Survey on European Studies of the Chemical Characterisation of Tattoo Ink Products and the Measurement of Potentially Harmful Ingredients

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Abstract

The results of the detection of carcinogenic aromatic amines in about 300 ink samples are discussed. All analysed inks contained at least one or more azo compound pigments, and the presence of aromatic amines could only have originated from these compounds through a chemical process named 'reductive cleavage'. Sometimes, aromatic amines were also present as impurities derived from the processing of the pigments. A systematic surveillance programme in Italy, promoted by the Italian Ministry of Health with the involvement of Italian regions, local public health authorities and Agenzia Regionale per la Protezione Ambiente del Piemonte (Environmental Protection Agency), has shown that about 40% of the monitored inks are not regular according to European Resolution ResAP(2008):1. The method utilised for the detection of aromatic amines has allowed the identification of other substances that are not carcinogenic but are toxic or have sensitisation properties that are derived from reductive cleavage or that are present as impurities.

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Introduction

Since the end of the 1990s, the tattoo fashion has spread widely all over the world. Due to this growth and the lack of information about the substances contained in tattoo inks, in 2006, Regione Piemonte, with the cooperation of Agenzia Regionale per la Protezione Ambiente del Piemonte (ARPA Piemonte), which is an Environmental Protection Agency, and some local public health authorities, promoted a programme for the surveillance of chemicals to ensure the safety of tattoos and permanent make-up.

Local public health authorities and ARPA Piemonte defined a protocol for the inspection of tattoo parlours in Piemonte and included sampling criteria for the products used for tattooing. After a monitoring period, this team found out that the information available for the ingredients contained in tattoo inks was very poor and confusing. A significant number of safety data sheets or technical data sheets that were available at the

monitored tattoo parlours were not updated with the current Italian legislations or did not correspond to the products present in the parlours. Analytical studies performed on 30 samples of tattoo inks in 2006–2007 showed that about 40% of the samples were not regular according to European resolution ResAP(2003):2 [1] on tattoos and permanent make-up.

Due to these analytical results, the Italian Ministry of Health has promoted Regione, a national, systematic surveillance programme that samples tattoo inks, since 2009. Surveillance is also conducted by other authorities, such as Nucleo Anti Sostituzioni – Carabinieri Force, custom agencies and local public health authorities. The analysis of samples is performed by a network of laboratories comprising the Istituto Superiore di Sanità (National Institute of Health) and specialised regional public laboratories according to different analytical methods and chemical matrices.

The legislative instrument utilised in Italy is EU Directive 2001/95/CEE [2], with application of the update for consumer protection (D. Lgs ‘Codice del Consumo’) n°206 of 2005. This legislation guarantees the safety of all products placed in the Italian market and allows for the application of Resolution ResAP(2008):1 [1] (which supersedes Resolution ResAP(2003):2 on tattoos and permanent make-up) and the development of a surveillance system coordinated by the Ministry of Health. In addition, the Registration, Evaluation, Authorisation and Restriction of Chemicals regulation n°1907/2006 and the Classification, Labelling and Packaging regulation n°1272/2008 provide some restrictions for dangerous substances as well as specific rules for the classification and labelling of substances, mixtures and articles.

The notification system Rapid Information System for Non-food Products is the instrument used in the EU for sharing information about the irregularity of products among local authorities, consumers, producers and users. This system is currently used in Italy.

Ink Characterisation

Tattoo inks consist of pigments combined with a carrier.

Tattoo inks are available in a range of colours that can be thinned or mixed to produce other colours and shades. Most professional tattoo artists purchase pre-made inks (known as pre-dispersed inks), while some tattooists mix their own ink using a dry pigment and a carrier.

Pigments are chemically derived from three main groups of vegetal, inorganic or organic origin.

Vegetal pigments are often derived from root plants or logwood, which is a heartwood extract from ‘*Haematoxylon campechianum*’ that is found in Central America and the West Indies. The most-used black inks are made of Carbon Black (CI 77266), but it is sometimes possible to find black inks made of dried vegetal algae dispersed in a carrier.

Inorganic pigments are made from minerals such as magnetite (Fe_3O_4 or FeO) and are often associated with brown and dark colours. Cinnabar (HgS) is used for red colours, and other kinds of inorganic compounds, such as various metal oxides or sulphides, are used for a wide range of colours. Titanium oxides (TiO_2) are commonly used as white pigments.

The organic pigments are the most variable group and include some different chemical classes that are dependent on characteristic functional groups:

- Nitro dye, associated with yellow colours.
- Xanthenic dye, associated with various colours, mainly various shades of yellow.
- Phtalocyanine compounds, associated with blue and green colours.
- Antraquinone dyes, associated with red and brown colours.
- Mono-azo and di-azo dyes, commonly known as azo dyes. These pigments are used for various colours such as yellow, red or orange.

The colorants (both dyes and pigments) are listed by their colour index numbers. The colour

Table 1. Colour index numbers

Structure	Colour index range
Nitroso	10000–10299
Nitro	10300–10999
Monoazo	11000–19999
Diazo	20000–39999
Stilbene	40000–41999
Diarylmethane	41000–41999
Triarylmethane	42000–44999
Xanthene	45000–45999
Acridine	46000–46999
Quinoline	47000–47999
Methine	48000–48999
Thiazole	49000–49399
Indamine	49400–49699
Indophenol	49700–49999
Azine	50000–50999
Oxazine	51000–51999
Thiazine	52000–52999
Aminoketone	56000–56999
Anthraquinone	57000–72999
Indigoid	73000–73999
Phtalocyanine	74000–74999
Natural dyes	75000–76999
Inorganic pigments	77000–77999

index numbers are grouped into ranges according to their chemical structure, as shown in table 1.

Aromatic Amines in Azo Dyes

Azo compounds are by far the most widely used synthetic organic colorants. The colour index lists more than 2,000 azo compounds. Azo dyes are generally synthesised starting from primary aromatic amines that are diazotised and coupled with phenols or secondary aromatic amines. In fact, the main chromophore group of the azo compounds is the diarylazo group ($\text{Ar-N} = \text{N-Ar'}$), which is derived from simpler molecules known as aromatic amines.

Commercial products often contain high levels of other components that are especially rele-

vant from a toxicological point of view: the aromatic amines, which are chemically characterised by a NH_2 group and are often found in inks as contaminants or reduction (cleavage) products.

According to some studies [3–6], aromatic amines can be formed by the reductive cleavage of azo compounds by human enzymes over a varying range of time beginning from the introduction of the pigments under the skin. Figure 1 shows a hypothetical example: pigment red 5 could be cleaved into three aromatic amines, two of which, p-cresidine and o-toluidine, are carcinogenic.

The chemical analysis discussed in this work focuses on inks containing at least one azo compound and on the determination of certain carcinogenic aromatic amines that could be derived from pigment decomposition by means of reductive cleavage.

The aromatic amines that were detected, with their classifications assigned according to regulations by Classification, Labelling and Packaging [7] and other institutions, such as the International Agency for Research on Cancer and the American Conference of Governmental Industrial Hygienists [8, 9], are listed in table 2.

According to Resolution ReSAP(2008):1, these aromatic amines ‘...particularly with regard to their carcinogenic, mutagenic, reprotoxic and sensitising properties, ...should neither be present in tattoos and PMU products nor released from azo-colorants’.

The limit of quantification of the method used for the determination of the aromatic amines is 1 mg/kg for each substance listed in table 2. Traces of aromatic amines below this limit of quantification are not considered.

Methodology

The chemical analysis carried out by Arpa Piemonte mainly focused on synthetic pigments specially derived from azo compounds. About

Fig. 1. Cleavage reduction of red 5.

Table 2. List of detected aromatic amines

Substances [CAS number]	Carcinogenic classifications		
	IARC	ACGIH	CLP
o-Toluidine [95–53–4]	2A	A3	Carc. 1B – H350
o-Anisidine [90–04–0]	2B	A3	Carc. 1B – H350
4-Chloroaniline [106–47–8]	2B	–	Carc. 1B – H350
6-Methoxy-m-toluidine [120–71–8]	2B	–	Carc. 1B – H350
2,4,5-Trimethylaniline [137–17–7]	3	–	Carc. 1B – H350
4-Chloro-o-toluidine [95–69–2]	2A	–	Carc. 1B – H350
4-Methyl-m-phenylenediamine [95–80–7]	2B	–	Muta. 2 – H341 Carc. 1B – H350
4-Methoxy-m-phenylenediamine [615–05–4]	2B	–	Muta. 2 – H341 Repr. 2 – H361f Carc. 1B – H350
2-Naphthylamine [91–59–8]	1	A1	Carc. 1B – H350
Biphenyl-4-ylamine [92–67–1]	1	A1	Carc. 1B – H350
4,4'-Oxydianiline [101–80–4]	2B	–	Carc. 1B – H350
Benzidine [92–87–5]	1	A1	Muta. 2 – H341 Repr. 2 – H361f Carc. 1B – H350
4,4'-Methylenedianiline [101–77–9]	2B	A3	Carc. 1B – H350
4,4'-Methylenedi-o-toluidine [838–88–0]	2B	–	Muta. 2 – H341 Carc. 1B – H350
3,3'-Dimethylbenzidine [119–93–7]	2B	A3	Carc. 1B – H350
4,4'-Thiodianiline [139–65–1]	2B	–	Carc. 1B – H350
3,3'-Dichlorobenzidine [91–94–1]	2B	A3	Carc. 1B – H350
4,4'-Methylenebis(2-chloroaniline) [101–14–4]	2A	A2	Carc. 1B – H350
3,3'-Dimethoxybenzidine [119–90–4]	2B	–	Carc. 1B – H350
5-Nitro-o-toluidine [99–55–8]	3	A3	Carc. 1B – H351
o-Aminoazotoluene [97–56–3]	2B	–	Carc. 1B – H351
4-Aminoazobenzene [60–09–3]	2B	–	Carc. 1B – H351

Table 3. Comparison of two methods

Inks	O-anisidine with ISO 17234 mg/kg	O-anisidine with ISO 14362 mg/kg
Yellow 1	318	46
Yellow 2	177	11
Yellow 3	242	78
Yellow 4	113	18
Yellow 5	69	32

300 ink samples of various colours were analysed for the determination of aromatic amines.

The analytical method was derived from EN ISO 17234 [10], which is normally used for the determination of aromatic amines in leather samples. The method consists of three stages:

(1) Chemical reduction of pigments with sodium dithionite as a reduction agent.

(2) Extraction of aromatic amines that could be formed by sodium dithionite by means of methyl tert butyl ether.

(3) Quantitative analysis by gas chromatography followed by mass spectrometry.

The method used for leather samples was adapted to ink samples and then compared to the method described in ResAP, which was derived from the determination of aromatic amines in textiles (ISO 14362 – Textiles. Methods for the determination of certain aromatic amines derived from azo colorants. Detection of certain azo colorants that are accessible with and without extracting the fibres) [11].

These two methods are rather similar, except for the first step, the pigment reduction. The concentration of sodium dithionite in ISO 17234 is about 200 mg/ml; 3 ml of this solution is added to the samples, and the samples are pre-heated for 20 min at 70°C in 17 ml of citrate buffer at pH 6 for other 20 min.

In the model method derived from ISO 14362, 5 ml of dithionite solution (5%) in phosphate buffer is added to the sample. Then, the reduction continues at 70°C for 90 min.

The leather method is more effective when compared to this method, but its application is in agreement with the directives of the Ministry of Health, which are based on a 'precaution principle'.

Table 3 shows the concentrations of o-anisidine in a group of samples, compared to the above-described methods. The classification of these samples does not conform to Resolution ResAP(2008):1 when both methods are considered.

Data Evaluation

The analytical results of the detection of carcinogenic aromatic amines in about 300 samples showed the presence of carcinogenic compounds in more than 40% of the inks that were considered [12].

Higher irregularities were found in yellow and red colours and related shades, such as orange, flesh and green (green colour is sometimes derived from blue pigment mixed with yellow pigment).

The aromatic amines that were frequently determined were o-toluidine, which was found in 24 samples; o-anisidine, which was found in 64 samples; 4-chloroaniline (also known as p-chloroaniline), which was found in 5 samples; 3,3'-dichlorobenzidine, which was found in 6 samples; 5-nitro-o-toluidine (also known as 2-methyl-5-nitroaniline), which was found in 5 samples; and 4-methyl-m-phenylenediamine (also known as 2,4-diaminotoluene), which was found in 4 samples. Often, the reduction split the pigments into more than one aromatic amine; therefore, two or more aromatic amines were detected in a significant number of irregular samples. In a red sample, 5 different carcinogenic aromatic amines were found.

The aromatic amines that were most frequently detected were o-toluidine, which is generally related to red colours, and o-anisidine, which is generally related to yellow colours.

The amounts of the aromatic amines that were found were quite variable, and a graphical representation is shown in figure 2.

Fig. 2. Statistical evaluation of the detected concentrations of aromatic amines.

A very large number of irregular samples contained a considerable amount of aromatic amines, and in about 44% of the irregular samples, these quantities exceeded 100 mg/kg (see fig. 3). The highest concentration was found in a shade of red: up to 700 mg/kg of o-toluidine. Furthermore, a shade of yellow had about 550 mg/kg of o-anisidine (see fig. 2).

Despite the strong reduction of the azo compound pigments, a significant percentage didn't show the presence of carcinogenic aromatic amines. This fact shows that it is possible to produce safe inks containing azo compound pigments.

Considerations

Our results led us to affirm that the determination of aromatic amines mostly depends on the specific lot number. In fact, some inks of the same brand and colour showed different results.

Table 4 shows some examples: the green, orange and yellow inks that were analysed could be either regular or irregular, based on the lot number.

The origin of the pigments is probably the key. Even though producers always use chemically equal pigments during the production of the various ink lots, the country of origin and the quality of the pigments could differ from one to the other.

One more test was carried out on two equal orange samples that had the same lot number. The analysis showed different results: one sample was regular, and the other one wasn't (see table 4, first row).

When investigating this peculiar case, the irregular sample that had a remarkable amount of o-toluidine was identified as a fake. The label was counterfeit and didn't correspond to the real product. It had been falsified. This situation represents a further danger in the production of tattoo inks and in the safety of customers.

Moreover, qualitative screening performed with the same method used for the determination

Fig. 3. Distribution of amounts of aromatic amines in different ranges of concentrations.

Table 4. Conformity by lot number

Orange ink – Brand A (Lot N° ABXXX10) Irregular 3,3'-dichlorobenzidine	Orange ink – Brand A (Lot N° ABXXX09) Irregular O-toluidine	Orange ink – Brand A (Lot N° ABXXX09) Regular
Green ink – Brand B (Lot N° CDXXX42) Irregular O-toluidine, O-anisidine	Green ink – Brand B (Lot N° CEXXX04) Regular	
Yellow ink – Brand C (Lot N° FGXXX88) Irregular O-anisidine	Yellow ink – Brand C (no lot number) Regular	

of carcinogenic aromatic amines revealed that other compounds, including aromatic amines that were not carcinogenic but were toxic (see table 5), were found in about 30% of the samples, both regular and irregular.

Future Developments

The evaluation of risks associated with the use of inks for tattoos carried out in recent years has allowed a frequent exchange of experiences at the Italian and European levels.

Table 5. H phrases about chemical substances found in the qualitative analyses

Substances [CAS number]	H phrases and notes from the American Conference of Governmental and Industrial Hygienists and the International Agency for Research on Cancer
Aniline [62–53–3]	H351 Suspected of causing cancer H341 Suspected of causing genetic defects H331 Toxic if inhaled H311 Toxic when in contact with skin H301 Toxic if swallowed H372 Causes damage to organs through prolonged or repeated exposure H318 Causes serious eye damage H317 May cause an allergic skin reaction H400 Very toxic to aquatic life ACGIH: Skin, A3
2-Ethoxyaniline [94–70–2]	H331 Toxic if inhaled H311 Toxic when in contact with skin H301 Toxic if swallowed H372 Causes damage to organs through prolonged or repeated exposure
3,4-Dichlorobenzeneamine [95–76–1]	H331 Toxic if inhaled H311 Toxic when in contact with skin H301 Toxic if swallowed H317 May cause an allergic skin reaction H318 Causes serious eye damage H400 Very toxic to aquatic life H410 Very toxic to aquatic life with long-lasting effects
4-Ethoxyaniline [156–43–4]	H341 Suspected of causing genetic defects H322 May be harmful if inhaled H312 Harmful when in contact with skin H302 Harmful if swallowed H319 Causes serious eye irritation H317 May cause an allergic skin reaction
N-Isopropyl-N'-phenyl-p-phenyldiamine (IPPD) [101–72–4]	H302 Harmful if swallowed H317 May cause an allergic skin reaction H400 Very toxic to aquatic life H410 Very toxic to aquatic life with long-lasting effects
Styrene [100–42–5]	H226 Flammable liquid and vapour H332 May be harmful if inhaled H319 Causes serious eye irritation H315 Causes skin irritation IARC NOTE: 2B (possibly carcinogenic)
3-Butenylbenzene [768–56–9]	H315 Causes skin irritation H411 Toxic to aquatic life with long-lasting effects
Biphenyl [92–52–4]	H319 Causes serious eye irritation H335 May cause respiratory irritation H315 Causes skin irritation H400 Very toxic to aquatic life H410 Very toxic to aquatic life with long-lasting effects

Table 5. Continued

Substances [CAS number]	H phrases and notes from the American Conference of Governmental and Industrial Hygienists and the International Agency for Research on Cancer
Thymol [89–83–8]	H302 Harmful if swallowed H314 Causes severe skin burns and eye damage H411 Toxic to aquatic life with long-lasting effects
1,2-Dichlorobenzene [95–50–1]	H302 Harmful if swallowed H319 Causes serious eye irritation H335 May cause respiratory irritation H315 Causes skin irritation H400 Very toxic to aquatic life H410 Very toxic to aquatic life with long-lasting effects
3,4-Dichlorobenzene [95–76–1]	H331 Toxic if inhaled H311 Toxic in contact with skin H301 Toxic if swallowed H318 Causes serious eye damage H317 May cause an allergic skin reaction H400 Very toxic to aquatic life H410 Very toxic to aquatic life with long-lasting effects
2,4,5-Trichlorophenol [95–95–4]	H302 Harmful if swallowed H319 Causes serious eye irritation H315 Causes skin irritation H400 Very toxic to aquatic life H410 Very toxic to aquatic life with long-lasting effects
1-Naphthol [90–15–3]	H312 Harmful in contact with skin H302 Harmful if swallowed H335 May cause respiratory irritation H315 Causes skin irritation H318 Causes serious eye damage

The goals of these collaborative efforts between institutions and laboratories in different countries are the more effective coordination of supervisory actions as well as the adoption of criteria that are as homogeneous as possible.

The purpose of the European Society of Tattoo and Pigment Research, in collaboration with producers and tattoo parlours, is the sharing of methods and objectives for improving the safety of these products.

The activities planned in Italy in the next few years will be oriented according to the following main guidelines:

a. Proposal of a single method, which is in accordance with guidelines of the national authorities, for the determination of different hazardous substances, among which are first-stage aromatic amines, polycyclic aromatic hydrocarbons and heavy metals.

b. Consideration of the adoption of limits for the concentrations of the aromatic amines listed in ResAP.

c. The extension of measures to restrict the levels of other hazardous aromatic amines, as a consequence of their high percentage of occurrence in the analysed samples, such as for the example substances listed in table 5.

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