A Method for Assessing Occupational Dermal Exposure to Permanent Hair Dyes

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Hairdressers have an increased risk of developing occupational skin diseases due to exposure to skin irritants and sensitizers. In the present work a method of assessing dermal exposure to permanent hair dyes was developed. The sampling performance characteristics of hand wash sampling with bag rinsing were studied for five hair dye compounds. The effect of residence time, sample load and different matrices were studied. Thirty volunteers were exposed to a reference solution of these compounds and to commercial hair dye products. The sampling efficiency after 5 min residence time was between 70 and 90% for the dye components in the hair dye products. Sampling efficiency decreases with increasing residence time, making the time of sampling an important factor. Hand wash sampling should be performed as soon as possible after the work task of interest. We conclude that the sampling efficiency is adequate for measurements of dermal exposure to permanent hair dyes. Hand wash sampling with bag rinsing is a useful tool for field studies of dermal exposure assessment in hairdressers.

Keywords: aromatic amines; hairdressers; hand wash; sampling efficiency; skin

INTRODUCTION

Hairdressing is a high risk occupation for occupational skin diseases (Uter et al., 1999a,b; van der Walle, 2000). Hairdressers are exposed to skin irritants and many contact allergens, e.g. thioglycolates in permanent wave solutions, aromatic amines in permanent hair dyes and persulfates in bleaches. Data on airborne occupational exposure to chemicals among hairdressers has been studied (Leino, 2001), but measurement of actual dermal exposure has been missing due to lack of methods.

The techniques for assessing dermal exposure can be divided into three categories (Brouwer et al., 1998; Cherrie et al., 2000) as follows.

1. Removal of chemicals deposited on the skin followed by chemical analysis of these compounds. Chemicals can be removed by washing, wiping with a piece of fabric or cotton swab, by brushing or by skin stripping using adhesive tape.
2. Tracer techniques, for example a fluorescent tracer in combination with image processing.
3. Surrogate skin. Skin patches, gloves or whole body overalls are used and the contaminant in the collected patches is measured.

Hand wash sampling has been employed internationally for a variety of pesticides in field studies and remains a standard procedure for assessing occupational exposure to pesticides in the USA (US EPA, 1986). When developing a method using hand wash sampling it is essential to study sampling performance characteristics (Brouwer et al., 2000). Sampling efficiency can be affected by sample load, i.e. the amount of contaminant on the surface contaminant layer, and by residence time, i.e. the elapsed time between contamination and the start of wash sampling. The removal efficiency is defined by Fenske and Lu (1994) as the percentage of chemical removed from the skin by the skin sampling procedure. Sampling efficiency is used by Brouwer et al. (2000) and also includes parameters that are determined by the
sampling strategy. The sampling efficiency must be determined for each chemical substance of interest since factors such as residence time and sample load may influence these parameters differently for each substance. The matrix in which the substance is present is also of importance, since compounds in the matrix may influence the solubility of the hair dyes in the sampling medium and may also influence the dermal uptake of hair dyes.

Permanent hair colorants represent the major segment of the world hair colouring market (Corbett, 1991). For these hair dyes the colouring process is complex; primary intermediates react with couplers forming larger molecules that become trapped in the hair fibre, thus making the colouring permanent. In the absence of couplers the primary intermediate still undergoes colour-forming oxidation. In the hair dying process the colour precursor is mixed with hydrogen peroxide prior to application to the hair. This triggers oxidation and also removes the natural (or synthetic) colours of the hair and enhances penetration of the hair dye product into the hair fibre. Different concentrations (6–12%) of hydrogen peroxide are used (Brown and Pohl, 1996).

There are several known health effects of chemicals included in permanent hair dye products. Among hair dye users, permanent hair dyes can cause scalp irritation, immediate hypersensitivity (urticaria) and delayed skin hypersensitivity (Engasser and Maibach, 1990). Apart from the local effect, systemic diseases can also follow exposure to the ingredients in permanent hair dyes. Studies of occupational exposure have found an increased risk of bladder carcinoma in hairdressers (Engasser and Maibach, 1990; Gago-Dominguez et al., 2001; Czene et al., 2003). Due to the risk of sensitization, the use of 1,4-phenylenediamine (PPD) was prohibited in hair dyes in Sweden between 1943 and 1992 and toluene-2,5-diamine (TDA) between 1943 and 1964. From 1964 to 1992 TDA was used in permanent hair dyes instead of PPD (TDA) between 1943 and 1964. From 1964 to 1992 TDA was used in permanent hair dyes instead of PPD (Wahlberg et al., 2002). In 1991, Sweden applied for membership of the European Union and the subsequent implementation of EU legislation lifted the national prohibition on the use of PPD.

The aim of this study was to develop and evaluate a sampling strategy. The sampling efficiency must be determined for each chemical substance of interest since factors such as residence time and sample load may influence these parameters differently for each substance. The matrix in which the substance is present is also of importance, since compounds in the matrix may influence the solubility of the hair dyes in the sampling medium and may also influence the dermal uptake of hair dyes.

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The aim of this study was to develop and evaluate a method of assessing hairdressers’ actual occupational skin exposure loading to permanent hair dyes through hand wash sampling with bag rinsing and analysis by HPLC.

**MATERIALS AND METHODS**

**Chemicals**

Five reference compounds were selected after a survey of the current literature, followed by an inventory of compounds in common commercial hair dye products in Sweden.

1.4-Phenylenediamine (PPD) (CAS no. 106-50-3, 97% pure), toluene-2,5-diaminesulphate (TDS) (CAS no. 615-50-9, 97% pure), 3-aminophenol (MAP) (CAS no. 591-27-5, 98% pure) and resorcinol (RES) (CAS no. 108-46-3, 99% pure) were obtained from Lancaster Synthesis (Lancaster, UK). 2-Methylresorcinol (MRE) (CAS no. 608-25-3, >98% pure) was from Tokyo Kasei Kogyo Co Ltd (Tokyo, Japan). L-(-)-Ascorbic acid p.a. (CAS no. 50-81-7, >99.7% pure), sodium tetraborate decahydrate (CAS no. 1303-96-4, >99% pure), methanol (Lichrosolv, HPLC grade >99.8% pure) and hydrochloric acid (37%) were obtained from Merck KGaA (Darmstadt, Germany). Ethanol (99.5%) was obtained from Kemetyl AB (Haninge, Sweden). Pure water (>18 MΩ/cm quality) was obtained using a MilliQ system with Q-PAK Purification Paks (Millipore Corp., Bedford, MA).

**Buffer solution.** The buffer solution, pH 8.0, was prepared by mixing 440 ml of 0.1 N hydrochloric acid and 560 ml of 0.05 M sodium tetraborate.

**Reference solution.** The reference solutions were 20 and 40 mM PPD, TDS, MAP, RES and MRE in buffer containing 10% ethanol. Ascorbic acid was added as an antioxidant to a final concentration of 0.2 M.

**Hand rinse liquid.** The solvent used for the hand rinse was 10% ethanol and 90% buffer with 0.2 M ascorbic acid.

**Sample preparation.** The samples were filtered prior to analysis using a 13 mm, 0.45 μm syringe filter GHP Acrodisk (Pall Life Sciences, Ann Arbor, MI) and analysed within 24 h.

**Hair dye products.** Two commercial hair dye products were used for laboratory tests of stability and for application to the hands of volunteers. Product A contained TDS, RES and MRE and product B contained PPD, MAP, RES and MRE (this product also contained 4-aminophenol).

**HPLC instrumentation**

The analytical method developed by Vincent et al. (1999) was used with some modifications. A different mobile phase and an isocratic system were used and the analyses were run at room temperature. For the chromatographic analyses the following equipment was used: a JASCO PU-980 one piston pump, a JASCO UV-975 UV/Vis detector (JASCO, Tokyo, Japan) and a CMA/240 autosampler (CMA Microdialysis AB, Stockholm, Sweden). The data were processed with the chromatography data system Elds Pro for Windows version 1.1 (Chromatography Data Systems Inc, Stockholm, Sweden). The column
was a Merck Lichrospher RP 60 Select B, 250 × 4 mm, 5 μm particle size. Sample volume was 10.0 μl. Each sample was analysed twice. The mobile phase was borate buffer with 10% methanol and was degassed prior to use in an ultrasonic bath for 6 min.

**Stability tests of the dye compounds**

**Reference compounds.** A test was performed to study sample stability when the hair dye was mixed with hydrogen peroxide. A 1 ml aliquot of a 10 mM solution of each of four reference compounds (PPD, TDS, MAP and RES) and 4 ml of 12% hydrogen peroxide were mixed and aliquots of this solution were analysed after 60 min.

**Hair dye product.** An aliquot of 1 g hair dye product A was mixed with 1 g oxidation cream containing 9% hydrogen peroxide. A sample of 0.1 g of this mixture was diluted in mobile phase and analysed 22, 44 and 69 min after mixing with oxidation cream. We then studied whether the oxidation process could be stopped. Ascorbic acid was added to the mixture to a final concentration of 0.2 M and this mixture was analysed repeatedly over 100 min. Since the oxidation is a continuous process that alters the chemical compounds, a sample was stored overnight at 4°C and analysed repeatedly over 100 min after 24–26 h.

**Hair dye component application on the hands of volunteers**

The hands of 30 healthy volunteers were exposed to two hair dye products and to a mixture of five reference compounds in a reference solution that was stabilized with 0.2 M ascorbic acid. The hair dye products are commercially available on the European market. Since the hair dye products vary in composition, the amount that was applied to the skin was chosen so that the amount of aromatic amines and resorcinol was of the same magnitude as in the reference solution.

**Hand rinse**

The test subjects washed their hands with soap and water before application of the hair dyes. When sampling the hair dyes, the hands were immersed in 50 ml of rinse liquid inside a 1 l polyethylene bag, which was sealed tightly above the wrist with a rubber band. The test persons shook their hands vigorously for 2 min and the rinse liquid was collected in 100 ml glass bottles. The hand rinse procedure was repeated once, 4 min after the start of the first sampling. The rinse liquid from the second hand rinse was collected in a separate bottle. The rinse liquids were analysed separately by HPLC within 24 h.

**Residence time**

The residence time is the time elapsed from application of the compound on the skin to the start of wash sampling. A solution containing 400 nmol of each of the five different reference compounds was applied to the index finger of one hand. A 20 mg sample of hair dye product A or 50 mg of hair dye product B was applied to the same location on the other hand for the same duration as the reference compounds. The experimental set-up is shown in Table 1. The subjects were asked to rub the thumb against the index finger five times back and forth (for ~10 s). The reference solution and the hair dye products were applied to the left or right hand alternately during different tests. The sampling started 5, 15 or 30 min after application of the compounds or hair dye product to the hands. The procedure was repeated three times for each residence time with different volunteers.

**Sample load.** Three different sample loads were studied. A solution with 400, 800 or 1600 nmol of each of the five different dye compounds was applied to one hand, the smaller amount on the index finger and the two larger amounts in the palm. Samples of 20, 50 or 100 mg hair dye product A or 50, 100 or 200 mg hair dye product B were applied to the same location on the other hand for the same duration as the reference compounds. When the reference solution or hair dye product was applied to the palm the subject was instructed to rub the fingers against the palm five times. The rinsing procedure was performed after 15 min residence time. For each sample load the procedure was repeated three times with different volunteers.

**Sampling efficiency**

Sampling efficiency is defined as the proportion of the amount of compound applied to the skin found in a sample. To determine the sampling efficiency, known amounts of reference compounds and hair dye products were applied to the hands, as described under the subheadings Residence time and Sample
load. For comparison, 50 ml of rinsing liquid was spiked with the same amounts of reference compounds as was applied to the hands. This liquid was then analysed and the amounts of reference compounds were compared with those found in the hand rinse samples. Secondly, the same amount of hair dye product as was applied to the hands, as described earlier, was put directly into a sample bottle and 5 ml of rinsing solution was added. The hair dye product was fully dissolved after 40 min stirring with a magnetic stirrer. This solution was then diluted 10 times to make the concentration in the hair dye product sample similar to that in the hand rinse sample. This liquid was then analysed and compared with the amount found in the hand rinse sample.

All subjects gave their informed consent and the study was approved by the Ethics Committee of Karolinska Institutet, Stockholm (KI 00-355).

RESULTS

Stability tests of the dye compounds

Reference compounds. Sixty minutes after mixing the reference solution with hydrogen peroxide, 2.5% of PPD, 3% of TDS, 17% of MAP and 80% of RES remained.

Hair dye product A. Twenty-two minutes after mixing the hair dye product with oxidation cream, 35% of TDS, 92% of RES and 91% of MRE in the cream remained. After 69 min the levels were down to 19, 40 and 32% of TDS, RES and MRE, respectively. In the second experiment where ascorbic acid was added to slow down the oxidation process, the sample remained stable over an observation time of 100 min. After 26 h 83% of the TDS remained and the levels of RES and MRE were unchanged.

Sampling efficiency

The effect of different residence time. The influence of residence time on sampling efficiency is shown in Fig. 1a–e. Sampling efficiency decreased with increasing residence time for the compounds in the hair dye products, while for the compounds in the reference solution it was not affected to the same extent. The amount found in the second hand rinse was between 0 and 10% of that in the first. The sampling efficiency for PPD in the hair dye product was reduced by 40% after 30 min residence time compared with 5 min. The sampling efficiency for TDS in the hair dye product was reduced by 30% over the same time. RES was present in both the hair dye products tested. The pattern is somewhat different than for PPD and TDS. The sampling efficiency was 20–30% higher for the hair dye products than for the reference solution for a residence time of 5 min. For product A, the sampling efficiency decreased by 21% after 30 min residence time compared with 5 min and for product B it decreased by 25%. MRE was also present in both the hair dye products tested. For product A, the sampling efficiency decreased by 16% and for product B, it decreased by 35% after 30 min residence time compared with 5 min. For MAP in the reference solution, the sampling efficiency decreased by 11% from 5 to 30 min residence time. The sampling efficiency for MAP in hair dye product B could not be evaluated due to co-eluting compounds shown in the analysis of hair dye products.

The effect of different sample load. The different sample loads had little or no effect on the sampling efficiency. For PPD the sampling efficiency in the reference solutions was 72–4% for the different sample loads and for hair dye product B ~50%. For TDS in the reference solution the sampling efficiency was 74–79% for the different sample loads and for TDS in hair dye product A, 59% for sample load 20 mg and 47% for sample load 100 mg. For RES in the reference solution, the sampling efficiency was 65% for sample load 400 nmol and 59% for sample load 1600 nmol. For product A the sampling efficiency was 78% for sample load 20 mg and 64% for sample load 100 mg and for product B the sampling efficiency was ~70% for the different sample loads. For MRE in the reference solution the sampling efficiency was 54–58% for the different sample loads. For product A the sampling efficiency was 71% for sample load 20 mg and 56% for sample load 100 mg and for product B the sampling efficiency was 54–63%. For MAP in the reference solution the sampling efficiency was 77% for sample load 400 nmol and 58% for sample load 1600 nmol. The sampling efficiency for MAP in hair dye product B could not be evaluated due to co-eluting compounds.

DISCUSSION

Our purpose was to develop a method to assess occupational dermal exposure loading to permanent hair dyes among hairdressers. We chose a removal technique, hand wash sampling with bag rinsing, and tested the sampling performance characteristics. The method was used earlier for assessment of pesticides (Fenske and Lu, 1994; Fenske et al., 1999). We studied the sampling efficiency for five compounds used in oxidative hair colouring. We tested the method on volunteers to determine the impact of parameters such as residence time and sample load in order to study their influence on sampling efficiency. After the studies on sampling performance characteristics, the logistics of measurement were also successfully performed in a hairdressing salon (data not shown).
We found that the sampling efficiency during hand wash sampling was adequate for assessment of dermal exposure to compounds in permanent hair dye products. An increasing residence time decreased the sampling efficiency for the compounds in the hair dye products. The reduction was faster for the compounds in the hair dye products than for those in the reference solution. This could be an effect of oxidation, since

Fig. 1. The effect on sampling efficiency of different residence times (min) for the hair dye compounds in the reference solution and for hair dye products A and B. The sample load is 400 nmol for the reference substances, 20 mg cream A and 50 mg cream B. The bars represent the arithmetic mean of three values for the creams and six values for the reference substances. Each individual value is represented by a dot. Black, reference substances; grey, cream A; white, cream B. (a) PPD; (b) TDS; (c) RES; (d) MRE; (e) MAP.
the compounds in the reference solution were stabilized with ascorbic acid. It may also be an effect of percutaneous absorption. Other compounds present in the hair dye products may enhance the penetration of the hair dye compounds. Fenske and Lu (1994) used the term hand wash removal efficiency, defined as the percentage of chemical removed from the skin by the hand wash procedure in one of the few studies available in the literature. They studied the removal efficiency after indirect exposures, in contrast to our study where we studied direct exposure. With regard to occupational dermal exposure of hairdressers, direct exposure is most relevant. Fenske and Lu found that an increased residence time decreased removal efficiency. These findings are consistent with our results. In our study the different sample loads had little or no effect on sampling efficiency. There was a slight tendency for a decrease in sampling efficiency with increasing sample load for certain compounds. No clear conclusions can be drawn from our results and it is plausible that different sample loads may affect sample efficiency (Brouwer et al., 2000). Our results may depend on removal/sampling efficiency being unaffected by sample load in the interval (1–5 times) studied. Fenske and Lu (1994) found a non-linear variation in sample efficiency over a 500-fold range of sample load, while over a 10-fold range of sample load they found no effect on sampling efficiency, which is consistent with our results.

Initially, laboratory tests with two different techniques for assessment of dermal exposure loading were performed. First we used an adhesive tape stripping sampling method. This method has previously been used to assess skin exposure loading for acrylates in the wood industry (Surakka et al., 2000). In our experiments the sampling efficiency for tape stripping was lower than for hand washing with bag rinsing. Another advantage of the bag rinsing method is that it allows assessment of the exposure loading of the whole hand. This is preferable because the exact exposure site on the hand is usually unknown. During tape stripping, there is a risk of underestimation of the exposure loading if the location chosen for stripping is different from the actual site of exposure. A disadvantage of hand rinsing is that the local dose cannot be measured or the dose/area calculated, which is of importance for sensitization.

The instability of the hair dye compounds is a factor that has to be considered. In our study, hydrogen peroxide was not added to our reference compounds or the hair dye products in order to be able to measure sampling efficiency. However, in hairdressing salons the hair dye product is mixed with an oxidation cream containing hydrogen peroxide to trigger oxidation, as mentioned in the Introduction. Due to this oxidation, the amounts of aromatic amines such as PPD and TDS decrease rapidly, which is evident from our results. This, of course, makes quantification of an exact exposure loading over time difficult. When using the method presented here to assess exposure loading in hairdressers, the measured value is the amount of hair dye compounds on the hand/hands at the precise moment of the hand rinse. Exposure loading measured in this way will most likely underestimate the total exposure loading from a work task since the amounts of dye compounds decrease after mixing with hydrogen peroxide. A preliminary field study on hairdresser work routines indicated that 15 min was a good estimate of average residence time. An appropriate sampling strategy would be to perform hand rinses directly or within 5 min after the work task of interest, but further studies are required to be able to suggest a standardized sampling strategy.

Percutaneous penetration can also influence sampling efficiency. Several authors have studied percutaneous penetration of hair dyes in vivo with animal or human tissue (Bronaugh and Congdon, 1984; Beck et al., 1993). There are only limited data from scalp penetration studies on humans after hair dyeing (Kiese and Rauscher, 1968; Maibach and Wolfram, 1981; Wolfram and Maibach, 1985; Goetz et al., 1988). The results of the latter studies indicate that <0.2% of the applied dose of PPD penetrates the skin of the hair-dye user under normal dyeing procedures. However, the major part of the hair dye is absorbed by the hair, making these results inapplicable for the assessment of dermal uptake in hairdressers.

Our conclusion is that hand wash sampling with bag rinsing is a useful tool for studying occupational dermal exposure to permanent hair dyes in hairdressers. The sampling efficiency during hand wash sampling with bag rinsing is adequate to measure the amount of hair dye on the hands at the time of sampling. This study will be followed by a field study of dermal exposure to hair dye compounds during work in hairdressing salons.

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