

Survey of chemical substances in kohl and henna products

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Contents

PREFACE	5
SAMMENFATNING OG KONKLUSIONER	7
SUMMARY AND CONCLUSIONS	9
1 INTRODUCTION	11
1.1 BACKGROUND	11
1.1.1 Kohl	11
1.1.2 Henna	12
1.2 USE	13
1.3 PRODUCT DESCRIPTION	13
1.3.1 Kohl	13
1.3.2 Henna	13
1.4 CONSUMER TARGET GROUP	15
2 SURVEY	17
2.1 INTRODUCTION	17
2.2 SURVEY	18
2.2.1 Kohl products	18
2.2.2 Henna products	18
2.2.3 Total consumption	18
2.3 PURCHASED PRODUCTS	19
2.3.1 Kohl products	19
2.3.2 Henna products	19
3 CHEMICAL ANALYSES	21
3.1 INORGANIC SUBSTANCES	21
3.1.1 Analyses	21
3.1.2 Methods	21
3.2 ORGANIC SUBSTANCES	22
3.2.1 Analyses	22
3.2.2 Methods	22
4 ANALYSES RESULTS	25
4.1 KOHL PRODUCTS	25
4.1.1 Analysis results - screening	25
4.1.2 Analysis results – quantitative measurements	27
4.2 HENNA PRODUCTS	30
4.2.1 Analysis results – screening of inorganic substances	30
4.2.2 Inorganic quantitative measurements	32
4.2.3 Organic quantitative measurements	33
5 CONCLUSION	35
5.1 KOHL PRODUCTS	35
5.2 HENNA PRODUCTS	36
5.3 SUMMARY CONCLUSION	38
6 REFERENCES	39

Preface

This report on survey of chemical substances in kohl and henna products is a project in the programme "Survey of chemical substances in consumer products" performed by the Danish Environmental Protection Agency.

The purpose of a survey of chemical substances in kohl and henna products is to monitor which undesirable chemical substances that may be contained in kohl and henna products besides the declared substances and to which the consumer may be exposed.

The project is performed by the Danish Technological Institute, Chemistry and Water Technology.

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The project has been divided into three phases. After each phase an interim report was prepared. In co-operation with the Danish Environmental Protection Agency the interim reports were used to select products for the following phase. The interim reports are included in the final report.

Phase 1

In phase 1, a survey of products on the market was performed and a suitable selection of the most common products were purchased.

Phase 2

In phase 2, chemical analyses (screening) of inorganic substances in kohl products and of both inorganic and organic chemical substances in henna products were performed.

Phase 3

Phase 3 consisted of quantitative analyses and report preparation.

The Chemical Inspection Service has dealt with violations, and illegal products are no longer on the market.

Sammenfatning og konklusioner

Som et led i Miljøstyrelsens kortlægning af kemiske stoffer i en række forbrugerprodukter ønskes viden om, hvilke stoffer der indgår i kohl- og hennaprodukter. Projektet Kortlægning af kemiske stoffer i kohl- og hennaprodukter er udført som en undersøgelse, der omfatter kortlægning af markedet samt kvalitative og kvantitative analyser af udvalgte produkters indhold af kemiske stoffer.

Undersøgelsen af markedet i Danmark er udført på basis af oplysninger, der er fremskaffet via:

- Søgning på Internet (telefonnumre og adresser på importører og forhandlere)
- Indkøb af kohl- og hennaprodukter hos detailhandlere og importører og samtidige interviews
- Ved kontakt med leverandører og importører om markedet, data på import, kendskab til andre osv.

Markedet for kohlprodukter omfatter enkelte produkter som pulvere, men de fleste er i fast form. Produktet anbringes omkring øjnene med pensel, stift eller "blyant". Anvendelsen er spredt i befolkningen. Baseret på en hollandsk rapport er der antaget et gennemsnitsforbrug på 10% af befolkningen. Forbruget antages størst hos kvinder generelt.

Markedet for hennaprodukter omfatter især hårfarvningsprodukter. Det professionelle marked har været vigende og er ifølge oplysninger fra SPT næsten ophørt. Det vil sige, at anvendelsen er udbredt til hjemmebrug. Produkterne er i pulverform, pasta eller shampoo.

Der er ikke fundet en separat statistik for hverken kohl- eller hennaprodukter.

Konklusionen var, at markedet i Danmark er rimeligt uoverskueligt. Der foregår ingen produktion af kohl- eller hennaprodukter i Danmark. Importen er spredt på et ukendt antal store og små importører. Import/køb via Internettet er antaget at forekomme, men en stor del hjembringes antageligt også via udlandsrejser eller tilsendes fra familie i udlandet. Opmærksomheden omkring problemer med hennaprodukter kan have betydet et fald i salget de senere år.

Et råt estimat af forbruget er omkring 1 ton kohlprodukter og omkring 10-15 tons hennaprodukter.

I analyserne af kohlprodukter blev bly fundet i hovedparten af de undersøgte produkter. Bly blev kun fundet i store koncentrationer (280 µg/g) i et produkt, som var indisk produceret. De fundne 280 µg/g svarer til ca. 0,028 % af produktet og er således langt under de koncentrationer, der er fundet i de udenlandske undersøgelser.

Foruden bly er der analyseret for en række grundstoffer ved screening. Her blev bl.a. fundet antimon (Sb), arsen (As), barium (Ba), cadmium (Cd), chrom (Cr) og thallium (Tl).

Udvalgte stoffer, der forekom i stor koncentration, blev målt kvantitativt. Her blev der fundet enkelte produkter med en stor koncentration af bor (3,2%), bismuth (7,5%) og zink (11,5%).

I hennaprodukterne blev bly fundet i 10 af de 17 undersøgte produkter. Indholdet i de 10 produkter blev målt i intervallet fra 0,5 mg/kg til 2,0 mg/kg.

p-Phenylendiamin blev fundet i 3 af de 17 hennaprodukter. Indholdet i prøve nr. 11 og 58 var henholdsvis 0,5% (m/m) og 0,003% (m/m), mens indholdet i prøve nr. 57 var 17% (m/m). Den højeste fundne koncentration på 17% (som svarer til 170 g/kg) var over den grænse, der er anført i bekendtgørelsen om kosmetiske produkter.

Lawson blev fundet i 14 ud af 25 analyserede hennaprodukter over detektionsgrænsen på 20 mg/kg. De 23 af de 25 prøver havde angivet at indeholde lawsonia inermis, henna ekstrakt eller "herbal henna". Et produkt angav ikke at indeholde *Lawsonia inermis*, men en anden planteart, og 1 produkt havde ikke indholdsdeklaration.

De største koncentrationer af lawson blev fundet i produkter med pulveriserede plantedele af *Lawsonia inermis*, hvor 6 produkter indeholdt over 1000 mg lawson/kg produkt svarende til >0,1% (m/m). Den største koncentration af lawson blev fundet i et produkt, som var udenlandsk import fra Indien og uden indholdsdeklaration. Den størst fundne koncentration var 3400 mg/kg svarende til 0,34% (m/m). Den næsthøjeste koncentration af lawson blev fundet i et produkt fra England med 3300 mg/kg, svarende til 0,33%. Et produkt fra samme producent indeholdt 2100 mg/kg svarer til 0,21%. De øvrige produkters indhold lå mellem 0,004 og 0,15% af produktet.

Sammenholdes denne analyses resultater med resultater fundet i litteraturen antydes en forskel på "vestligt" producerede og "østligt" producerede kohl produkter.

Det samme er tilsyneladende tilfældet for henna, hvor der blev fundet størst koncentration af barium, p-phenylendiamin og lawson i østasiatiske produkter.

Summary and conclusions

In the Danish Environmental Protection programme on survey of chemical substances in consumer products, a project was initiated to analyse which chemicals were contained in kohl and henna products. The project survey of chemical substances in kohl and henna products has been performed as a study including a survey of the market and qualitative and quantitative analyses of the content of chemical substances in selected products.

The study of the Danish market is performed from information gathered via:

- Search on the Internet (telephone numbers and addresses on importers and dealers)
- Purchases of kohl and henna products at retailers and importers and simultaneous interviews
- Contacting retailers and importers for information on the market, data on imports, sales and knowledge on other dealers, etc.

The market for kohl products includes some products as powders but most products are in solid forms. The product is placed around the eyes with a brush or hard or soft pencil. The use is wide spread in the population. The consumption is assumed to average 10% of the population based on a Dutch report. The consumption is assumed to be primarily by women.

The market for henna products includes mostly hair dyeing products. The professional market has been declining and according to the Danish retail organisation on cosmetics almost non-existing. This indicates that the primary use is by private consumption. The products are found as powders, pasta or shampoo.

No specific statistics on kohl or henna products were found.

The conclusion was that the Danish market is fairly chaotic. No kohl or henna products are manufactured in Denmark. The import is scattered among an unknown number of small and large importers. Import and purchase via the Internet is assumed to take place. A large part is assumed to be brought in via travellers or sent by families in foreign countries. The attention around problems with the use of henna products may have influenced the decline in sales within recent years.

The consumption was estimated to approximately 1 tonnes of kohl products and approximately 10-15 tonnes of henna products.

In the analyses of kohl products, lead was measured in the major part of the examined products. Lead was only found at high levels in 1 product (280 µg/g) that was of Indian origin. The measured 280 µg/g is equivalent to ca. 0.028 % of the product and thus far below the concentrations reported in foreign studies.

Besides lead analyses for several elemental substances were performed by screening. Among others antimony (Sb), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr) and thallium (Tl) were found.

Substances found in substantial amounts in the screening were measured quantitatively. Single products were found with high concentrations of boron (3.2%), bismuth (7.5%) and zinc (11.5%).

In henna products, lead was found in 10 out of 17 studied products. The contents in the 10 products were measured in the range from 0.5 mg/kg to 2.0 mg/kg.

p-Phenylenediamine was observed in 3 out of 17 henna products. The content in sample no. 11 and 58 was 0.5% (m/m) and 0.003% (m/m), respectively, while the content in sample no. 57 was 17% (m/m). The highest concentration of 17% (170 g/kg) exceeded the limit stated in the Directive on cosmetics.

Lawsone was found in 14 out of 25 analysed products above the detection limit. The 23 of the 25 samples had declared to contain lawsone (*Lawsonia inermis*, henna extract or herbal henna). One product did not declare to contain *Lawsonia inermis* but a different plant species. One product did not have an ingredients list.

The highest concentrations of lawsone were found in henna products of powdered plant parts of *Lawsonia inermis* where 6 products contained more than 1000 mg lawsone/kg product corresponding to more than 0.1% (m/m). The highest concentration of lawsone was found in a product that was a foreign import from India and without an ingredients list. The highest measured concentration was 3400 mg/kg corresponding to 0.34% (m/m). The next highest concentration of lawsone was found in a product imported from United Kingdom that contained 3300 mg/kg corresponding to 0.33% (m/m). A product from the same manufacturer contained 2100 mg/kg corresponding to 0.21% of the product. The concentrations in the remaining products were between 0.004 and 0.15% (m/m) of the product.

A comparison between this analysis and the results from literature research indicate a difference between "Western" and "Eastern" manufactured kohl products.

The same appears to be the case for henna products where for instance the highest concentrations of barium, p-phenylenediamine and lawsone were measured.

1 Introduction

1.1 Background

The purpose of the project is to perform a survey of kohl and henna products on the Danish market.

Foreign studies on kohl and henna products have shown that the products may contain harmful substances, which are prohibited in cosmetic products. For instance, in a study by Lekouch *et al.* (2001) a high concentration of lead in kohl products: 54 to 89%, and a lower concentration in henna products: 0.2 to 1.2%, was measured. It could also be mentioned that Hardy *et al.* (2004) and Illes (2000) found that besides galena (lead sulphide, PbS) stibnite (antimony sulphide, Sb_2S_3) was a major component in kohl products.

The purpose is also to clarify whether the consumers are exposed to lead or other metals based on a screening survey. The study starts with a quantitative analysis of lead and a screening for other elements such as e.g. antimony, arsenic, cadmium, gold, iron, copper, cobalt, mercury, manganese, nickel, sulphur, and zinc in kohl and henna products.

1.1.1 Kohl

The traditional eye cosmetic to be put around the eyes is commonly known as kohl. Other names may be used such as kajal, al-kahl or surma. In Western cultures, the name eye liner may be more common although names as kohl and kajal often are included in the product name.

The kohl products previously analysed for their contents have typically been local products from for instance Marocco, Egypt, Saudi Arabia, India and Pakistan (e.g. Al-Ashban *et al.* 2004, Al-Hazzaa and Krahn 1995, Hardy *et al.* 1998 and 2004, Lekouch *et al.* 2001, Nir *et al.* 1992, Parry and Eaton 1991).

Al-Ashban *et al.* (2004) collected 107 kohl samples from different regions of Saudi Arabia and analysed for the presence of lead. Lead levels up to 53% (530 mg/g) were detected in some kohl samples.

Al-Hazzaa and Krahn (1995) analysed the elements in 21 kohl products from Saudi Arabia, India and the Middle East. Seven products did not contain lead while 4 contained between 2.9 and 34.1% lead and 10 products contained more than 84% lead.

Hardy *et al.* (2004) analysed 18 kohl samples, all purchased in Cairo and eleven of them originated in Egypt. The main component of six samples (4 from Egypt and 2 from India) was found to be galena (PbS). For a further ten samples, the main component was found to be one of the following: amorphous carbon, calcite ($CaCO_3$), cuprite (copper(I)oxide, Cu_2O), goethite ($FeO(OH)$), elemental silicon or talc ($Mg_3Si_4O_{10}(OH)_2$). For the last two samples the main component of each was an unknown amorphous organic compound.

In Oman, 47 samples of kohl products were collected, 15 contained lead. Of the lead containing samples 4 originated from India. The remaining 11 samples from India contained, besides lead, amorphous carbon, iron, zinc and calcium (Hardy *et al.* 1998).

Several studies have found increased levels of lead in the blood of infants to whom kohl was applied (e.g. Alkhawajah 1992, Al-Saleh *et al.* 1999, Nir *et al.* 1992, Rahbar *et al.* 2002, Shaltout *et al.* 1981). Also in adult users of kohl, increased lead blood levels have been observed following the use of kohl (Warley *et al.* 1968, Al-Ashban *et al.* 2004).

Some of the inorganic or organic colorants present or potentially present in the products are prohibited in cosmetic products. Inorganic colorants may contain heavy metals, which besides the already mentioned lead, for instance could be antimony, arsenic, cadmium, chromium, mercury, selenium, strontium lactate, -nitrate and -polycarboxylate, tellur, thallium and zirconium.

The toxic effects of lead to humans are well studied. The recognition that children are especially sensitive to very low levels of lead has caused several studies in situations where children could be exposed to lead (e.g. Mojdehi and Gurtner 1996, Rahbar *et al.* 2002, Shaltout *et al.* 1981). Chronic exposure to lead that could result in as low blood lead levels as e.g. 10 µg/dl blood or less may cause mental deterioration, learning disabilities or behavioural abnormalities in children (Vaishnav 2001).

It has been observed that while adults absorb approx. 11% of the lead that reaches the stomach-intestine system it is 30 to 75% for children. By exposure via inhalation approx. 50% is absorbed while less than 1% of lead is absorbed by skin contact (Farley 1998).

In 1992, WHO has set a provisional tolerable weekly intake value (PTWI) of lead to 25 µg/kg body weight for both children and adults. The PTWI value was previously 50 µg/kg bodyweight for adults but was reduced based on the desire to reduce the lead load to unborn children.

In USA, kohl is not allowed as cosmetic. In USA, kohl is defined as colour additive and not allowed to be used in cosmetics (FDA 1996, 2001). However, it is noted that products may use the name kohl without containing kohl and FDA recommends the consumer carefully to check the ingredients list (FDA 2001).

1.1.2 Henna

Henna like kohl is used traditionally in the decoration of women. Extracts from dried powdered leaves of the henna plant (shrub) has been used for centuries by women in the East for the dying of hair. Since 1890 the use has been widely distributed to Europe for the dying of hair. Most common is the use for dying of hair where many shades may be obtained by mixing with leaves from other plants such as e.g. indigo (*Indigofera* sp.). For the coloration of skin and nails, the powder can be mixed with lucerne or catechu, which is a brown coloured substance made from the sap of the Indian tree Black catechu (*Acacia catechu*). The powder is mixed and made into a paste with hot water. The paste is applied to the area desired to be dyed and left on for a few hours (Grieve 1971).

In the series of henna products, some mixed products exist containing among others p-phenylenediamine (CAS no. 106-50-3) and lawsone (2-hydroxy-1,4-naphthoquinone, CAS no. 83-72-7).

p-Phenylenediamine is used with an oxidation agent in oxidatively based hair dyeing preparations. p-Phenylenediamine has by the Scientific Committee on Cosmetics been evaluated to be a strong contact allergen (SCCNFP 2002a).

Lawsone naturally occurs in henna and is evaluated to be mutagenic *in vitro* and *in vivo* in some animal studies and, therefore, to fulfil the criteria to be classified as a category 3: mutagen (SCCNFP 2002b). The Scientific Committee evaluating the studies concluded that lawsone had a genotoxicity/mutagenicity potential and that therefore no safe threshold for lawsone could be established (SCCNFP 2004).

The henna products are therefore also analysed for their content of p-phenylenediamine and lawsone.

In Denmark, henna products have previously been analysed for their content of p-phenylenediamine and lawsone. Four henna products were analysed p-Phenylenediamine was not present in any of the products and lawsone was found in only 1 product at the concentration 0.24% (Rastogi *et al.* 2003).

1.2 Use

Kohl and henna products are both product groups within cosmetics that traditionally have been widely used in Southern Europe, North Africa, the Middle East and parts of Asia among women, children and babies as cosmetic and for medical purposes. The use of both product types are assumed to be widely distributed among the Danish population. Based on a Dutch study (Bremmer *et al.* 2002) it is assumed that an average of 10% of the population uses kohl or eyeliner on a daily basis.

1.3 Product description

1.3.1 Kohl

Kohl is an eye makeup product. The Arabian name for eye makeup is "kohl". Kohl exists as a black or greyish powder, cake or liquid aimed to be applied on the skin in close vicinity to the eyes (Illes 2000). Kohl is often mixed with other chemical substances and is applied to eyebrows, skin area around the eyes or in the treatment of skin diseases in infants (Lekouch *et al.* 2001, Hardy 1998 and 2004).

Kohl products entail a few products as powders but most products exist in a more or less solid form. The product is applied around the eyes with a brush, stick or pencil.

1.3.2 Henna

Henna is a powder or a plant extract from plants or the genus *Lawsonia*. Typically *Lawsonia inermis* is used but *Lawsonia alba* is also mentioned in this context. The henna plant is a small shrub existing naturally in Western Asia

and North Africa. The leaves are picked, dried and grinded to powdered henna. The colouring agent in the henna leaves is called lawsone.

Some henna products claim to be of pure botanical origin and as lawsone is a natural constituent of the plant the substance will be present. The concentration is usually stipulated as less than 0.5 to 1% of lawsone in so-called natural products. Some of the marketed products may be added chemical substances such as phenylenediamines or the entire henna product may be synthetically manufactured.

Henna used in hair care and for hair dyeing is often used as an alternative to permanent chemical hair dyeing. However, the colour nuances of henna is normally limited to the darker shades.

During the survey products were obtained that presented as "black Henna" with the notion that another plant was included (Indigo: *Indigofera tinctoria*). Indigo is claimed not to contain lawsone. At the other end of the colour scale with a minimum of colour was obtained a "Henna neutral" declared to contain the plant *Cassia auriculata*, which also is claimed not to contain lawsone.

The henna powder usually consisting of grinded dry powdered plant parts is used by mixing the powder with hot boiling water stirred to a paste. After a certain cooling period the hot paste is massaged into the dry hair and skull of the head where it is left for approximately 45 to 90 minutes before flushing / washing. Sometimes it is recommended to use a special balsam for a following fixation of the colour making it last longer. Hair dyed with henna usually keeps the colour for the duration of approximately 30 days.

Henna as colorant in temporary tattoos

Henna is traditionally red but for temporary henna tattoos (skin stains) a black henna is used. This henna is added other colorants such as para-phenylenediamine which results in the black colour. These temporary tattoos are not included in the project although both the trade association SPT and the Scientific Committee SCCNFP (SCCNFP 2003) has the opinion that the use of temporary tattoos may be the cause to problems with henna hair dyeing. The argument is supported by several studies (e.g. Läuchl *et al.* 2001, Marcoux *et al.* 2002, Neri *et al.* 2002, Schultz and Mahler 2002, Temesvari *et al.* 2002).

Lawsone

The substance lawsone (INCI name lawsone, CAS no. 83-72-7) exists as mentioned in the dried leaves of *Lawsonia inermis* (CAS no. 84988-66-9) at a concentration up to 1%. The substance may also be chemically manufactured as: 2-hydroxy-1,4-naphthoquinon.

Lawsone is suggested to be used as a non-oxidising colouring agent for hair dyeing at a maximum concentration of 1.5% (typical concentration 1.26%) in the finished cosmetic product (SCCNFP 2001).

The Scientific Committee on Cosmetic products has evaluated several studies on the substance and concluded that lawsone was not suitable for use as a non-oxidising colouring agent for hair dyeing and gave lawsone a classification 2A: the available data support the conclusion that the substance constitutes a health hazard (SCCNFP 2002b).

1.4 Consumer target group

The consumers of kohl products are usually women. The use of black decorations around the eyes is often observable in Danish women and is very widely used by ethnic women who traditionally use such products.

The users of henna products are likewise often women. The use is assumed widely distributed among the entire population.

2 Survey

2.1 Introduction

The survey was performed traditionally by searching in official statistics and approaching importers and retailers, and seeking information from the trade association SPT (Association of Danish Cosmetics, Toiletries, Soap and Detergent Industries), speciality shops and beauty shops.

Statistics Denmark does not have a separate commodity identification number for neither kohl nor henna products. The total trade statistics on consumption (import minus export) for cosmetic products is approximately 20,000 tonnes. In the inventory of Statistics Denmark on import and export, a commodity number CN 3304.20.00 is found that covers import and export of "cosmetics to eyes". Besides eyeliner the presented number include mascara, eye shadows, etc. without any possibility of segregation. According to this statistics the consumption of cosmetics to the eyes is 377 tonnes (or 91 million DKK).

The trade statistics from the trade association SPT is given in Danish currency (cf. figure below). Based on an average price the stated amount indicate a consumption of approximately 20 tonnes. The statistics are, however, uncertain due to the mixing with other commodity groups.

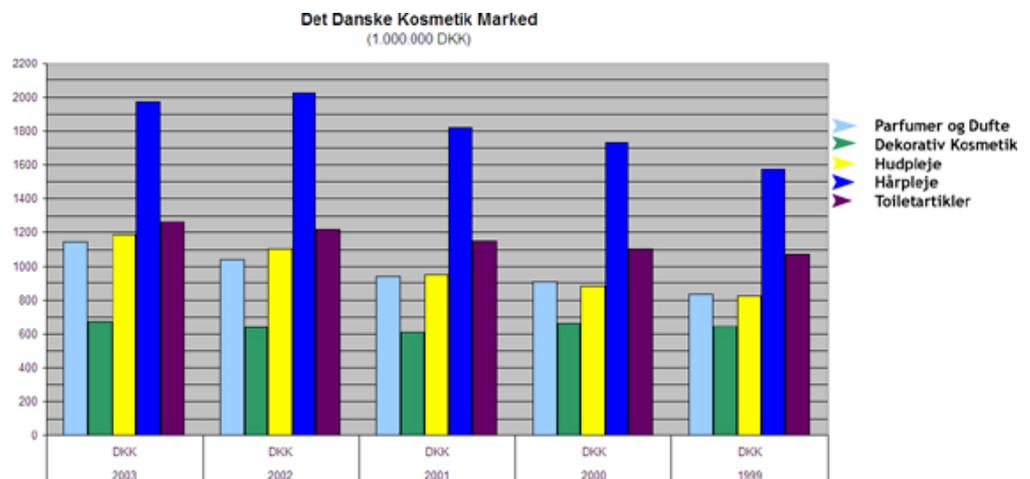


Figure 1. SPT Trade statistics on the Danish market of cosmetics (SPT 2004). Columns represent in order of appearance: perfumes and fragrances, Decorative cosmetics, skin care products, hair care products, and toiletries

Due to a wide use of personal import and no specific registration in Statistics Denmark the assessment of the consumption is based on individual importers and information on their import that most identified importers have made available.

The survey was made difficult as no strict structure with a few importers and a network of retail dealers existed. Besides retailers marketing the established

brands, many retailers perform their own import and consumers buy via the Internet or purchase cosmetics on travels, vacations, etc.

2.2 Survey

Several retail dealers has been visited and questioned to track the importers. The identified importers has been approached by a questionnaire.

2.2.1 Kohl products

Regarding to the kohl products, import and sale data from the sources that wished to participate were variable. The data varied for individual importers from a few hundred to thousands of kohl sticks or pencils per year. The retailers stated the sale of kohl products in numbers and not in weight (kg). Assuming an average weight of approximately 3 grams each, an annual consumption of approximately 350 kg could be accounted for.

An alternative estimate is performed using information from TGD (2003), The Scientific Committee on Cosmetics and Non-Food Products (SCCNFP 2000) and a Dutch report on cosmetics. In the TGD (2003) and SCCNFP (2000) is stated that eye liners are used 1 time daily at an amount of 5 mg each time. In the Dutch report (Bremmer *et al.* 2002) is assumed that kohl (eyeliner) is used 1 time daily by 10% of the population in amounts of 5 mg each time. The exposed skin area is calculated as a line over and under both eyes at a length of 4 cm and 2 mm wide, i.e. in total $4 \times 4 \times 0.2 = 3.2 \text{ cm}^2$. Using a population in Denmark of 5.4 million inhabitants the estimated consumption is $5 \text{ mg} \times 5.4 \cdot 10^6 \text{ persons} \times 0.1 (10\%) \times 365 \text{ days} = 986 \text{ kg/year}$.

2.2.2 Henna products

The sale of henna was considered to be stopped or at best diminishing. SPT states that approximately 8 million hair dyeings were performed in Denmark annually but the number of henna dyeing was unknown. According to SPT Danish hair dressers do not use powdered henna indicating that the consumption may well be solely by the consumers. At any rate the questionnaires could account for an annual consumption of approximately 10 tonnes of henna.

An estimate based on e.g. 100,000 hair dyeings using 100 g henna powder results in approximately 10 tonnes of henna powder/year.

It is assessed from the interviews that a large part is imported directly by the individual retailers from the manufacturing countries or via the Internet. The latter may be performed by both retailers and consumers.

The retailers were especially ethnic shops (Indian, Pakistani, etc), health diet shops, single retail shops and chains of retail stores (Matas, Føtex, etc.). Besides, single retailers may take kohl and henna products in as casuals or cash commodities.

2.2.3 Total consumption

Based on interviews with importers and retailers and their information from questionnaires is concluded that the surveyed market accounted for 0.4 tonnes of kohl and approx. 10 tonnes of henna products. Assuming that minor

retailers and consumers own import is approximately 1 to 2 times the identified import, the total consumption is thus estimated to be approximately 1 tonne of kohl products, which is equivalent to the estimate performed using the Dutch method, and approximately 10 to 15 tonnes of henna products per year.

2.3 Purchased products

2.3.1 Kohl products

Kohl products used traditionally by some groups of immigrants were purchased in ethnic shops. Besides products with a similar designation were found in larger Danish retail stores such as Matas, Føtex, Magasin, etc. where they usually were known as "eyeliner".

2.3.1.1 Collected types

The collected samples are distributed as:

- 1) Powders. Kohl as powder was observed in ethnic shops. They are applied using a brush or moistened with oil or similar.
- 2) Solid forms of small lump on a stick to be moistened before application.
- 3) Stick
- 4) Pencil/crayon with a soft interior

The main part of the products had black, grey or brown colours but violet and blue colours were also available.

According to the Danish Statutory Order on Cosmetics (Bkg. 489, 2003) all cosmetic products must be labelled with an ingredients list. A list of ingredients mentioned in a descending order according to weight at the time they are added to the cosmetic preparation. The list starts with the word "Ingredients".

Of the 37 collected samples, 18 are selected for screening analysis.

The sampling stopped as repetitions became frequent.

2.3.1.2 Selection of products for analysis

The following kohl products is selected based on their ingredients, type (stick / pencil / brush / powder), origin, and market occurrence:

Lab. no. 31431-25, -30, -31, -33, -35, -42, -43, -49, -51, -52, -56, -59, -60, -61, 62, 64, 65, and -66.

2.3.2 Henna products

The henna products were mostly intended for hair dyeing. Henna products were observed in ethnic shops and larger retail stores (Matas, Føtex, etc.).

2.3.2.1 Collected types

Of the collected samples the products are distributed among powders, paste and shampoo.

The henna products were found in ethnic stores and in other retail stores.

The products had a large selection of shades but mostly of a reddish brown nuances.

Of the 25 collected samples, 17 are selected for screening analysis.

The sampling stopped as repetitions became frequent.

2.3.2.2 Selection of products for analysis

The following henna products are selected based on their ingredients, type (powder / paste), the range in assortment and origin:

Lab. no.: 31341-1, -2, -3, -4, -5, -8, -9, -10, -11, -12, -14, -20, -21, -23, -57, -58 and -63.

3 Chemical analyses

3.1 Inorganic substances

3.1.1 Analyses

In co-operation with the Danish Environmental Protection Agency, 18 kohl and 17 henna products were selected for quantitative analysis of lead and screening for elements and among others heavy metals.

The screening analyses covered all elements from atomic mass 6 (lithium) to mass 238 (uranium). The analyses were performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after microwave induced preparation of the products with nitric acid in autoclaves. Selected elements including heavy metals were quantified subsequently by ICP-MS and/or Inductively Coupled Plasma Atom Emission Spectrometry (ICP-AES).

Lead (Pb) was quantified in all products and the same were elements that were measured at a concentration above 10 mg/kg by the screening analysis.

The detection limits in the screening analyses were in the range from 0.05 to 10 mg/kg.

In relation to the quantification of selected elements, duplicate determinations were performed and the %RSD calculated.

The found content of elements is compared to the regulation in the Statutory Order on cosmetics if the substance is regulated.

3.1.2 Methods

3.1.2.1 Test preparation

Approximately 500 mg sample – precisely weighed – was prepared by microwave induced heating in a PFA autoclave with 20 ml 7 M HNO₃ (subboiling quality). The resulting solution was filtered and then diluted to 50 ml using demineralised water (Milli-Q Plus).

Duplicate preparations were performed.

Blanks were prepared in the same way.

3.1.2.2 Standards

Standards and control samples were prepared from a Perkin-Elmer and a Merck multi-element standard solution, respectively, by dilution with 2.8 M HNO₃. The internal standard solution was prepared from Perkin-Elmer single element standards of Ge, Rh and Re by dilution with 0.14 M HNO₃.

3.1.2.3 Apparatus

A Perkin-Elmer Sciex Elan 6100 DRC Plus ICP Mass Spectrometer with a FIAS 400 flow injection system and autosampler AS 93 Plus and a Perkin-Elmer Optima 3300 DV inductively-coupled-plasma atomic emission spectrometer with autosampler AS-90 plus were used.

3.1.2.4 Screening analysis

Adding germanium, rhodium and rhenium as internal standards "on-line", the prepared solutions were screened for content of trace elements by inductively-coupled-plasma mass spectrometry (ICP-MS) using the expert programme TotalQuantIII that from an instrument response curve for the elements from mass 6 (Li) to mass 238 (U) quantifies the content. The instrument response curve was updated by a multi-element standard containing 30 elements that covers the total mass range. The elements Br, C, Cl, F, I, N, O, P and S are not quantified due to interferences.

3.1.2.5 Quantitative analysis

All prepared solutions were analysed quantitatively for lead by ICP-MS and the same were elements which by the screening were measured at a concentration higher than 10 mg/kg (Zn 50 mg/kg). Cobalt, copper, nickel and vanadium were analysed by ICP-MS and boron, barium, bismuth, manganese, strontium and zinc by ICP-AES.

3.2 Organic substances

3.2.1 Analyses

Selected henna products were analysed quantitatively for the content of p-phenylenediamine and lawsone.

The analysis for p-phenylenediamine was performed by capillary gas chromatography using mass spectrometric detection (GC-MS) after extraction of the substance with dichloromethane (DCM). The detection limit for p-phenylenediamine was in the range from 4 to 20 mg/kg.

The analysis for lawsone was performed by liquid chromatography using mass spectrometric detection (LC-MS) after extraction of the substances with ethanol/water. For lawsone the detection limit was 20 mg/kg.

Duplicate determinations were performed and the %RSD is calculated.

3.2.2 Methods

3.2.2.1 Test preparation of p-phenylenediamine

Approximately 1 g sample – precisely weighed – in a 50 ml vial was added 20 ml extraction solution containing naphthalene-d₈, aniline-d₅ and phenol-d₅ as internal standards in dichloromethane. The sample was first ultrasonically extracted for 1 hour and then mechanically extracted for 1 hour further and finally centrifuged.

Duplicate determinations were performed.
Blanks were prepared in the same way.

3.2.2.2 Test preparation of lawsone

Extraction and sample preparation performed according to Bakkali *et al.* (1997). Approximately 50 mg sample – precisely weighed – in a 50 ml vial was added 50 ml extraction solution. The sample was extracted for 16 hours under constant agitation, then centrifuged, added 50 µl acetic acid and extracted with 3 x 10 ml chloroform. The extracts were dried over sodium sulphate, evaporated to dryness and re-dissolved in methanol and added water

corresponding to the eluent composition. Blanks and recovery controls were made according to the same procedure. The recovery was 83 %.

3.2.2.3 Quantitative analysis for p-phenylenediamine

External standards were made from p-phenylenediamine (Sigma P6001/033K3482, CAS no. 106-50-3) in the extraction solution.

The analysis was performed using GC-MS.

3.2.2.4 Quantitative analysis for lawsone

External standards were made from lawsone (2-hydroxy-1,4-naphthoquinone, Sigma H-0508 lot no. 072K3652, CAS no. 83-72-7) in the extraction solution.

The analysis was performed using LC-MS with methanol/water as eluent.

Analyte	DL µg/g	%RSD	Sample no.: 31341-								
			52	56	59	60	61	62	64	65	66
			µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Cs	0.1	5.3%	8.2	0.33	-	0.39	-	-	-	-	-
Ba	0.1	4.8%	38	1.4	5500	11	2.7	1.2	1.5	1.5	88
La	0.05	2.1%	-	0.08	0.08	1.0	0.31	0.25	0.16	0.12	0.15
Ce	0.05	3.1%	0.06	0.16	0.06	1.7	0.24	0.20	0.11	0.09	0.29
Pr	0.05	2.5%	-	-	-	0.28	-	0.05	-	-	-
Nd	0.05	2.3%	-	0.1	-	1.5	0.11	0.12	0.08	0.06	0.16
Sm	0.05	11%	-	-	-	0.57	-	-	-	-	-
Eu	0.05	10%	-	-	0.52	0.12	-	-	-	-	-
Gd	0.05	10%	-	-	-	0.91	-	-	-	-	-
Tb	0.05	3.6%	-	-	-	0.14	-	-	-	-	-
Dy	0.05	3.6%	-	-	-	1.0	-	-	-	-	-
Ho	0.05	5%	-	-	-	0.30	-	-	-	-	-
Er	0.05	4.9%	-	-	-	1.1	-	-	-	-	-
Tm	0.05	7.3%	-	-	-	0.20	-	-	-	-	-
Yb	0.05	7.1%	-	-	-	1.6	-	-	-	-	-
Lu	0.05	5.5%	-	-	-	0.33	-	-	-	-	-
Hf	0.05	8.7%	0.08	0.25	-	-	-	-	-	-	-
Ta	0.05	16%	-	-	-	-	-	-	-	-	-
W	0.05	20%	3.4	-	-	-	-	-	-	-	-
Os	0.05	-	-	-	-	-	-	-	-	-	-
Ir	0.05	-	-	-	-	-	-	-	-	-	-
Pt	0.05	-	-	-	-	-	-	-	-	-	-
Au	0.05	-	-	-	-	-	-	-	-	-	-
Hg	0.5	-	-	-	-	-	-	-	-	-	-
Tl	0.05	11%	0.28	-	-	0.05	-	-	-	-	-
Pb	0.05	4.1%	3.1	0.43	0.76	280	3.8	2.9	0.48	0.75	4.0
Bi	0.05	9.6%	4.9	0.7	-	0.11	-	-	0.14	0.1	0.32
Th	0.05	5.5%	-	-	-	0.06	-	-	-	-	-
U	0.5	-	-	-	-	-	-	-	-	-	-

DL : Detection limit

%RSD presents the percentage relative standard deviation made as common estimate based on the relative standard deviation from duplicate determinations.

4.1.2 Analysis results – quantitative measurements

The analyses results from the quantitative measurements for inorganic substances in the selected kohl products is presented in the tables below.

It should be noticed that deviations may occur when comparing the quantitative results with the screening results. In that case the quantitative results are the preferred values as they consider circumstances that may affect the analysis which is not the case to the same extent in the screening.

Table 2. Quantitative measurements of lead (Pb) in kohl products

Sample number	Pb mg/kg
25	0.30
30	0.31
31	-
33	-
35	0.81
42	0.71
43	0.39
49	-
51	0.86
52	2.8
56	0.36
59	0.78
60	280
61	4.2
62	3.1
64	0.53
65	0.84
66	4.2
%RSD	6.6

"-" denotes measurement below detection limit of 0.1 mg/kg.

%RSD presents the percentage relative standard deviation made as common estimate based on the relative standard deviation from duplicate determinations.

Further quantitative determinations on elements were performed when a content higher than 10 mg/kg were observed in the screening with the exception of normally occurring elements such as aluminium, calcium, iron, magnesium, silicon and titanium and the alkali metals. The results are listed in the table below.

Table 3. Quantitative measurements of other elements in kohl products

Sample number	B mg/kg	Ba mg/kg	Bi mg/kg	Co mg/kg	Cu mg/kg
25	420	-	-	28	-
30	-	-	-	30	-
31	-	-	-	47	-
33	-	-	-	28	-
35	-	-	-	13	-
42	-	-	-	26	-
43	-	-	-	37	-
49	-	-	-	-	-
51	-	-	74900	-	-
52	-	53	-	33	-
56	-	-	-	25	-
59	-	6700	-	-	-
60	32000	14,5	-	-	950
61	NA	-	-	-	-
62	-	-	-	-	-
64	-	-	-	-	-
65	-	-	-	-	-
66	NA	NA	-	-	-
%RSD	3.1	3.7	0.6	3.5	7.3

(the table continues)

Table 3. continued

Sample number	Mn mg/kg	Ni mg/kg	Sr mg/kg	V mg/kg	Zn mg/kg
25	465	31	-	-	-
30	377	33	-	-	-
31	710	23	-	-	-
33	334	29	-	-	-
35	270	37	-	-	-
42	344	26	-	-	-
43	421	41	-	-	-
49	554	15	-	-	-
51	490	-	-	-	-
52	474	38	-	-	-
56	363	39	-	-	-
59	297	17	120	-	-
60	190	65	280	74	115000
61	-	55	-	62	-
62	-	24	-	-	-
64	-	16	-	-	-
65	-	-	-	-	-
66	-	-	NA	-	-
%RSD	1.6	7.6	2.3	1.4	1.1

∴ Below detection limit

NA: Not analysed

%RSD presents the percentage relative standard deviation made as common estimate based on the relative standard deviation from duplicate determinations.

It is noticed that:

- Lead (Pb) occurs in 15 out of 18 products above the detection limit of 0.1 mg/kg. The concentrations ranges 0.3 to 280 mg/kg. Lead occurs at the highest concentration in product no. 60 (280 mg/kg), cf. table 2.
- Boron (B) occurs in 2 products and at the highest concentration in product no. 60 (32000 mg/kg corresponding to 3.2%), cf. table 3.
- Barium (Ba) occurs in 3 products but only at high concentration in product no. 59 (6700 mg/kg).
- Bismuth (Bi) occurs in product no. 51 at high concentration (74900 mg/kg corresponding to 7.5%).
- Cobalt (Co) occurs in 9 out of 18 analysed products at the range 13 to 47 mg/kg.
- Manganese (Mn) occurs in 13 out of 18 products at the range 190 to 710 mg/kg.
- Nickel (Ni) occurs in 15 out of 18 products at the range 15 to 65 mg/kg.
- Strontium (Sr) occurs in 2 products at the range 120 to 280 mg/kg.
- Vanadium (V) occurs in 2 products at the range 62 to 74 mg/kg.
- Zinc (Zn) occurs in product no 60 at a high concentration (115000 mg/kg corresponding to 11.5% m/m).

Analyte	Name	DL µg/g	%RSD	Sample no.: 31341-							
				1	2	3	4	5	8	9	10
				µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
W	Tungsten	0.05	20%	-	0.06	-	-	-	-	-	-
Os	Osmium	0.05	-	-	-	-	-	-	-	-	-
Ir	Iridium	0.05	-	-	-	-	-	-	-	-	-
Pt	Platinum	0.05	-	-	-	-	-	-	-	-	-
Au	Gold	0.05	-	-	-	-	-	-	-	-	-
Hg	Mercury	0.5	-	-	-	-	-	-	-	-	-
Tl	Thallium	0.05	11%	-	-	-	-	-	-	-	-
Pb	Lead	0.05	4.1%	-	1.2	0.76	-	1.8	1.4	-	-
Bi	Bismuth	0.05	9.6%	-	-	-	-	-	-	-	-
Th	Thorium	0.05	5.5%	-	0.73	0.45	-	1.1	0.3	-	-
U	Uranium	0.5	-	-	-	-	-	-	-	-	-

Analyte	DL µg/g	%RSD	Sample no.: 31341-								
			11	12	14	20	21	23	57	58	63
			µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Li	0.5	4.6%	-	2.9	-	2.3	-	5.9	1.5	1.4	1.6
Be	1	14%	-	-	-	-	-	-	-	-	-
B	5	4.6%	-	31	-	30	-	63	42	25	22
Na	1	1.9%	3900	1200	>	2900	4100	3600	3000	3600	1200
Mg	1	1.7%	1.3	4100	22	2700	1.3	5600	5500	8600	2600
Al	1	4.7%	4.5	1300	2.5	1400	7.8	3400	770	510	460
Si	10	6.3%	13	710	11	720	12	1000	380	330	580
K	5	2.8%	20	>	74	5800	41	7300	2800	2600	5700
Ca	5	2.8%	13	15000	10	11000	6.2	16000	7500	6700	12000
Sc	0.5	4.1%	-	-	-	0.53	-	1.1	-	-	-
Ti	0.5	6.5%	-	57	-	56	-	160	27	29	29
V	0.5	4.4%	-	2.9	-	2.5	-	8.7	2.3	2.0	1.5
Cr	0.5	2.2%	-	3.3	-	10	-	6.0	3.2	5.7	5.7
Mn	0.1	0.99%	-	78	-	68	-	170	35	46	95
Fe	10	8.2%	-	1500	-	1600	-	2400	810	640	600
Co	0.1	5.6%	-	0.90	-	0.7	-	2.0	0.59	0.62	0.78
Ni	0.5	4.4%	-	3.9	-	3.0	-	8.7	2.1	1.9	1.9
Cu	0.1	3.6%	0.20	7.8	0.14	4.9	0.17	12	4.4	5.1	8.1
Zn	0.5	6.8%	1.4	20	19	11	3.0	22	76	71	14
Ga	0.1	8.0%	-	0.45	-	0.47	-	0.97	0.30	0.21	0.22
As	1	17%	-	-	-	-	-	2.3	-	-	-
Se	1	29%	-	-	-	-	-	-	-	-	2.4
Rb	0.1	2.9%	-	5.1	-	9.6	-	3.3	2.3	1.5	9.6
Sr	0.1	2.1%	-	180	0.13	91	-	210	180	210	120
Y	0.1	2.4%	-	0.62	-	0.80	-	1.5	1.1	0.89	0.36
Zr	0.1	11%	-	-	-	0.68	-	0.60	0.20	-	0.14
Nb	0.1	3.1%	-	0.19	-	0.26	-	0.20	-	-	-
Mo	0.1	4.9%	-	1.7	-	0.75	-	0.60	0.17	0.15	0.29
Ru	0.1	-	-	-	-	-	-	-	-	-	-
Pd	0.1	-	-	-	-	-	-	-	-	-	-
Ag	0.1	1.8%	-	-	-	-	-	-	-	-	-
Cd	0.05	12%	-	0.06	-	-	-	0.07	-	-	0.07
In	0.05	19%	-	-	-	-	-	-	-	-	-
Sn	0.5	4.8%	-	-	-	-	-	-	-	-	-
Sb	0.05	6.0%	-	-	-	-	-	-	-	-	-
Te	0.1	30%	-	-	-	-	-	-	-	-	-
Cs	0.1	5.3%	-	0.30	-	0.43	-	0.46	0.28	0.21	0.19
Ba	0.1	4.8%	0.61	32	0.30	15	0.32	15	160000	140000	51
La	0.05	2.1%	-	0.72	-	1.6	-	1.3	1.3	1.1	0.43
Ce	0.05	3.1%	-	1.6	-	3.2	-	2.8	1.3	0.86	0.88
Pr	0.05	2.5%	-	0.20	-	0.38	-	0.36	0.17	0.11	0.11
Nd	0.05	2.3%	-	0.84	-	1.5	-	1.6	0.7	0.46	0.45
Sm	0.05	11%	-	0.19	-	0.31	-	0.36	0.71	0.65	0.11
Eu	0.05	10%	-	-	-	-	-	0.09	12	12	-
Gd	0.05	10%	-	0.21	-	0.30	-	0.42	0.17	0.12	0.11
Tb	0.05	3.6%	-	-	-	-	-	0.06	-	-	-
Dy	0.05	3.6%	-	0.14	-	0.19	-	0.33	0.12	0.08	0.08

Analyte	DL µg/g	%RSD	Sample no.: 31341-									
			11	12	14	20	21	23	57	58	63	
			µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Ho	0.05	5%	-	-	-	-	-	-	0.06	-	-	-
Er	0.05	4.9%	-	0.06	-	0.09	-	-	0.16	0.06	-	-
Tm	0.05	7.3%	-	-	-	-	-	-	-	-	-	-
Yb	0.05	7.1%	-	-	-	0.06	-	-	0.14	0.05	-	-
Lu	0.05	5.5%	-	-	-	-	-	-	-	-	-	-
Hf	0.05	8.7%	-	-	-	-	-	-	-	-	-	-
Ta	0.05	16%	-	-	-	-	-	-	-	-	-	-
W	0.05	20%	-	0.1	-	0.17	-	-	-	-	-	-
Os	0.05	-	-	-	-	-	-	-	-	-	-	-
Ir	0.05	-	-	-	-	-	-	-	-	-	-	-
Pt	0.05	-	-	-	-	-	-	-	-	-	-	-
Au	0.05	-	-	-	-	-	-	-	-	-	-	-
Hg	0.5	-	-	-	-	-	-	-	-	-	-	-
Tl	0.05	11%	-	-	-	-	-	-	-	-	-	-
Pb	0.05	4.1%	-	1.1	-	1.3	-	-	1.5	0.62	0.46	1.3
Bi	0.05	9.6%	-	0.09	-	-	-	-	-	-	-	-
Th	0.05	5.5%	-	0.51	-	1.8	-	-	0.43	0.55	0.36	0.32
U	0.5	-	-	-	-	-	-	-	-	-	-	-

DL: Detection limit

%RSD presents the percentage relative standard deviation made as common estimate based on the relative standard deviation from duplicate determinations.

4.2.2 Inorganic quantitative measurements

The quantitative measurements of inorganic substances in the selected henna products are shown in the tables below.

Table 5. Quantitative measurements of Lead (Pb) in henna products

Sample number	Pb mg/kg
1	-
2	1.37
3	0.89
4	-
5	2.0
8	1.5
9	-
10	-
11	-
12	1.24
14	-
20	1.5
21	-
23	1.7
57	0.57
58	0.47
63	1.3
%RSD	6.7

"-" denotes measurement below detection limit of 0.1 mg/kg.

%RSD presents the percentage relative standard deviation made as common estimate based on the relative standard deviation from duplicate determinations.

Correspondingly, quantitative determinations of elements, which at the screening were measured at a concentration above 10 mg/kg, were performed. However with the exception of normally occurring elements such as aluminium, calcium, iron, magnesium, silicon and titanium and the alkali metals, which were included. The results are listed in the tables below.

Table 6. Quantitative measurements of other elements in henna products

Sample number	B mg/kg	Ba mg/kg	Mn mg/kg	Sr mg/kg
1	-	-	-	-
2	112	37	189	407
3	157	47	230	710
4	-	-	-	-
5	110	31	144	406
8	48	24	78	219
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	60	49	104	272
14	-	-	-	-
20	57	26	91	145
21	-	-	-	-
23	111	24	234	307
57	53	178000	45	296
58	35	170000	65	357
63	43	65	122	180
%RSD	3.0	6.7	2.2	2.1

"-" denotes measurement below detection limit of 0.1 mg/kg.

%RSD presents the percentage relative standard deviation made as common estimate based on the relative standard deviation from duplicate determinations.

It is noted that:

- Lead (Pb) occurs in 10 out of 17 products at the range 0.5 to 2.0 mg/kg.
- Boron (B) occurs in 10 out of 17 products at the range 35 to 157 mg/kg.
- Barium (Ba) occurs in 10 out of 17 products at the range 24 to 178000 mg/kg. Barium was found at high concentration in product no. 57 (178000 mg/kg corresponding to 17.8% m/m) and at almost the same concentration in product no 58. The remaining products were found at the range 24 to 65 mg/kg.
- Manganese (Mn) occurs in 10 out of 17 products at the range 45 to 234 mg/kg.
- Strontium (Sr) occurs in 10 out of 17 products at the range 145 to 710 mg/kg.

4.2.3 Organic quantitative measurements

The primarily selected 17 henna products were analysed quantitatively for the content of para-phenylenediamine and lawsone.

p-Phenylenediamine was found in 3 products: In product no. 58 at a low concentration near the detection limit. In product no. 11 in a tube containing paste, p-phenylenediamine was found at 5000 mg/kg corresponding to 0.5% m/m. In product no. 57, 170000 mg/kg was found, corresponding to 17% m/m, cf. table 7.

Based on the results of the lawsone analyses, the Danish Environment Protection Agency decided that also the remaining henna products should be analysed for lawsone.

The results are presented below.

Table 7. Quantitative measurements of p-phenylenediamine and Lawsone in henna products

Sample number	p-phenylenediamine mg/kg	Lawsone mg/kg
1	< 20	< 20
2	< 2	310
3	< 2	40
4	< 2	< 20
5	< 2	3300
8	< 2	1300
9	< 2	< 20
10	< 2	< 20
11a (bag with paste)	< 2	
11b (tube with paste)	5000	< 20
12	< 10	1100
14	< 2	390
20	< 20	160
21	< 2	< 20
23	< 20	1500
57	170000	50
58	27	190
63	< 20	3400
%RSD*	11	26
Supplementary measurements for lawsone:		
4		< 20
6		2100
7		730
13		< 20
15		< 20
17		< 20
18		91
22		< 20
%RSD		11

*: %RSD presents the percentage relative standard deviation made as common estimate based on the relative standard deviation from duplicate determinations.

Lawsone was found in 11 out of the 17 initially analysed products above the detection limit of 20 µg/g (= mg/kg). The 16 of the 17 samples had declared to contain lawsone (*Lawsonia inermis*). Only product no. 3 had not declared to contain lawsone or *Lawsonia inermis* but a different plant species (*Indigofera tinctoria*).

The highest concentration of lawsone was found in product no. 63 that was a foreign import from India and without an ingredients list. The highest found concentration was 3400 mg/kg corresponding to 0.34% (m/m). The next highest concentration of lawsone was found in product no. 5 manufactured in the United Kingdom. The product contained 3300 mg/kg corresponding to 0.33% (m/m). The remaining products contained lawsone below the detection limit (6 products) or had concentrations between 38 and 1500 mg/kg, i.e. between 0.004 and 0.15% of the product.

In the supplementary analyses, a further 8 products were analysed. Five of the products contained lawsone below the detection limit while 3 products were above the detection limit containing 91, 730 and 2100 mg/kg, respectively, cf. table 7. The concentration of lawsone in product no. 6 of 2100 mg/kg corresponds to 0.21% (m/m). Product no. 6 is produced by the same manufacturer in United Kingdom as the aforementioned product no. 5.

5 Conclusion

5.1 Kohl products

According to the Danish Statutory Order on Cosmetics (Bkg. 489, 2003) lead must not be added intentionally in cosmetics.

Lead was found in the main part of the analysed products above the detection limit of 0.05 µg/g, in 10 products between 0.30 and 1 µg/g, and in 4 products between 1 and 4 µg/g. Lead was only found in a high concentration (280 µg/g) in product no. 60 that was manufactured in India. The measured 280 µg/g corresponds to approx. 0.028 % of the product and is thus far below the concentrations measured in the foreign referenced studies.

According to the Danish Statutory Order on Cosmetics no. 489 (2003) besides lead the cosmetic products are not allowed to contain:

- Antimony (Sb) and its compounds. Antimony was found in the screening at trace levels below 3 times the detection limit of 0.05 mg/kg in 3 products (ranging 0.09 to 0.15 mg/kg).
- Arsenic (As) and its compounds. Arsenic was found in the screening in kohl product sample no. 60 at a concentration of 6 times the detection limit of 1 mg/kg (6.1 mg/kg).
- Barium salts with the exception of barium sulphate, barium sulphide under the conditions laid down in Annex 3 in the Statutory Order, and lakes, salts and pigments prepared from the coloring agents listed with the note (3) in Annex 4 (i.e. must pass the test for insolubility which will be determined by the procedure laid down in the EU regulation). Barium was found unspecified as barium in 3 samples by the quantitative analysis between 14.5 and 6700 mg/kg.
- Cadmium (Cd) and its compounds. Cadmium was found in the screening in kohl product sample no. 60 at 1.8 mg/kg. Further 2 products were found to contain cadmium around the detection limit of 0.05 mg/kg at 0.06 and 0.08 mg/kg.
- Chromium; chromic acid and its salts. Chromium was found unspecified as chromium in the screening in all kohl products at the concentration interval from 0.6 to 18 mg/kg. The detection limit was 0.5 mg/kg.
- Thallium (Tl) and its compounds. Thallium was found in the screening of kohl product samples no. 35, 51, 52 and 60 but only at trace levels below 5 times the detection limit of 0.05 mg/kg.

The amendment to the Directive on cosmetics (Directive 2003/15/EC, EC 2003) prohibits the use in cosmetic products of substances classified as carcinogenic, mutagenic or toxic for reproduction (CMR), of category 1, 2

and 3, under Annex I to Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, but allows the use of substances classified in category 3 pursuant to Directive 67/548/EEC subject to evaluation and approval by the SCCNFP (Directive 2004/93/EC, EC2004).

The Directive mentions besides “lead and its compounds” also:

- Nickel and certain of its compounds. Nickel was found in the screening of all kohl products at the interval between 1.4 and 56 mg/kg and in 10 out of 17 henna products at the interval between 1.7 and 8.7 mg/kg.
- Cobalt dichloride and cobalt sulphate. Cobalt was found in the screening of 16 out of 18 kohl products at the interval between 0.11 and 51 mg/kg and in 10 out of 17 henna products at the interval between 0.59 and 1.1 mg/kg.
- Potassium bromate. Bromine was not included in the analysis.

Based on the inorganic analyses a difference between “Western” i.e. European and American manufactured and “Eastern” i.e. from the Middle East and Asia manufactured kohl products was indicated.

5.2 Henna products

Lead was found in 10 out of the 17 analysed products. The contents of lead in the 10 products were measured at the interval from 0.5 mg/kg to 2.0 mg/kg.

According to the Statutory Order on Cosmetics no. 489, 2003, cosmetic products are not allowed to contain:

- Antimony (Sb) and its compounds. Antimony was found in the screening of henna product sample no. 2 close to the detection limit of 0.05 mg/kg.
- Arsenic (As) and its compounds. Arsenic was found in the screening of henna products sample no. 2, 3, 5 and 23 at concentrations below 3 times the detection limit at the range 1.3 to 2.5 mg/kg.
- Barium salts with the exception of barium, barium sulphide under the conditions laid down in Annex 3 in the Statutory Order, and lakes, salts and pigments prepared from the coloring agents listed with the note (3) in Annex 4 (i.e. must pass the test for insolubility which will be determined by the procedure laid down in the EU regulation). Barium was found unspecific as barium in 10 samples by the quantitative analysis: In 8 samples at the interval between 24 and 65 mg/kg and in 2 samples, sample no. 57 and 58, at concentrations of 178000 mg/kg (17.8% m/m) and 17000 (17.0% m/m), respectively.

- Cadmium (Cd) and its compounds. Cadmium was found in the screening of henna product samples no. 2, 12, 23 and 63 at trace levels close to the detection limit of 0.05 mg/kg at the range 0.05 to 0.07 mg/kg.
- Chromium; chromic acid and its salts. Chromium was found unspecified as chromium in 10 of the products in the screening at the concentration range from 2 to 10 mg/kg. The detection limit was 0.5 mg/kg.

p-Phenylenediamine was found in 3 of the 17 products. The content in sample no. 11 and 58 was 0.5% (m/m) and 0.003% (m/m), respectively, while the content in sample no. 57 was 17% (m/m). The highest found concentration of 17% corresponding to 170 g/kg was above the limit of 6% given in the Statutory Order on cosmetics (Bkg. 489, 2003).

According to the Statutory Order (Bkg. 74, 14th of January 2005) p-phenylenediamine is placed in Annex 3 (i.e. allowed to be used in cosmetic preparations) with the following notes (cf. table below):

Table 8. Notations to p-phenylenediamine in the Statutory Order on Cosmetics, Annex 3

Reference no. (Annex, part and reference no. in Directive 76/768/EEC and later amendments)	46 III, 8
Substance	p-Phenylenediamine CAS-no. 106-50-3. Benzenediamines (1,4-benzenediamine, nitrogen substituted compounds of 1,2-, 1,3- and 1,4-benzenediamine and salts of 1,3- and 1,4-benzenediamine) (4), except those compounds mentioned elsewhere in this annex (4 = These substances may be used individually or mixed depending that the sum of the concentration of each substance expressed as a fraction of the highest allowed concentration of the substance does not exceed 1)
Field of application and/or use	Oxidising colouring agents for hair dyeing a) general use b) professional use
Maximum authorised concentration in the finished cosmetic product	6% calculated as free base
Other limitations and requirements	-
Conditions of use and warnings which must be printed on the label	a) Can cause allergic reaction (sensitivity test recommended). Do not use to dye eyelashes or eyebrows. Contains phenylenediamines b) For professional use only. Can cause allergic reaction (sensitivity test recommended). Contains phenylenediamines. Wear suitable gloves.

It is noted that p-phenylenediamine was found on the ingredients list to the products sample no. 57 and 58 but not in product no. 11.

Lawsonia was found in 14 out of 25 analysed products above the detection limit. The 23 of the 25 samples had declared to contain lawsonia (*Lawsonia inermis*, henna extract or herbal henna). One product (sample no. 3) did not declare to contain *Lawsonia inermis* but a different plant species. One product (no. 63) did not have an ingredients list.

How large a fraction of the products the ingredients *Lawsonia inermis*, henna extract or herbal henna actually covers is not given in the ingredients lists. The highest concentrations is therefore expected in products of powdered plant parts of *Lawsonia inermis*. This also appears to be the case for products no. 5, 6, 7, 8, 12, 23 and 63. The products all consisted of powdered plant parts and contained more than 1000 mg lawsone/kg product. The remaining 6 out of 12 powdered henna products contained between 40 and 730 mg lawsone/kg.

The highest concentration of lawsone was found in product no. 63 that was a foreign import from India and without an ingredients list. The highest measured concentration was 3400 mg/kg corresponding to 0.34% (m/m). The next highest concentration of lawsone was found in product no. 5 imported from United Kingdom and containing 3300 mg/kg corresponding to 0.33% (m/m). Product no. 6 from the same manufacturer in UK contained 2100 mg/kg corresponding to 0.21% of the product.

The concentrations in the remaining products were between 0.004 and 0.15% (m/m) of the product.

5.3 Summary conclusion

Comparing the results from this analysis to results found in the literature indicate a difference between "Western" and "Eastern" manufactured kohl products. Thus lead was not found at concentrations above 0.028% in the kohl products but the highest concentration was measured in a product from India. The highest concentration of the remaining analysed inorganic substances were mainly found in products made by "Eastern" manufacturers (e.g. boron, barium, copper, strontium and zinc).

The same apparently applies to henna where the highest concentration of barium, p-phenylenediamine and lawsone was measured in products of "Eastern" origin. The high concentrations of lawsone in products manufactured in the United Kingdom may be explained by import of raw materials ("natural henna") from Asia.

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