-dye that decomposes, it may cause a reaction. In addition, several PAAs are proven allergens and as such might thus be responsible for the local reactions even though the mechanism is unknown.

Despite that the mechanism is unknown nickel and chromium are allergens and could as such be responsible for the local reactions:

Regarding nickel, concerning the ink with the highest concentrations (which are only trace amounts!) no local skin reactions have been observed, while the ink where the local reactions have been observed only contains very low nickel concentrations.

Table 9. Ink with high Ni content

Ink no.	**Ink**	**Concentration of Ni**

Ink no. 20	(orange)	18 µg/g Ni
Ink no. 1	(red)	7,3 µg/g Ni
Ink no. 46	(white)	3,8 µg/g Ni
Ink no. 53*	(red)	3,4 µg/g Ni
Ink no. 62	(blue)	3,0 µg/g Ni

*Local skin reactions have been observed

Table 10. Content of Ni in inks where local skin reactions were observed

Ink no.	**Ink**	**Concentration of Ni**
Ink no. 53*	(red)	3,4 µg/g Ni
Ink no. 18	(red)	0,31 µg/g Ni
Ink no. 24	(red)	0,28 µg/g Ni
Ink no. 35	(purple)	1,8 µg/g Ni
Ink no. 36	(yellow)	0,27 µg/g Ni
Ink no. 37	(purple)	0,44 µg/g Ni
Ink no. 48	(red)	0,18 µg/g Ni
Ink no. 49	(red)	1,4 µg/g Ni
Ink no. 57	(brown)	1,0 µg/g Ni

For chromium, as with nickel and aluminium, there are no indications that local skin reactions should be related to the concentration of chromium.

Table 11. Ink with high content of Cr

Ink no.	**Ink**	**Concentration of Ni**
Ink no. 20	(orange)	31 µg/g Cr
Ink no. 26	(light green)	12 µg/g Cr
Ink no. 1	(red)	11 µg/g Cr
Ink no. 43	(black)	8,9 µg/g Cr
Ink no. 49*	(red)	6,9 µg/g Cr
Ink no. 53*	(red)	6,4 µg/g Cr
Ink no. 27	(yellow)	5,9 µg/g Cr

*Local skin reactions have been observed

Table 12. Contents of Cr in ink where local skin reactions have been observed

Ink no.	**Ink**	**Concentration of Ni**
Ink no. 18	(red)	0,63 µg/g Cr
Ink no. 24	(red)	3,8 µg/g Cr
Ink no. 35	(purple)	1,3 µg/g Cr
Ink no. 36	(yellow)	0,33 µg/g Cr
Ink no. 37	(purple)	1,2 µg/g Cr
Ink no. 48	(red)	1,8 µg/g Cr
Ink no. 57	(brown)	1,5 µg/g Cr

Thus if the local skin reaction relates to the content of any of the chemical substances analysed for in the report, the best guess would be the PAAs.

Lead

Ink no. 4 (white) has the highest concentration of lead at 10 μg/g lead (10 ppm). In Table 12, it is calculated how large a percentage of absorption is needed to exceeded the DMEL value of 0.000 5 mg/kg d (from RAC opinion 2011 on lead in jewellery). Data shows that a concentration of 10 ppm does not exceed the DMEL value. It should be noted that calculations show the DMEL value is exceeded at 15 ppm.

Table 13.

Substance	Critical effects	DNEL/ DMEL mg/kg/d	Tolerable daily dose (x 70 kg) mg/d	Tolerable daily dose over a period of 42 days (x 42 d) mg	Absorption for achieving DN(M)EL exposure % A) Average tattoo (1.09 g ink) B) Worst case tattoo (10.3 g ink) .. if the ink contained 100% of the substance	(Absorption required to exceed the DN(M)EL value) .. for the measured max concentration of the substance in the ink (%)
Lead (impurity)	**Neuro-toxic effects**	**DMEL:** **0.00005 mg/kg/d**	**0.0035 mg/d**	**0.147 mg**	**A: (0.147 mg/1.09 g = 0.00014) 0.014%** **B: (0.147 mg/10.3 g = 0.000014) 0.0014%**	**(10 ppm = 0.001%)** **1400 %** **140 %**

Other inks with high lead levels were ink no. 6 (light green) at 9.3 μg/g lead and ink no. 8 (blue) at 5,7 μg/g lead. These two inks are also estimated to not pose an unacceptable increased risk.

Nickel

For nickel, the NOAEL and DMEL value taken from the EU risk assessment report of nickel, indicating the tolerable internal dose of nickel, is the most relevant dose parameter to compare with calculations that focuses on how large a proportion that must be absorbed (i.e. internal dose) in order for the exposure to exceed the DN(M)EL value.

Table 14

Sub-stance	**Critical effect**	**NOAEL/ LOAEL** **mg/kg/d**	**DNEL/ DMEL** **mg/kg/d**	**Tolerable daily intake** **(x 70 kg)** **mg/d**	**Tolerable dose over 42 days** **(x 42 d)** **mg**	**Absorption required to exceed the DN(M)EL value** **%** **A) Average tattoo (1,09 g ink)** **B) Worst case tattoo (10,3 g ink)** **.. if the ink contained 100% of the substance**	**(Absorption required to exceed the DN(M)EL value) .. for the measured max concentration of the substance in the ink** **%**
Nickel (impurity)	**Repro-toxic effects**	**NOAEL: 1.1 mg/kg/d external dose corresp. 0.055 mg/kg/d internal dose (EU-RAR on Nickel)**	**DNEL** **0.00027 m/kg/d (EU-RAR)**	**0.0189 mg/d**	**0.79 mg**	**A: (0.79 mg/1.09 g = 0.0007) 0.07%** **B: (0.79 mg /10.3 g = 0.00008) 0.008%**	**(18 ppm = 0.0018%)** **3500%** **400%**

Formateret: Dansk

Calculations show that nickel in tattoo ink does not form an unacceptable increased risk in terms of reprotoxic effects.

Considering sensitizing effects a deposition load of 9.4 mg ink/cm^2 is considered as worst case. With a nickel content of 18 ppm this would result in a nickel exposure of 0.17 µg/cm^2. The nickel Directive allows a release of 0.5 µg Ni/cm^2/week to the outer surface of the skin which is considered to protect against sensitisation. However, it is not possible to conclude to which extent an intradermal nickel exposure of 0.17 µg Ni/cm^2 in relation to tattoo ink would still pose a risk for skin sensitisation towards nickel.

Cadmium and Barium

The calculation scheme for cadmium and barium shows no risks have been found.

Table 15

Sub-stance	Critical effect	NOAEL/ LOAEL mg/kg/d	DNEL/ DMEL mg/kg/d	Tolerable daily intake (x 70 kg) mg/d	Tolerable dose over 42 days (x 42 d) mg	Absorption required to exceed the DN(M)EL value % A) Average tattoo (1.09 g ink) B) Worst case tattoo (10.3 g ink) .. if the ink contained 100% of the substance	(Absorption required to exceed the DN(M)EL value) .. for the measured max concentration of the substance in the ink %
Cadmium	**Renal effects**	**LOAEL:** **0.0006 mg/kg/d**	**DNEL:** **0.0002 mg/kg/d**	**0.014 mg/d**	**0.59 mg**	**A: (0.59 mg/1.09 g = 0.0005) 0.05 %** **B: (0.59mg /10,3 g = 0.00006) 0.006 %**	**(0.3 ppm)** **150000 %** **18000 %**
Barium	**Cardio-vascular effects**	**NOAEL: 0.21 mg/kg/d**	**DNEL: 0.021 mg/kg/d**	**1.47 mg/d**	**62 mg**	**A: (62/1088 = 0.06) 6%**	**(1800 ppm = 0.18 %)** **3300 %**

						B: (62/10268 = 0.006) 0.6%	**330 %**

Phthalocyanine

Phthalocyanine is, unlike the other substances discussed in the memo, a pigment and not an impurity or a degradation product. This means that only 68% systemic absorption is presumed possible. In addition, the phthalocyanine is of extremely low solubility and consequently they are most likely absorbed to a much lesser degree. Furthermore, the report underline the uncertainty in the DNEL estimation.

Ink no. 15 (blue) has the highest content of phthalocyanine and contains 18.9 %. Table 15 shows the calculation of how large the percentage uptake must be for the ink to pose a risk. According to the calculations, the ink does not pose a risk.

Table 16

Sub-stance	Critical effect	NOAEL/ LOAEL mg/kg/d	DNEL/ DMEL mg/kg/d	Tolerable daily intake (x 70 kg) mg/d	Tolerable dose over 42 days (x 42 d) mg	Absorption required to exceed the DN(M)EL value % A) Average tattoo (1,09 g ink) B) Worst case tattoo (10.3 g ink) .. if the ink contained 100% of the substance	(Absorption required to exceed the DN(M)EL value) .. for the measured max concentration of the substance in the ink %
Phthalo-cyanine	**Effect on red blood cells**	**NOAEL: 200 mg/kg/d**	**DNEL: 2 mg/kg/d**	**140 mg/d**	**5.88 g**	**A: (5.88/1.09 = 5.4) 540%**	**A:2857%**

						B: (5.88/10.3 = 0.57) 57%	**B: 301 %**

The remaining inks in which a high content of phthalocyanine has been found is ink no. 8 (blue), which contains 174.000 µg/g i.e. 17.4 %, ink no. 7 (dark green) at 11.2 % and ink no. 25 (light blue) at 4.7 %. It is estimated that the remaining inks do not pose a risk as their content is lower than ink no. 15.

The conclusion is that, regarding green and blue inks, phthalocyanine represents a viable alternative to azo-dyes, which may degrade to PAA.

Nanomaterials

Since the pigments in the inks are particles and since a fraction of these particles may be in nanoscale (i.e. less than 100 nm or 100 x 10^{-9} m in diameter) addressing health risks posed by exposure to nanosized particles may be relevant. At present, there is however limited knowledge to which extent pigments in nanosize constitute other or increased hazards compared to larger particles. Therefore, currently no conclusions can be drawn regarding effects due to pigments they may occur in nanoscale.

Conclusion

For the carcinogenic substances BaP, aniline, o-anisidine and 3,3'-dichlorbenzidine, it is calculated that the DMEL value can be exceeded over a period of 42 days due to possible absorption of the substances. The DMEL value may be exceeded by using ink no. 11, 18, 20, 24, 26, 27, 34, 37, 48, 49, 53, 57 and 65. These inks thus pose an unacceptable increase in risk of cancer. The content of the PAAs is considered to be the largest problem with respect to the carcinogenic risk.
Another serious hazard with tattoo inks is, however, considered to be local reactions in the tissue, which has been found in some cases. The findings suggest that the local reactions for - which it is impossible to establish any safe threshold for exposure - may be related to the content of reactive PAAs and thus the content of azo-dyes.

Table 17

	Unacceptable increase in risk of cancer due to content of one of the below listed substances				Skin reactions
Ink no.	BaP	Aniline	o-Anisidine	3,3'-Dichlorbenzidine	
11	X				
18			X		X
20		X			
24		X		X	X
26			X		
27			X		

34			X		
37		X			X
48		X	X		X
49			X		X
53		X		X	X
57		X			X
65		X			