DANISH MINISTRY
OF THE ENVIRONMENT
Environmental
Protection Agency

Colorants in transferable picture tattoos for the skin

Senior research scientist, Dr. Phil. Suresh C. Rastogi
and Senior technician, Gitte Hellerup Jensen
National Environmental Research Institute

Research director, Dr. Med. Jeanne Duus Johansen
National Allergy Research Centre

Survey of Chemical Substances in Consumer Products, No. 61 2005

The Danish Environmental Protection Agency will, when opportunity offers, publish reports and contributions relating to environmental research and development projects financed via the Danish EPA.

Please note that publication does not signify that the contents of the reports necessarily reflect the views of the Danish EPA.

The reports are, however, published because the Danish EPA finds that the studies represent a valuable contribution to the debate on environmental policy in Denmark.

Contents

Summary

The use of transferable picture tattoos for the skin is popular among children. As these picture tattoos are used on the skin, they should comply with the Danish Statutory Order on Cosmetic Products/EU Cosmetic Directive. There is very little knowledge of the colorants that may be present in the picture tattoos.

Picture tattoos are sold as such, or they can be attached to different types of products such as toys, food, candies and chewing gums etc. The aims of the present investigation were to perform a survey of the colorants that may be present in the transferable picture tattoos for children, to check the compliance of these products with the Cosmetic Directive, and to derive general conclusions concerning the safety of the these products with respect to their colour contents.

Forty-two products of picture tattoos or other products with attached picture tattoos were purchased in Denmark. Of these, 22 products were selected for the chemical analysis of colorants, so that most of the colorants present in the collected picture tattoo samples were represented.

The colorants in the picture tattoos were extracted in suitable solvents and analysed by high performance liquid chromatography. The presence of 129 cosmetic colorants, permitted for the use in cosmetic products, was checked in the picture tattoos. Only 11 of these colorants could be identified in the analysed samples. The content of seven of these colorants (CI 15850, CI 11920, CI 45220, CI 75300, CI 13015, CI 45100, CI 15525) was >12 ppm. Risk assessment of these seven colorants has been performed on the basis of the information available in the scientific literature.

The Danish EPA has received a few complaints about children getting skin reactions from the use of picture tattoos. The reason for these skin reactions from picture tattoos is not known. No cases of allergic skin reaction from transferable picture tattoos have been reported in the scientific literature, from selected Dermatology Departments (a query was made), or from the Danish Consumer Association. There is no information on animal testing for sensitisation or allergic reactions in humans by the colorants CI 45220, CI 13015 and, CI 15525. However, there are reports of a few cases of allergic reactions caused by CI 15850 and 75300 in humans, but never by the use of picture tattoos. QSAR studies based on the physical chemical properties have indicated that CI 11920 may be a moderate/strong allergen and CI 45100 may be a weak allergen. Further studies are required to confirm whether these colorants are potential allergens in practice. It is possible that the sensitisation potential of the colorants has been evaluated in animal tests without the results being published. Similarly, there may be unpublished cases of skin allergy from these colorants.

On the basis of the present investigation, it is concluded that the potential risk of allergic reactions from the colorants in transferable picture tattoos is limited. .

All colorants found in the picture tattoos are permitted for the use in cosmetic products. However, the colorants CI 45100 and CI 45200 should only be used in the products, which remain in contact with the skin for a short period. The colour contents were not labelled in most of the investigated picture tattoos.

Before publication, the report has been sent in hearing to manufacturers and retailers of picture tattoos included in the report. The Danish EPA have asked the manufacturers and retailers for their comments on the results of the analysis and have drawn attention to the current rules and regulations. Several of them have since approached the Danish EPA with their comments and stated that they will in future make sure the rules and regulations are followed, while others have stated that they will no longer sell this type of products.

1 Introduction

Decoration of the body with different types of tattoos, permanent tattoos, temporary tattoos, stickers, transferable tattoo pictures for the skin etc. has become a fashion. In permanent tattoos, colorants are implanted into different layers of skin, so that it becomes a permanent art on the skin. Painting the body with suitable materials, for example henna has been used for this purpose for many years in various cultures create temporary tattoos. The temporary tattoos are washed off/worn off in few weeks. Sometimes, the chemical substance p-phenylene-diamine (PPD) is incorporated in henna powder used for creating temporary tattoos on the skin. PPD is a potent allergen, and it can produce strong allergic reactions (1-3). This application of PPD is illegal. Other tattoo types available to consumers are: stickers or figures made of plastic pearls that can be glued on the body, and some special paints for the decoration of the body which can be washed off by soap and water.

Present investigation concerns picture tattoos that can be transferred on to the skin, normally with the use of water. These transferable pictures are used for a short period and they can be washed off. This type of tattoo is also popular among small children. The Danish Environmental Protection Agency (Danish EPA) has received a few complaints from parents, whose children got skin reactions from the use of picture tattoos. There is only sparse knowledge about the colorants used in the transferable picture tattoos. As these products are used on the skin, they should comply with the Danish Statutory Order on Cosmetic Products/EU Cosmetic Directive (4)

The aims of the present project are to perform a survey of the colorants that are commonly used in the picture tattoos for children, and to investigate whether the contents of colorants in these products fulfil the requirements laid down in the Cosmetic Directive.

The project is sub-divided in three phases: 1) Collection of the products (both those, which are on the market for a short and long period) and check compliance of the labelling on the products with the Cosmetic Directive; ii) perform qualitative and semi-quantitative analysis of colour content in a series of samples selected in co-operation with the Danish EPA and to check the compliance of the colour content with the permitted colorants in cosmetic products listed in the Annex 4 of the Cosmetic Directive; and iii) perform risk assessment of the identified colorants with respect to their potential of causing skin allergy.

2 Sample Collection

2.1 Market survey

A large number of retail shops were visited to find transferable picture tattoos. Thus, the possibility of buying picture tattoos in supermarkets, hobby shops, toy shops, shops for children articles, general merchants and hardware shops, book shops, kiosks, amusement parks, camping sites and some special shops was envisaged. The shops, especially in Copenhagen and Roskilde areas were visited. In the supermarkets and kiosks, the food and other products were looked at carefully to find out, if picture tattoos were attached to certain products, or sold separately. Furthermore, the availability of picture tattoos was enquired in all shops. The market survey revealed that picture tattoos for children are attached to certain products during specific campaigns.

Traders of picture tattoos were also searched on the internet, search: tattoo and tattooing. Hundreds of homepages were found that provided information on various types of tattoos and tattoo articles, network of persons with tattoos, workshops for permanent tattooing, sale of various articles for tattooing, tattoo stencils, stickers and transferable picture tattoos. On the Internet, no Danish trader was found with information on and sale of transferable picture tattoos.

2.2 Legislation

Picture tattoos are applied to the skin for the decoration of the body. The tattoo stickers are in contact with the skin and are used for changing the appearance of the body. For this reason they are regarded as cosmetic products according to § 3 of the Danish Statutory Order on Cosmetic Products. Picture tattoos are thus covered by Statutory Order on Cosmetic Products. The marketed cosmetics in Denmark including picture tattoos must comply with the Danish Statutory Order on Cosmetic Products/EU Cosmetic Directive. Therefore, the picture tattoos should contain only those colorants that are listed in Annex IV of the Cosmetic Directive and under the conditions as laid down in the Annex IV. Furthermore, requirements concerning the content of other ingredients, according to the Cosmetic Directive, should also be fulfilled by the picture tattoos marketed in Denmark/EU. Among other requirements is the labelling of complete ingredients list of the cosmetic products. This means that the picture tattoos should also be labelled for the complete ingredient list, including the colour content, according to chapter 3 of the Danish Statutory Order on Cosmetic Products (4).

2.3 Labelling check

The purchased picture tattoos as well as the products with attached picture tattoos are described in Table 1. Other tattoo products (use of which may resemble that of picture tattoos) used for body decoration are also described in the Table 1. Most of the products are bought in retail outlets, kiosks and special shops. Some products are bought in an amusement park, and at a camping site. One of the picture tattoos, which was attached to a Swedish comic

magazine, is also included in the Table 1. Picture tattoos attached to comic magazines were not found in Denmark, in the period April-July 2004.

Information on the manufacturer/importer of picture tattoos in Denmark, declaration of colorant content, the recommended age group and declaration for eventual risk by the use, according to the labelling on the products, is described in Table 1. The colour content was declared in a number of products, especially food products. Possibly, the labelling of colour content in these products concerns the colour content in the food and not the picture tattoos attached with the food products. The content of colorants was declared on two of the picture tattoos, sample no. 2-0286, and 4-0382.

2.4 Samples for the analysis of colorants

Among the collected products, picture tattoos especially for children, were selected for the chemical analysis. Furthermore, the tattoo pictures, which can be used both by children and adults, were also included in the samples for analysis. Thus, 22 products of picture tattoos or picture tattoos attached with other products were analysed. Each sample contained 1-10 picture tattoos of different figures composed of different colours. One-to-three picture tattoos from each sample were selected for the analysis in a way so that most of the colorants in all subsamples were represented. All-in-all 36 picture tattoos were analysed in the present project.

Table 1: Identification of the purchased products.

NERI No.	Product/Product with attaches sticker(s)	Declaration
4-0223	Enclosed chewing gum	E100*, E122*
4-0224	Enclosed chewing gum	
4-0225	Enclosed chewing gum	E120 (CI75470)*
4-0226	Enclosed chewing gum	E120*
4-0027	Enclosed chewing gum	Titan dioxid/E171*
4-0228	Enclosed chewing gum	
4-0229	Enclosed chewing gum	E162* (or E102)*
4-0230	Enclosed chewing gum	
4-0231	Enclosed chewing gum	E120*, E100*
4-0232	Enclosed chewing gum	E122*
4-0233	Enclosed cookies	
4-0234	Enclosed cookies	Sugar granules: E104*, E124*, E129*, E131*
4-0235	Enclosed cookies	
4-0241	Nail Tattoo	Keep protected from children
4-0242	Nail Tattoo	Keep protected from children
4-0243	Body Tattoo	Keep protected from children
4-0244	Body Tattoo	Keep protected from children
4-0245	Body Tattoo	Keep protected from children
4-0246	Body Tattoo	Keep protected from children
4-0247	Body Tattoo, Butterfly	Keep protected from children
4-0248	Body Tattoo, Dragon-fly	Keep protected from children
4-0249	Body Tattoo, flowers	Keep protected from children
4-0250	Body tattoo	Not suitable for children below 3 years because of small parts which, can be swallowed
4-0251	Body Tattoos	Safe & non-toxic
4-0252	Body Glitter Tattoo	Sensitive skin don't use, Stop use if skin rash occurs
4-0253	Body Glitter Tattoo	Sensitive skin don't use, Stop use if skin rash occurs
4-0286	Enclosed yoghurt	CI74160, CI72266 CI15850, CI77891 (declaration is attached to the sticker)
4-0287	Enclosed chewing gum	
4-0288	Enclosed chewing gum	
4-0294	Tattoos for textiles	Wash-off, Non-toxic. Not suitable for children<3 year. Jeans-transfer is not a tattoo. Therefore, do not use on the skin
4-0347	Enclosed comic	
4-0348	Enclosed chewing gum	E100*, E122*
4-3049	Body tattoo	Not suitable for children below 3 years because of small parts, which can be swallowed
4-0350	Body tattoos	Not suitable for children below 3 years because of small parts, which can be swallowed
4-0351	Enclosed chewing gum	
4-0352	Body tattoo	
4-0353	Body tattoo	
4-0354	Enclosed chewing gum	E160a*
4-0355	Enclosed chewing gum	E 120*, E 133*, E 141*, E 150c*, E 162*
4-0382	Body tattoo	Acryl resin, CI 77499, CI 19140, CI 15985, CI 42090, CI 15850
4-0459	Body tattoo	Choking Hazard, UTA-100
4-0460	Body tattoo	Choking Hazard, TA-w00

*Colorants, which are possibly used for manufacturing the product but not for the tattoo stickers

3 Analysis

3.1 Chemicals and reagents

All-in all 129 reference colorants were obtained through various suppliers, as described in an earlier study (5) on the contents of colorants in cosmetic products. Acetonitrile (ACN, Lichroslov, for chromatography) and tetrabutyl ammonium hydroxide (TBAH, 40%) was purchased from E. Merck, tetrahydrofuran (THF, HPLC grade) from Rathburn, and hydrated citric acid was purchased from M&B.

HPLC Buffer: In a one litre volumetric flask, dissolve 1.4 g citric acid, 6.0 g TBAH in water, adjust pH to 9.0 with ammonia and fill up to the mark with water.
HPLC solvent: HPLC buffer/ACNl/THF (75:12,5:12,5, v/v/v)

3.2 Sample preparation

Weigh the picture tattoos, including the paper, in a centrifuge glass, add 5 ml HPLC solvent and ultrasonicate the mixture for 15 min. Scrape off carefully the coloured material, which still remains on the paper, into the solvent. The paper is removed, dried overnight at room temperature, and finally weighed. The extract is filtered through a Whatman No. 2 filter paper and analysed by HPLC. To the filter paper with undissolved coloured material, 2 ml THF is added and the solution is filtered again through a Whatman No. 2 filter paper, and analysed by HPLC.

3.3 High performance liquid chromatography (HPLC)

All sample extracts and calibration standard solutions are analysed in duplicate by HPLC as described below:

Instrument

HPLC pump	Gradient pump (Waters 616 pump with 600 controller)
Autosampler	Autosampler/Visp (Waters 717)
Detector	PDA detector (Waters 996)
PC-Software sys-	Waters EMPOWER software for PC-control of HPLC
tem	
HPLC column	Precolumn PLRP-S Guard cartridge, 5 mm x 3 mm and analytical column PLRP-S 100 Å, 5 µm, 150 mm x 4,6 mm from Polymer laboratories.
Column temperature	25 °C

HPLC analysis

Run	Gradient
Flow	0,8 ml/min
Mobile phase	As described in the table below
Injections volume	20 µl
Analysis time	35 min
Data collection	In the range of 275 nm-760 nm, 1 spectrum/s, resolution 4.8 nm

Gradient program

Time	*Flow ml/min*	*% HPLC buffer*	*% ACN*	*% THF*	*Curve*
0	0,8	75	12	13	
2,5	0,8	75	12	13	
25	0,8	5,0	47	48	Linear
30	0,8	5,0	47	48	
35	0,8	75	12	13	Linear

3.4 UV-Visible spectra library of reference colorants

HPLC analysis of all reference colorants dissolved in a suitable solvent (5) is performed as described above. Chromatographic data on each colorant is treated with EMPOWER software to generate a ”maxplot” chromatogram, where all substances eluted from the HPLC column show a chromatographic peak at their respective λ_{max}.

3.5 Identification and semi-quantitative determination

A maxplot chromatogram of each sample t is prepared from the chromatographic data on respective sample extract. Identification of colorants in the sample extracts is performed by matching the retention times and spectra of the HPLC peaks with the retention times and spectra of reference colorants analysed under the same conditions (5).

The content of the identified colorants is determined using one point calibration curve for each colorant. The concentrations of the colorants used for the preparation of calibration curves, 0.5-3.0 ppm, varied depending upon the chromatographic response of the respective colorants.

4 Results

All-in-all 42 products of picture tattoos (or products to which picture tattoos were attached) and similar products were purchased from the Danish retail outlets. Two of the products 4-0224 and 4-0225 were purchased twice (4-0287 and 4-0288 respectively) by different persons in the different part of the country. Often, the picture tattoos in each product were different figures composed of different colours. Therefore, 36 picture tattoos (22 products) were selected for the analysis, so that the most of the colorants used in the collected tattoo stickers were represented.

In a preliminary investigation, 6 different picture tattoos were extracted with various solvents (acetone, methanol, hexane, dichloromethane, acetonitrile, tetrhydrofuran, HPLC-solvent and water). Furthermore, both shaking and ultrasonic treatment was used to investigate the dissolution/release of the tattoo colorants into the solvent. The preliminary study revealed that ultrasonic treatment of the samples (picture tattoos sticking on a paper) in a solvent, followed by gently scraping off the remaining coloured material from the paper into the solvent, was the most suitable method for extracting the colorants from picture tattoos into the solvent. Most of the colorants (but not all) were soluble in HPLC-solvent and THF. But some colorants, especially Black and Blue, were not soluble in the solvents used. To perform a systematic investigation of colorants in all picture tattoo samples, the method described in 3.2 was chosen.

Both HPLC-solvent and THF extracts were analysed by HPLC. The colorants in the sample extracts were identified by matching the retention time and spectrum of each HPLC peak with the retention times and spectra of reference colorants analysed under the same conditions. The identification of colorants in some of the sample extracts is shown in Figures 2-5. As shown in Figures 2C and 5B, the spectrum of sample peak does not match 100% with the spectrum of reference colorant. However, this is not unusual when the concentration of the colorant is very low.

The colorants identified in the picture tattoos are described in Table 2. The unidentified colours in the sample extracts as well as the undissolved colours of the samples are also described in Table 2. The unidentified colorants may be those, which are not included in our library of colours or the concentration of these may be below the detection limit (0.5 ppm).

The colorant(s) in two out of the three Black picture tattoos could not be dissolved in the solvents used in the present study. The black colour in the third product (4-0460) was soluble in ACN:THF (1:1).However, the dissolved black colour from this sample could not be identified by the method used in the present investigation (no HPLC peak). Most of the remaining picture tattoos (31 out 33) were composed of colorant CI15850. Other colorants presents in the investigated picture tattoos were: CI 11920 in 2 out of 33 samples, CI 45220 in 10 out of 33 samples, CI 75300 and CI 74180 each in 4 out of 33 samples, CI 13015 and CI 45100 each in 3 out of 33 samples, and CI 14815 in 2 out of 33 samples. CI 15225, CI 16255 and CI 42170 were present only in one picture tattoo each.

The Blue colour CI 74180 was identified in four of the analysed tattoo stickers. However, the Blue colour in several of the samples could not be extracted. This may also be the reason that green (blue+yellow) and violet (blue+red) colours could not be identified in several of the investigated samples. Sample No. 4-0382 is declared to contain CI 19140 (yellow), CI 15985 (yellow) and CI 42090 (blue), but these colorants could not be identified in the subsamples investigated. It is possible that the content of these colorants in the investigated subsamples is below the detection limit (0.5 ppm) of these.

The contents (semi-quantitative determination) of the identified colorants in the investigated picture tattoos are described in Table 3. The content of the colorants is described as total content of these found in HPLC- solvent and THF extracts. In some samples, the content of certain colorants could not be determined because of the interference from other HPLC-peaks, very close to the HPLC-peak of the target colorant in the sample extract. Furthermore, in some samples the content of certain identified colorants could not be determined due to a very small HPLC-peak. In such cases, approximate content (<2 ppm) is given, calculated on the basis of detection limits of these colorants. The contents of the identified colorants in the investigated samples were: 6-4479 ppm CI 11920, <2 -2926 ppm CI 15850, <2- 800 ppm CI 13015, <2-103 ppm CI 45220, <2-35 ppm CI 75300 and 217 ppm CI 15525. The contents of the remaining identified colorants in the tattoo stickers were <12 ppm.

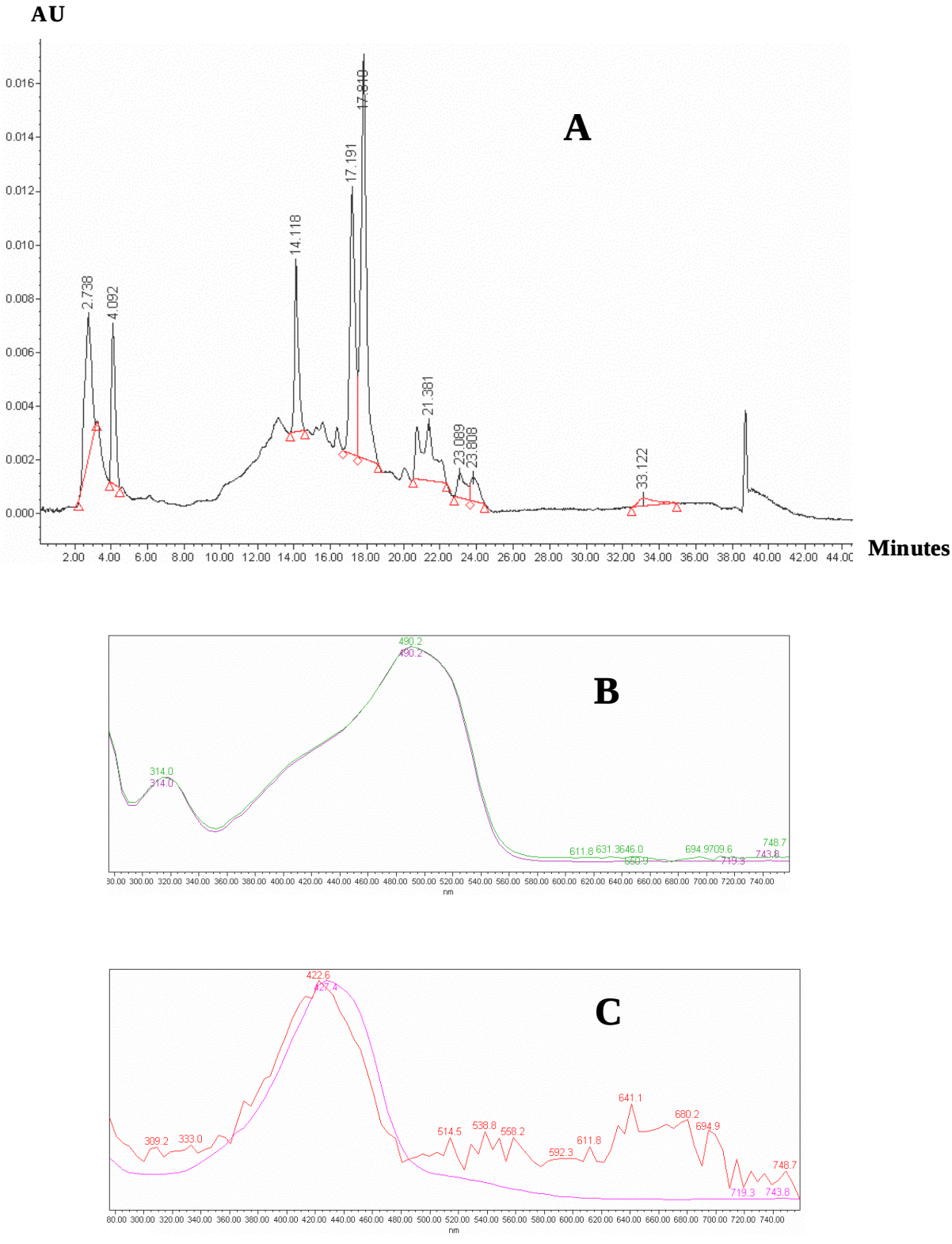

Figure 2: Identification of colorants in the HPLC-solvent extract of sample no. 225-2. A: HPLC chromatogram, B: spectrum of 14,118 min chromatographic peak with the spectrum of CI 15850, C: spectrum of 33,122 min chromatographic peak with the spectrum of
CI 75300.

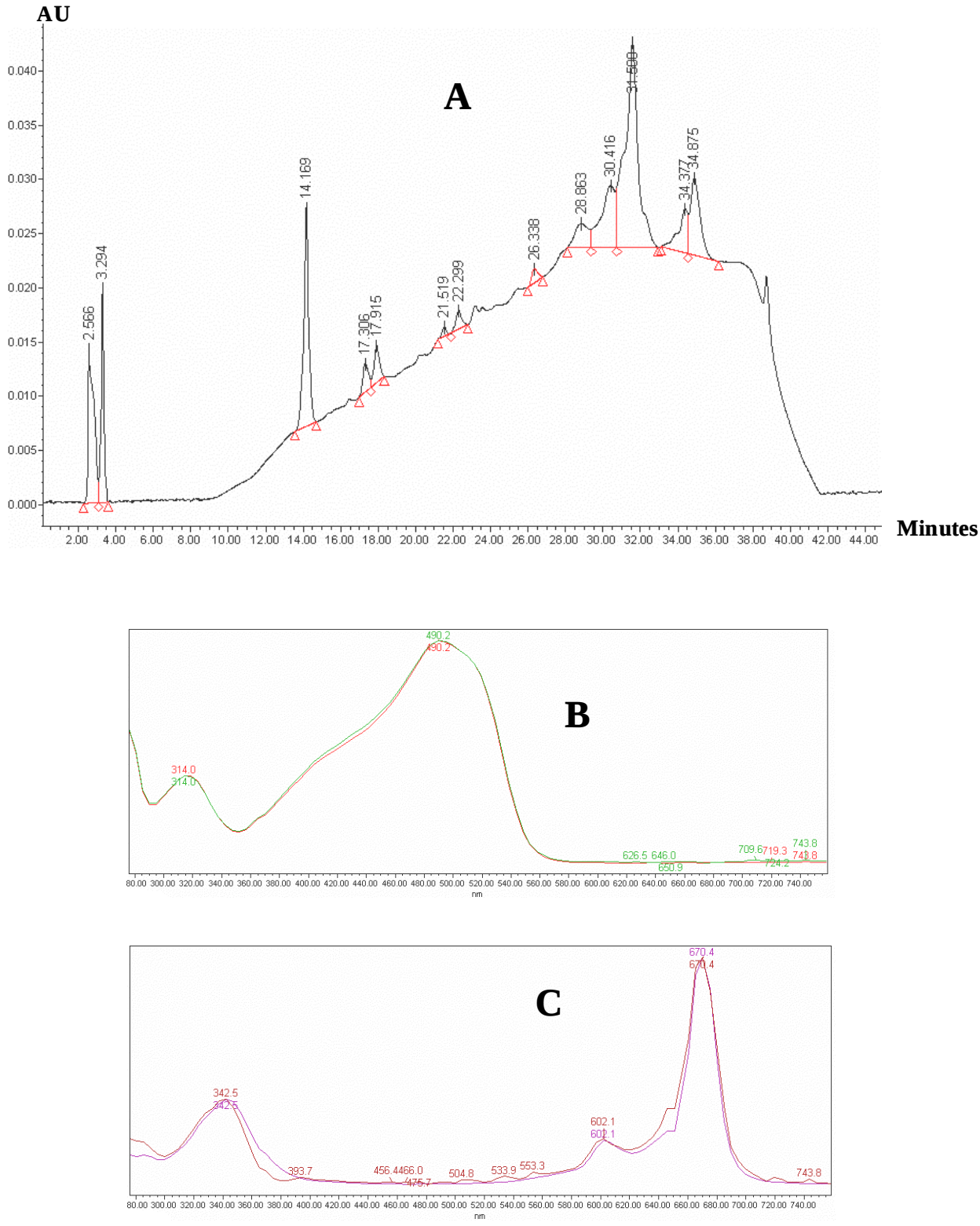

Figure 3: Identification of colorants in THF extract of sample no. 226-2. A: HPLC chromatogram, B: spectrum of 14,169 min chromatographic peak with the spectrum of
CI 15850, C: spectrum of 21,519 min chromatographic peak with the spectrum of CI 74180.

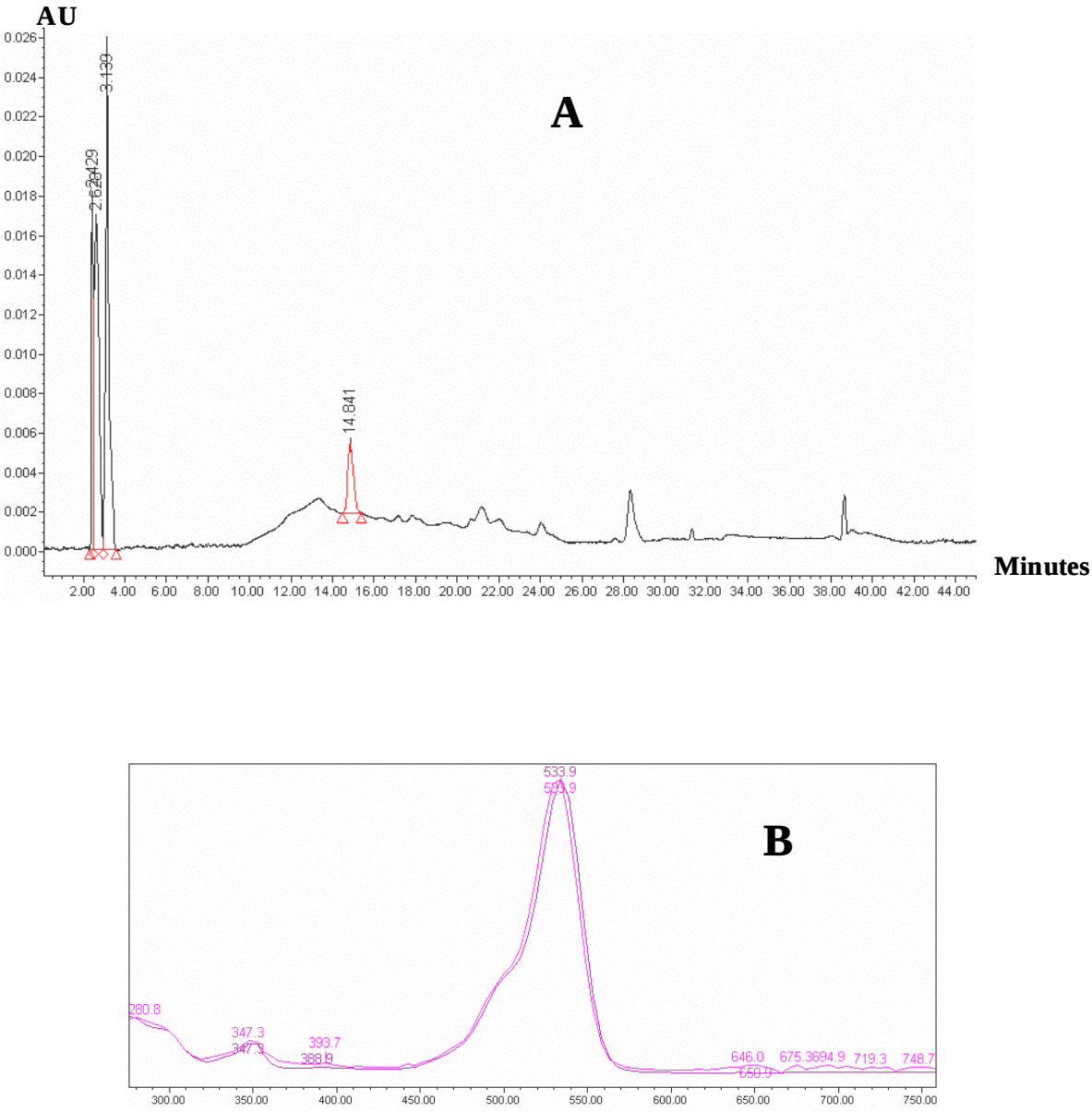

Figure 4: Identification of colorants in the THF extract of sample no.229-2. A: HPLC chromatogram, B: spectrum of 14,841 min chromatographic peak with the spectrum of CI 45220.

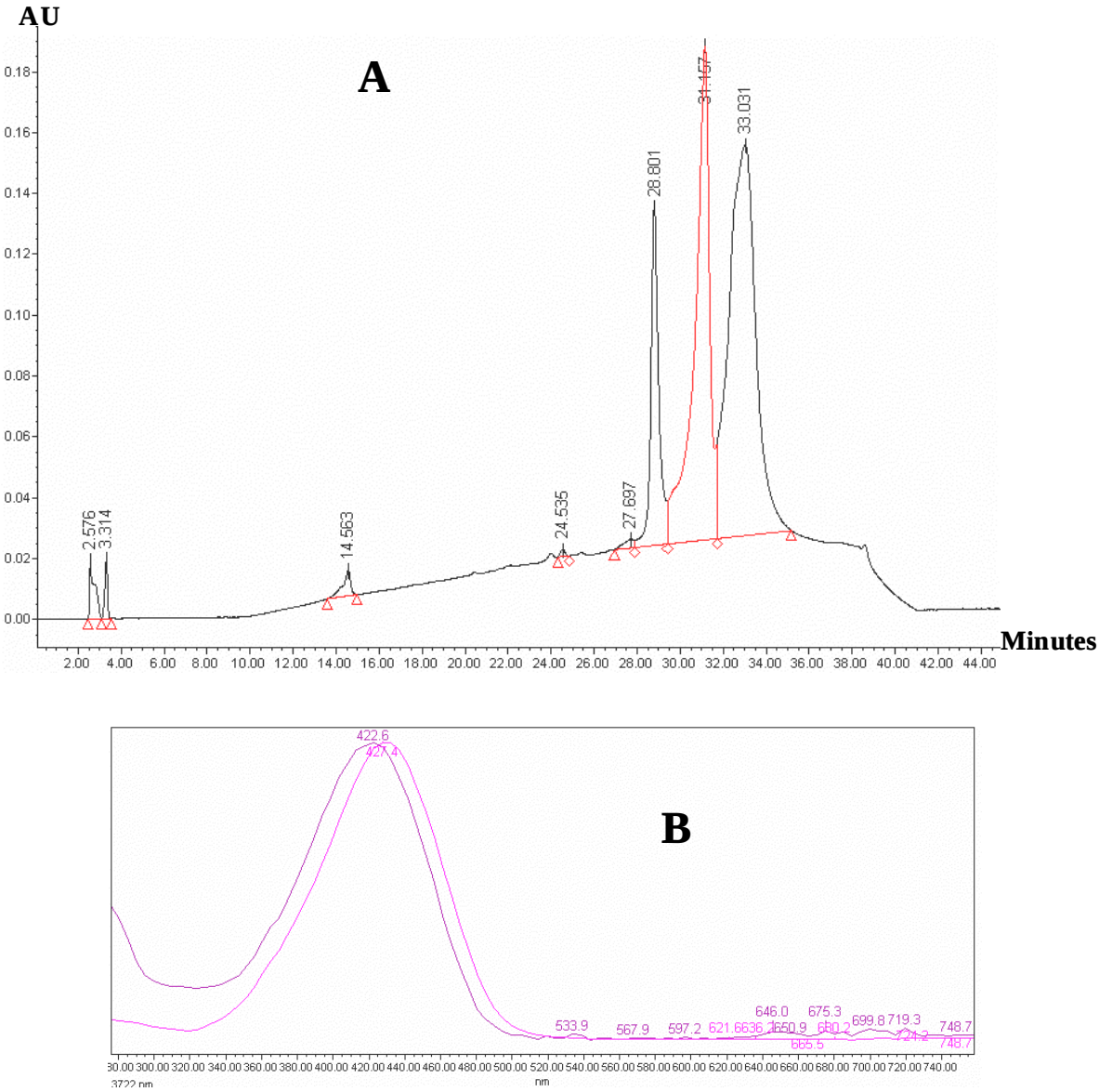

Figure 5: Identification of colorants n the THF extract of sample no. 250-2. A: HPLC chromatogram, B: spectrum of 24,535 min chromatographic peak with the spectrum of CI 11920.

Table 2: Identification of colorants in picture tattoos

Sample No.	Tattoo sticker	Extraction solvent	Identified colorants	Unidentified colorants on picture tattoos	Comments
4-0223-1	Sitting eagle	HPLC	CI 15850		
4-0223-1	Sitting eagle	THF	CI 74180		
4-0223-2	Flying eagle	HPLC	CI 15850		
4-0223-2	Flying eagle	THF	CI 15850, CI 74180		
4-0224-1	Dynamite stick	HPLC	CI 15850		
4-0224-1	Dynamite stick	THF	CI 15850, CI 75300	Blue	
4-0224-2	Cat	HPLC	CI 15850, CI 14815		
4-0224-2	Cat	THF	CI 15850, CI 75300	Blue	
4-0225-1	Rabbit on roller-skate	HPLC	CI 15850		
4-0225-2	Rabbit on roller-skate	THF	CI 15850, CI 14815	Blue, Yellow	
4-0225-1	Rabbit on rostrum	HPLC	CI 15850		
4-0225-2	Rabbit on rostrum	THF	CI 15850, CI 75300	Blue	
4-0226-1	Barbie with Pink hand bag	HPLC	CI 15850		
4-0226-1	Barbie with Pink hand bag	THF	CI 15850, CI 74180		
4-0226-2	Barbie (Pink trousers+Blue T-shirt)	HPLC	CI 15850		
4-0226-2	Barbie (Pink trousers+Blue T-shirt)	THF	CI 15850, CI 74180		
4-0228-1	Woman with fire	HPLC	CI 45220, CI 45100		
4-0228-1	Woman with fire	THF	-	Blue	Undissolved Blue/Black
4-0228-2	Skull with horns	HPLC	CI 45220, CI 45100		

4-0228-2	Skull with horns	THF	CI 15850, CI 75300	Blue	Undissolved Blue/Black
4-0229-1	Mummy	HPLC	CI 15850		
4-0229-1	Mummy	THF	CI 45220	Blue	
4-0229-2	Woman on chair with Sun	HPLC	CI 15850		
4-0229-2	Woman on chair with Sun	THF	CI 45220	Blue, Green	

Table 2: Continued.

Sample No.	Tattoo sticker	Extraction solvent	Identified colorants	Unidentified colorants on tattoo stickers	Comments
4-0231-1	Blue flower	HPLC	CI 15850		
4-0231-1	Blue flower	THF	CI 15850	Blue	
4-0231-2	Red flower	HPLC	CI 15850		
4-0231-2	Bred flower	THF	CI 15850		
4-0232-1	Grasshopper	HPLC	CI 15850		
4-0232-1	Grasshopper	THF	CI 11920	Blue/Violet, Green	
4-0232-2	Larva	HPLC	CI 15850		
4-0232-2	Larva	THF	CI 45220, CI 11920	Blue	
4-0233	Vulcan (lava stone)	HPLC	CI 15850		
4-0233	Vulcan (lava stone)	THF	CI 15850, CI 11920		
4-0234	Dragon (dragon egg)	HPLC	CI 15850		
4-0234	Dragon (dragon egg)	THF	CI 15850, CI 11920	Green	
4-0235	Canon (Canon balls)	HPLC	CI 15850		
4-0235	Canon (Canon balls)	THF	CI 15850, CI 11920	Blue	Undissolved Blue/Black
4-0250-1	Red/Orange Butterfly	HPLC	CI 15850		
4-0250-1	Red/Orange Butterfly	THF	CI 15850, CI 16255, CI 11920		
4-0250-2	Violet butterfly	HPLC	CI 15850		
4-0250-2	Violet butterfly	THF	CI 15850, CI 11920	Violet/Blue	
4-0250-3	Blue butterfly	HPLC	CI 15850		
4-0250-3	Blue butterfly	THF	CI 15850, CI 11920	Blue	
4-0251	“Wild”	-	-	Black	Undissolved Black
4-0294	Dragon	HPLC	CI 15850, CI11920		

4-0294	Dragon	THF	CI 15850	Blue, Violet, Green,	
4-0350	Flower branch	HPLC	CI 15850, CI15525, CI 11920, CI 42170		
4-0350	Flower branch	THF	CI 15850, CI 15525, CI 11920, CI 42170		

Table 2: Continued

Sample No.	**Tattoo sticker**	**Extraction solvent**	**Identified colorants**	**Unidentified colorants on tattoo stickers**	**Comments**
4-0351-1	Man with green mask	HPLC	CI 15850, CI 45220		
4-0351-1	Man with Green mask	THF	CI 15850	Green, Blue	
4-0351-2	Woman with Red dress	HPLC	CI 15850, CI 45220		
4-0351-2	Woman with Red dress	THF	CI 15850	Green, Violet	
4-0352-1	Indian with feather	HPLC	CI 15850, CI 45220		
4-0352-1	Indian with feather	THF	CI 15850	Blue	
4-0352-2	Heart with arrow and butterfly	HPLC	CI 15850, CI 45220		
4-0352-2	Heart with arrow and butterfly	THF	-		Many small (mixed) chromatographic peaks
4-0353	Snake (Black)	-	-	Black	Undissolved Black
4-0354-1	Snake (Green)	HPLC	CI 15850		
4-0354-1	Snake(Green)	THF	CI 15850	Green	Undissolved Blue/Black
4-0354-2	Snake + rose	HPLC	CI 15850		
4-0354-2	Snake + rose	THF	CI 15850	Green	Undissolved Blue/Black
4-0382-1	Yellow + Green birds	HPLC	CI 15850		
4-0382-1	Yellow + Green birds	THF	CI 15850, CI	Green	Undissolved Blue/Black

			11920, CI 13015		
4-0382-2	Red + Blue birds	HPLC	CI 15850		
4-0382-2	Red + Blue birds	THF	CI 15850, CI 11920, CI 13015	Blue	Undissolved Blue/Black
4-0459	Scorpion	HPLC	CI 45220, CI 45100		
4-0459	Scorpion	THF	CI 13065	Blue	
4-0460	Jing/jang (Black/Grey)	ACN/THF	-	Balck/silver	Dissolved in ACN:THF (1:1), no chroma-tographic peak

Table 3: Colorants contents (semi-kvantitativt) in picture tattoos

Sample No.		**Colorant content in ppm**										
	Color-ant	**CI 15850**	**CI 11920**	**CI 45220**	**CI 75300**	**CI 74180**	**CI 13015**	**CI 45100**	**CI 14815**	**CI 15525**	**CI 16255**	**CI 42170**
4-0223-1		580				5						
4-0223-2		556				6						
4-0224-1		76			<2							
4-0224-2		74			<2				<2			
4-0225-1		47							<2			
4-0225-2		72			14							
4-0226-1		161				7						
4-0226-2		314				5						
4-0228-1		-		27				52				
4-0228-2		4		29	35			55				
4-0229-1		982		4								

4-0229-2		561		<1								
4-0231-1		54										
4-0231-2		230										
4-0232-1		25	19									
4-0232-2		69	20	<1								
4-0233		276	4479									
4-0234		82	2907									
4-0235		114	6									
4-0250-1		633	81								5	
4-0250-2		815	99									
4-0250-3		594	125									
4-0251		-										
4-0294		1496	*									
4-0350		36	*							217		<2

Table 3: Continued.

Sample No.		Colorant content in ppm										
	Color-ant	CI 15850	CI 11920	CI 45220	CI 75300	CI 74180	CI 13015	CI 45100	CI 14815	CI 15525	CI 16255	CI 42170
3-0351-1		<3		<1								
3-0351-2		868		11								
3-0352-1		48		96								
3-0352-2		58		103								
3-0353												
3-0354-1		58										
3-0354-2		332										
3-0382-1		8	888				<2				12	
3-0382-2		2391	150				800					
3-0459		-		4			3	6				
4-0460		-										

5 Risk assessment

The present investigation concerns transferable picture tattoos for the skin. They are sold as tattoo pictures fixed on a paper, where from they usually are transferred on to the skin with the help of water, without use of glue, and these can be washed off. As the picture tattoos are in contact with the skin, they can cause contact allergy if they contain allergenic substances, i.e. colorants within the context of the present investigation. Skin contact with the picture tattoos can last for hours or days.

The Danish EPA has received a few complaints about children getting skin reactions by the use of picture tattoos on the skin. The reason for such reactions is not known. A survey of the scientific literature in PubMed did not reveal any information on skin allergy from picture tattoos. Furthermore, no cases of skin reactions to tattoo stickers have been registered, so far, at the Dermatology Departments at University Hospitals in Gentofte and Odense (personal communication). Normally, only the severe cases of skin allergy are referred to the Dermatology Departments in the hospitals. Similarly, only severe cases of allergy are reported in scientific journals. So, there may be undocumented cases of skin reactions to transferable picture tattoos. The skin reactions may be both allergic as well as non-allergic type. However, the Danish Consumer Association, which has direct contact with the general population, has also not registered or notified any case of skin reactions to tattoo stickers (personal communication). Thus, it is stipulated that the potential of tattoo stickers to induce skin reactions is very limited.

As described earlier (se 2.2 Legislation), transferable picture tattoos are cosmetic products, and therefore, they should comply with the Danish Statutory Order on Cosmetic Products/EU Cosmetic Directive (4).

The colorants, which are permitted for the use in the formulation of cosmetic products, are listed in the Annex IV, part 1 of the Cosmetic Directive. For an unequivocal identification of the colorants, they are described in the Annex IV as Colour Index (CI) Numbers. In the present investigation, the contents of 7 of the colorants in the picture tattoos were > 12 ppm. A literature survey on these 7 colorants, with respect to their potential for causing allergic skin reaction, has been performed. The literature survey was performed in PubMed and in Toxline using both their CI No. and CAS No. with the search codes: allergy and/or sensitisation. Furthermore, reference books on allergy were used (6,7).

The most commonly used colorant in the picture tattoos, i.e. CI 15850 was present in 31/33 (94%) of the samples at a concentration up to 2391 ppm. The EU Scientific Committee of Cosmetic Products and Non-Food Products (SCCNFP) has performed a risk assessment of this colorant in May 2004. The summary of an unpublished study on the evaluation of sensitisation potential of CI 15850 by use of " The Local Lymph Node Assay (LLNA)" in mice is described in the SCCNFP Opinion (8). The Committee found that the study was satisfactorily performed according to OECD Guideline 429, and concluded on the basis of the results of the study that CI 15850 was not a sensitiser. Further literature search revealed that the sensitisation potential of

CI 15850 had been studied in two additional investigations. In an animal study in mice employing a modified LLNA (not an official standard method), CI 15850 was shown to cause sensitization under the experimental conditions used (9). CI 15850 is also used as pigment (pigment 57). In a Japanese report, single cases of allergy lipstick containing this pigment have been described (10). In one case the allergic reaction was due to impurities in the pigment.

The colorant CI 11920 was present in 12 out of 33 picture tattoos (36%) at a concentration up to 4479 ppm. The literature search did not reveal any investigation concerning sensitisation potential of this colorant in animals, or allergic reactions from the colorant in humans. However, CI 11920 is classified as moderate to strong sensitiser in a QSAR (Quantitative Structure Activity Relationships) study based on the chemical structure of the substance (11). Further studies are required to confirm the allergenic potential of the colorant.

Ten of the 33 picture tattoos were found to contain CI 45220 at a concentration up to 103 ppm. No animal study concerning sensitisation potential of this colorant, or any study concerning allergic reaction by the colorant in humans were found in the literature. According to the Cosmetic Directive, CI 45220 should only be used in those cosmetic products, which remain in contact with the skin for a short period.

CI 75300, also known as curcumin (12), was present in 4 of the 33 investigated tattoo stickers at a concentration up to 35 ppm. This colorant is widely used in food, where it is a component of curry powder. Curcumin is also used for the coloration of the disinfecting liquids that are used before surgery. There are a few reports of allergy from this colorant in association with food/spice production (13, 14). In a study in which 25 patients were investigated for allergy to anaesthetics, allergy in two patients was recorded due to curcumin (CI 75300) present in the disinfecting liquid that was used before the surgery. The concentration of the colorant in the disinfecting liquid causing allergic reaction was 0.05% (500 ppm) (15). The maximum concentration of CI 75300 in the picture tattoos was found to be 35 ppm, which is at least 10 times lower than the concentration of the colorant in disinfecting liquid responsible for allergic reaction. There is no report of curcumin allergy by the use of picture tattoos, possibly due to less frequent use and low concentration of this colorant in picture tattoos compared to that in the disinfecting liquids that are used before surgery.

Both CI 13015 and CI 45100 were present in 3 out of 33 (9%) picture tattoos. Literature search did not reveal any information concerning allergy to CI 13015.
The SCCNFP has performed the risk assessment of CI 45100 (16). In the Opinion of the SCCNFP, a summary of an unpublished animal study of Guinea Pig Maximisation test (GPMT) is described. Negative results were obtained in this study, which was performed according to OECD Guideline 406. The SCCNFP considered that CI 45100 was not a sensitiser. In a QSAR study, based on the chemical structure of hair dyes, CI 45100 was classified as a weak allergen (11). CI 45100 should, according to the Cosmetic Directive, only be used in those cosmetic products, which remain in contact with the skin for a short period (4). No further information on this colorant was found in the literature.

CI 15525 was found at a concentration level of 215 ppm, but only in one of the investigated tattoo stickers. The literature search did not reveal any information on the allergenic potential of this colorant.

Colorant	No. Product the colorant is found in	Max conc. level (ppm)	Risk assessment in relation to contact allergy
CI 15850 (red)	31	2391	Evaluated not to be a sensitiser based on the existing data, but-single cases of allergy lipstick containing this pigment have been described.
CI 11920 (orange)	12	4479	No investigation on animals or human. However based on models, it is classified as moderate/strong sensitising
CI 45220 (red)	10	103	Evaluated not to be a sensitiser based on the existing data. According to the Cosmetic Directive, CI 45220 should only be used in those cosmetic products, which remain in contact with the skin for a short period.
CI 75300 (yellow)	4	35	Few reports of allergy from this colorant in association with food/spice production and anaesthetics. There is no report of allergy by the use of picture tattoos, possibly due to less frequent use and low concentration of this colorant in picture tattoos compared to that in the disinfecting liquids.
CI 13015 (yellow)	3	800	Evaluated not to be a sensitiser based on the existing data.
CI 45100 (red)	3	55	The SCCNFP considered that CI 45100 was not a sensitiser. In a QSAR study, based on the chemical structure of hair dyes, CI 45100 was classified as a weak allergen. CI 45100 should, according to the Cosmetic Directive, only be used in those cosmetic products, which remain in contact with the skin for a short period. No further information on this colorant was found in the literature.
CI 15525 (red)	1	217	The literature search did not reveal any information on the allergenic potential of this colorant

6 Discussion

Transferable picture tattoos for children (and adults) are used for the decoration of the body, and therefore, they should comply with the Cosmetic Directive. Picture tattoos can be purchased in special shops, and they may also be attached to weekly/monthly magazines, food sold in kiosks and also in supermarkets, during some special campaigns.

The chemical analysis of 36 picture tattoos stickers, bought in Denmark, revealed that only a limited number of colorants were used in the formulation of these products. Thus, only 11 of the 129 organic colorants investigated in the present study were identified in the picture tattoos. All of the identified colorants are permitted for the use according to the Cosmetic Directive. The blue colour in several samples could not be identified. Thus, green and violet colours, which may be composed of blue together with yellow and red respectively, in several samples, could also not be identified. A dark blue/black residue, found after extraction of these samples in HPLC-solvent and THF, may indicate that the blue colour was bound with the matrix (synthetic polymers), and therefore, it was insoluble in the solvents. Another possibility is that the unidentified blue colour is Pigment Blue 29 (CI 77007), which is permitted for the use in cosmetic products. This colorant ($Na_2Al_6Si_6O_{24}S_2$), which is also used for the composition of green, violet and pink pigments, is insoluble in water and organic solvents; and therefore, it is often used in the formulation of decorative cosmetics (12).

The black colour could not be identified in three picture tattoos, which was composed of only this colour (visible). As p-phenylenediamine (PPD) is used as colouring agent in black temporary tattoos and hairs dyes, one of the products (4-02519) was investigated for the content of PPD employing a previously described method (3). PPD was not detected in the sample, at a detection limit of 2 ppm.

The content of the colorants was labelled only in one of the investigated products. However, The declared colorants (CI 15985, CI 19140 and CI 42090) in this sample, could not be identified. It is possible that the contents of these colorants in the investigated sample may be below the detection limit (0.5 ppm), or the colorants were bound with the acryl resin (matrix), which is not soluble in the solvents used for the extraction.

A semi-quantitative determination of the 11 identified colorants in the picture tattoos revealed that the content of 7 of the colorants in the samples was ≥35 ppm (maximum 4479 ppm). Risk assessment of these seven colorants, with regard to their allergenic potential has been performed in the present study. The content of remaining identified colorants in the picture tattoos was maximum 12 ppm.

Among the seven colorants, CI 15850 and CI 75300 have been shown to cause allergic reaction in a few cases, but never in association with the use of picture tattoos. Furthermore, negative results were obtained when the sensitisation potential of CI 15850 and CI 45100 was tested in animal experiments according to OECD guidelines. However, CI 15850 was found to be a skin sensitiser in a test, which was performed using a non-validated method. The

reliability of the results of this test is uncertain due to use of a non-validated method. Two of these seven colorants, i.e. CI 11920 and CI 45100 are also used as hair-dye ingredients, and in this respect, they have also been analysed by QSAR (based of their physical-chemical properties) to determine whether these can induce allergic reactions in humans. The chemical structure of CI 11920 indicates that it may be a moderate to strong allergen. However, further studies are required to confirm the sensitisation potential of this colorant. In the scientific literature, there is no information on allergic reactions in humans from the use of CI 11920. CI 45100 has been classified as a weak allergen on the basis of physical chemical characteristics, and it produced negative results in animal tests for the sensitisation potential employing a validated official method.

The literature survey did not reveal any information concerning allergenic potential of the colorants CI 45220, CI 13015, CI 15525, neither in animal tests nor in humans. These colorants might have been investigated, but the results are not published. Similarly, there may be cases of allergy by the use of these substances, but not reported. A proper risk assessment is always limited to the published reports that are available in the scientific literature. There are published reports of allergic reactions to a number of colorants (6, 7), which are permitted for the use in cosmetic products (Annex IV of the Cosmetic Directive), but none of were found to be present in the investigated picture tattoos. The sparse information on the colorants identified in the present investigation should, therefore, not be considered as the allergenic potential of the colorants, in general, is not investigated.

On the basis of the present investigation, it is concluded that the potential risk of allergic reactions from the identified colorants in transferable picture tattoos is limited. .

The Danish EPA has received a few complaints about children getting skin reactions from the use of picture tattoos. The reason for these skin reactions from picture tattoos is not known. No cases of allergic skin reaction from transferable picture tattoos have been reported in the scientific literature, from selected Dermatology Departments, or from the Danish Consumer Association.

Taking into account the contents of colorants in picture tattoos as well as the risk assessment as described above, allergy problem from the use of picture tattoos seems to be limited.

All identified colorants in the investigated picture tattoos are permitted for the use in cosmetic products. However, the colorants CI 45100 and CI 45220 should only be used in products, which come in contact with the skin for a short period. In most of the investigated products the content of colorants was not declared.

7 References

1. Chung W-H, Chang Y-C, Yang L-J, Hung S-I, Wong W-R, Lin J-Y, Chan H-L. Clinicopathologic features of skin reactions to temporary tattoos and analysis of possible causes. *Arch Dermatol* 2002: 138: 88-92.

2. Sidbury R, Storrs F J. Pruritic eruption at the site of a temporary tattoo. *Am J Contact Derm* 2000: 11: 182-183.

3. Avnstrop C, Rastogi, S C, Menné T. Acute fingertip dermatitis from a temporary tattoo and quantitative chemical analysis of the product *Contact Dermatitis*, 2002: 47: 119-120.

4. Statutory Order on Cosmetic Products, No. 489 of 12. June 2003, Ministry of Environment, Denmark; later replaced by Statutory Order on Cosmetic Products, No. 74 of 14. January 2005, Ministry of Environment, Denmark.
Council Directive 76/768/EEC of 27th July on the approximation of the laws of Member States relating to cosmetic Products. EC Official J., No. L262, 27.9.1976, p.169.

5. Rastogi S C, Barvick V J, Carter S.V. Identification of organic colourants in cosmetics by HPLC-diode array detection. *Chromatographia* 1997: 45: 215-222

6. Rycroft RJG, Menné T, Frosch PJ, Lepoittevin JP. Textbook of contact dermatitis. 3.ed. Springer Verlag 2001.

7. DeGroot AC, Weyland JW, Nater JP. Unwanted effects of cosmetics and drugs used in dermatology. 3.ed Elsevier Science 1994.

8. Opinion of the Scientific Commitee on Cosmetic Products and Non-Food products intended for consumers. Pigment red 57. Adopted by the SCCNFP during the 28th plenary meeting of 25 May 2004.

9. Ikarashi Y, Tsuchiya T, Nakamura A. Application of sensitive mouse lymph node assay for detection of contact sensitization capacity of dyes. Journal Applied Toxicology 1996:16:349-354.

10. Hayakawa R, Fujimoto Y, Kaniwa M. Allergic pigmented lip dermatitis from Lithol Rubine BCA. American Journal Contact Dermatitis 1994:5:34-37.

11. Sosted H, Basketter DA, Estrada E, Johansen JD, Patlewicz GY. Ranking of hair dye substances according to predicted sensitization potency – quantitative structure-activity relationships. Contact Dermatitis 2004: accepted.

12. Deutsche Forschungsgemeinschaft DFG. Kosmetische Färbemittel Colours for Cosmetics. Verlag Chemie GmbH, Weinheim 1984.

13. Goh CL, Ng SK. Allergic contact dermatitis to Curcuma longa (turturmic). Contact Dermatitis 1987:186-187.

14. Hata M, Sasaki E, Ota M, Fujimoto K, Yajima J, Shichida T, Honda M. Allergic contact dermatitis from curcumin (turmeric). Contact Dermatitis 1997:36:107-108.

15. Fischer AL, Agner T. Curcumin allergy in relation to yellow chlorhexidine solution used for skin desinfection prior to surgery. Contact Dermatitis: 2004:

16. Opinion of The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers. Acid Red 52. Adopted by the SCCNFP 23 April 2004 by means of written procedur