

Unconsumed precursors and couplers after formation of oxidative hair dyes

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Contact allergy to hair dye ingredients, especially precursors and couplers, is a well-known entity among consumers having hair colouring done at home or at a hairdresser. The aim of the present investigation was to estimate consumer exposure to some selected precursors (*p*-phenylenediamine, toluene-2,5-diamine) and couplers (3-aminophenol, 4-aminophenol, resorcinol) of oxidative hair dyes during and after hair dyeing. Concentrations of unconsumed precursors and couplers in 8 hair dye formulations for non-professional use were investigated, under the conditions reflecting hair dyeing. Oxidative hair dye formation in the absence of hair was investigated using 6 products, and 2 products were used for experimental hair dyeing. In both presence and absence of hair, significant amounts of unconsumed precursors and couplers remained in the hair dye formulations after final colour development. Thus, up to 1.1% *p*-phenylenediamine (PPD), 0.04% toluene-2,5-diamine, 0.02% 3-aminophenol and 0.02% resorcinol were found in the hair dye formulation after the required colour was developed. The consumers are thus exposed to precursors and couplers of oxidative hair dyes, both during and after hair dyeing, when the hair is washed. Furthermore, the consumers are also expected to be exposed to intermediates of oxidative hair dyes. The allergenic potential of oxidative hair dyes as well as the intermediates of these remains unknown.

Key words: *m*-aminophenol; *p*-aminophenol; oxidative hair dyes; PPD; precursors and couplers; resorcinol; toluene-2, 5-diamine. © Blackwell Munksgaard, 2006.

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An oxidative hair dye product contains a precursor/coupler part (colour gel) and a hydrogen peroxide part (developer). When the two parts are mixed, the precursor molecules are oxidized to transient quinonediimine intermediates, which react with the couplers present in the hair dye formulation to produce hair dye molecules. Depending upon the coupler type, a dimer or trimer (or higher oligomers) of hair dye molecules may be produced. An example of oxidative hair dye produced by coupling of toluene-2,5-diamine and *m*-aminophenol is shown in Fig. 1.

Contact allergy to hair dye ingredients, especially precursors and couplers, is a well-known entity among consumers having hair colouring done at home or at a hairdresser (1–3). Furthermore, surveys show that people with allergic contact dermatitis from hair dyeing can react to own or others' dyed hair (4–6). Consumer exposure to the amount of unconsumed precursors and couplers in hair dye for-

mulations during and after hair dyeing is not known. The aim of the present investigation was to estimate consumer exposure to precursors and couplers of oxidative hair dyes during and after hair dyeing under conditions reflecting hair dyeing with the commercial formulations.

Materials and Methods

Target precursors and couplers

Target substances in the present investigation were *p*-phenylenediamine (PPD), toluene-2,5-diamine (2,5-TDA), 4-aminophenol (4-AP), 3-aminophenol (3-AP) and resorcinol.

Chemicals

Target precursors and couplers of 97% to >99% purity were purchased from Sigma-Aldrich, Brøndby, Denmark. All other chemicals used in the present study were of >99.5% purity and

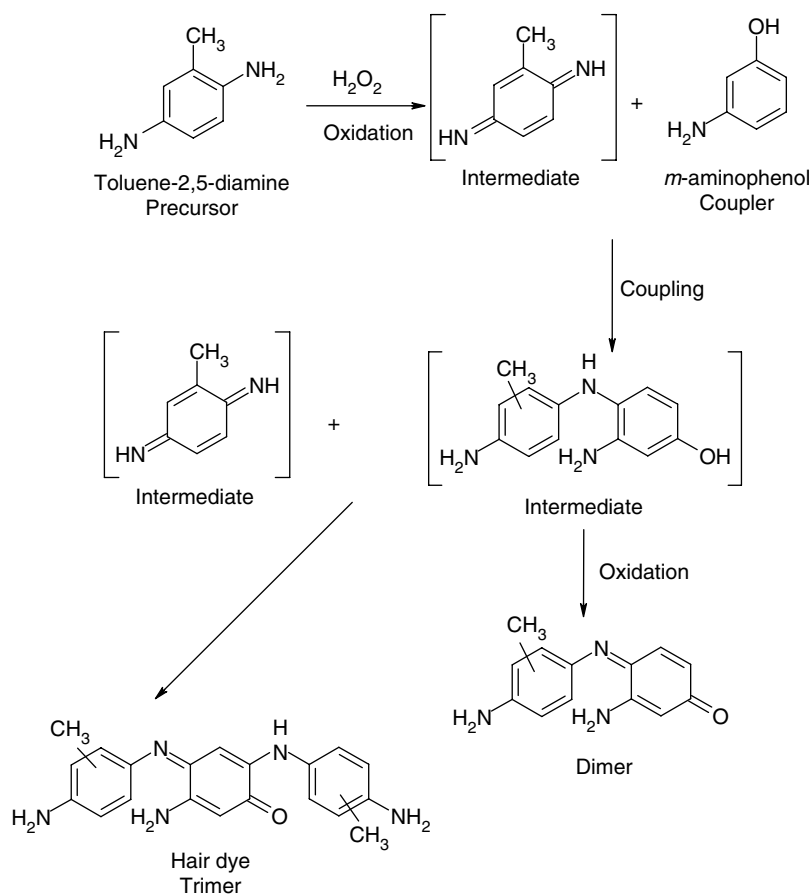


Fig. 1. Hair dye formation by oxidative coupling of toluene-2,5-diamine and *m*-aminophenol.

they were also from Sigma-Aldrich. None of the target precursor/coupler was detected in any of these chemicals. An ordinary hair shampoo for normal hairs was used to wash hairs.

Hair dye products

The investigation included 8 oxidative hair dye products for personal use, purchased in European retail shops during 2003–2005. The products containing different combinations of precursor and couplers and/or different manufacturers were used. 6 of the products were used for the colour formation in the absence of hair and 2 of the products, containing PPD, were used for the colour formation in the presence of hair. All products also contained a hair-conditioning solution to be used after hair dyeing.

Colour formation without hair

The use instructions described on the hair dye packages were followed for the development of colours, i.e. colour gel/cream containing precursor(s) and coupler(s) were mixed with the developer containing peroxide in a ratio 1:1 for colour development at 30°C for the prescribed time period. Approximately 0.5 g of colour gel was mixed

with approximately 0.5 g of peroxide developer, in a screw capped bottle, and the mixture was incubated at 30°C for 30–45 min as described in the 'instructions for use' of the products. Unincubated (T_0) mixtures of colour gel and developer of each product served as blanks for respective colour formulations. After the incubation period (blank at time T_0), the oxidative coupling of precursor and coupler was stopped by acidifying the mixtures with 10 ml of 0.1% aqueous formic acid. This was followed by the addition of 40 ml of 0.25 M phosphate buffer containing 0.1% 1-hepatanesulfonic acid/acetonitrile (90/10) and mixed thoroughly using a magnetic stirrer followed by ultrasonication for 10 min. The mixture was filtered through a 0.2- μ m filter. All experiments were performed in duplicate. The contents of precursors and couplers in the filtrates were analysed by high-performance liquid chromatography (HPLC) with diode array detector (7).

Colour formation in the presence of hair

A 38-year-old woman, who had not coloured her hair the last 5 years, volunteered to provide hair for the investigation. 2 hair dye formulations containing PPD were used for colouring the

hair following use instructions. A flow chart of the experimental hair-dyeing process is shown in Fig. 2. Approximately 5 g of hair (prewashed with shampoo and water) were mixed thoroughly with a colour formulation composed of 10 g of colour gel and 10 g of developer. The mixture was incubated at 30°C for 30 min. A blank was prepared by mixing the colour gel, developer and hair but that was not incubated (T_0). The T_0 represents the time required for mixing the colour gel with the developer and further treatment until analysis. In our hands, T_0 was 5–7 min. To stop the colour formation, the mixture was acidified by the addition of 10 ml of 0.1% aqueous formic acid and mixed thoroughly. Thereafter, 40 ml of 10% aqueous methanol/5 mM ammonium acetate buffer (10:90) was added, mixed thoroughly and treated with ultrasound for 10 min. The mixture was filtered through a prewashed cotton-wool plug, and hair was washed with 2 × 400 ml of water (ordinary tap water). The filtrate and wash water were collected in a 1-l volumetric

flask and made up to the mark with water. The coloured hair was transferred into a beaker and mixed thoroughly with 5 g of conditioning solution supplied with the hair dye product. After 2 min, the mixture was filtered through a prewashed cotton-wool plug and washed with 2 × 250 ml of water. The filtrate and washes were collected in a 1-l volumetric flask and made up to the mark with water. PPD content in all filtrates/washes was determined, as fast as possible, by HPLC-mass spectrometry (MS)-MS employing a BDS Hypersil C18 analytical column (250 × 2.1 cm, particle size 5 µm, from Thermohypersil-Keystone, Kleinostheim, Germany) and a gradient mobile phase composed of ammonium acetate buffer and methanol. Electrospray in positive ionization mode was employed, and ions with masses 102 and 93 were monitored for the detection and determination of PPD. The detection limit of PPD was 10 p.p.b. under the experimental conditions.

The coloured/conditioned hairs were dried at room temperature and washed by mixing in a

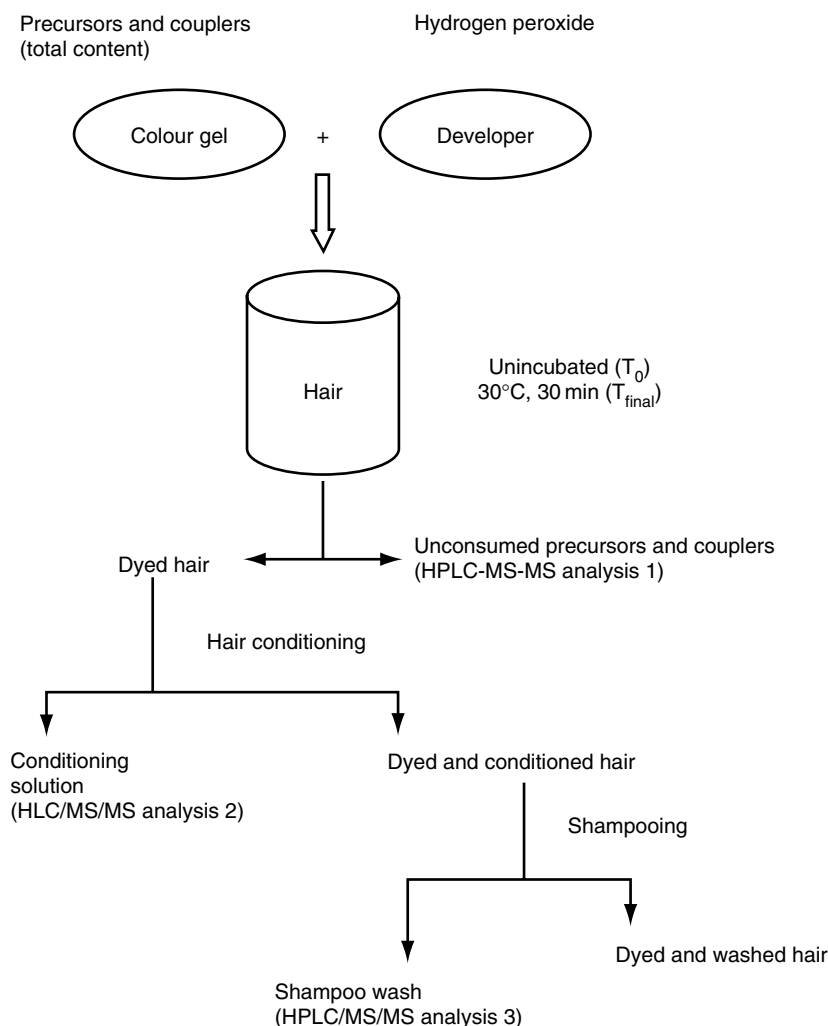


Fig. 2. Flow chart of experimental hair dyeing and analysis.

beaker with 200 ml of aqueous shampoo solution (5 g/l), filtered and washed with 500 ml of water. The filtrate and the wash were collected together in a 1-l volumetric flask and made up to the mark with water. The hair was dried at room temperature and washed once again with shampoo as before. The content of PPD in both wash waters was determined by HPLC-MS-MS as described above.

Results

The contents of precursors and couplers in the colour formulations at T_0 were significantly less than the amounts of these present in the respective colour gels used for the colour formulations (Table 1). At the completion of the reaction (T_{final}), according to the use instructions, the contents of precursors/couplers were much lower compared to that at T_0 , but they were

still present in significant amounts in most cases (Table 1).

In the absence of hair, $\geq 50\%$ of the initial amount of PPD remained unconsumed in the colour formulations after the colour development according to use instructions. Thus, 0.6% and 1.1% PPD remained unconsumed in the mixtures containing 1.2% and 1.7% PPD, respectively (Table 1). Similarly, unconsumed 2,5-TDA in colour formulations was found to be 10–60% of the initial concentration, after final colour development. The concentrations of unconsumed 2,5-TDA in the hair dye formulations after final colour development were 0.0014–0.088%. The concentrations of unconsumed couplers resorcinol/3-aminophenol/4-aminophenol in hair dye formulations after final colour development were 0–35% of the initial concentrations.

In the presence of hair, the concentrations of unconsumed PPD in the colour formulation after hair dyeing (Fig. 2: HPLC/MS/MS analysis 1)

Table 1. Contents of target precursors and couplers in oxidative hair dye formulations

Sample no.	Precursor/ coupler	Time for colour development (min)		Content, % (W/W)		
		Recommended	Used	Total ^a	T_0	T_{final}
1	2,5-TDA	30–45	40	0.1229	0.0486	0.0393
	Resorcinol			0.0925	0.0331	0.0193
2	2,5-TDA	30–45	45	0.0529	0.0334	0.03332
	4-AP			0.0066	0.0058	–
3	Resorcinol	15–20	30	0.0040	0.0031	0.0019
	PPD			1.7556	1.2393	1.1023
4	3-AP	30	30	0.0423	0.0231	0.0166
	Resorcinol			0.7158	0.3193	0.2826
5	PPD	40–45	45	1.2554	0.9447	0.6490
	Resorcinol			0.7351	0.2501	0.1388
6	2,5-TDA	15–20	30	0.1890	0.188	0.0880
	4-AP			0.0149	0.0028	0.0014
	Resorcinol			0.0149	–	–
				0.6763	0.5159	0.0529

^aTotal content in colour gel before mixing with developer.

Table 2. Amount of unconsumed PPD after hair colouring according to instructions

Sample no.	Total PPD content % (w/w)	PPD used in the colour formulation for hair dyeing mg	Unconsumed PPD in the				
			Colour formulation after 30-min ^a incubation mg	%	Wash-water after conditioning the hairs ^b mg	%	Wash-water after shampooing the coloured hair ^c
7	1.61	155.9	20.9	13.5	4.0	2.6	Wash 1, <10 µg Wash 2, <10 µg
8	1.11	112.0	68.0	60.7	8.0	7.1	Wash 1, <10 µg Wash 2, <10 µg

^aFig. 2: HPLC/MS/MS analysis 1.

^bFig. 2: HPLC/MS/MS analysis 2.

^cFig. 2: HPLC/MS/MS analysis 3.

were found to be 13.5% and 60.7% of their respective initial concentrations (Table 2). The conditioning of the coloured hair extracted further 2.6% and 7.1% PPD from the respective hair (Fig. 2: HPLC/MS/MS analysis 2). No PPD was detected (detection limit 10 p.p.b.) in the shampoo/wash water (Fig. 2: HPLC/MS/MS analysis 2), which means that <10 p.p.m. PPD may be present in 1 l shampoo/wash water.

Discussion

The concentrations of unconsumed precursors such as PPD and 2,5-TDA in the colour formulations, both in the absence and in the presence of hair, were found to be 10–65% of the initial amounts used for the colour development according to use instructions. The concentrations of unconsumed target couplers in hair dye formulations after final colour development were 0–35% of their respective initial concentrations. A large variation in the unconsumed amounts of precursors and couplers may reflect the composition of the colour gels, i.e. the amounts and types of precursors, couplers and other ingredients present in the products, as well as the final colour required. All products contained other ingredients in addition to target precursor/couplers in this study; for example, see Table 3.

A significant decrease in the content of precursors and couplers in the colour formulation at T_0 indicates that the colour forming reaction starts immediately after the 2 components of an oxidative hair dye product are mixed. The colour development continues with time and is stopped, when the required colour nuance is achieved. Thus, the consumer (scalp) is exposed to the precursor and couplers of oxidative hair dyes during the whole hair-dyeing period usually for 30–45 min. The scalp and other parts of the body are later exposed to unconsumed precursors/couplers, when conditioning and washing the hair as part of the hair-dyeing procedure. The unconsumed amounts of precursors and coupler

after colour development are relatively high; e.g. PPD was found in more than 1% concentration after 30 min of development (Table 1, sample 4) and in 0.66–0.21% after development in the presence of hair (Table 2, sample 7–8). Practical experience and epidemiological investigations show that exposure to ordinary hair dye products/ingredients causes sensitization (1, 2). Epidemiological studies in Thailand suggest a rate of 2.7% PPD-allergic individuals amongst the adult population, and an incidence of 1.3% sensitization with monthly application of hair dye over 6 months (8, 9). Further, under patch test conditions, 1% PPD elicited reactions in 50% of sensitized patients after exposure for 30 min (10).

In some products, the time for colour development is recommended as a minimum time and a longer time is required, if a higher intensity of the colour is wanted. This indicates that the intermediates of hair dye are also present in the hair dye formulations after the colour development for a defined period of time. Conditioning and washing of the dyed hair may thus be a source of human exposure to intermediates of oxidative hair dyes. Skin reactions to intermediate products, present in the oxidative hair dye mixtures, are not known.

From the kinetics of oxidative hair dye formation, it should be possible to calculate exact amounts of precursors and couplers needed for the formation of required colours and their intensities. The reason for using a large excess, up to 60% more than required, of precursors and couplers in the hair dye products is not known. Regulations of the amount of residual monomer of potent allergens such as PPD may be one way of preventing hair dye allergy.

The marketed oxidative hair dye products may contain various combinations of more than 100 different precursors/couplers (11), which may produce many different hair dyes. Although the precursors and couplers may have been tested for the allergic reaction, no information is available on the allergenic potential of the oxidative hair dyes.

Table 3. An example of composition of an oxidative hair dye product for non-professional use

Colour gel: Aqua, isopropyl alcohol, Laureth-2, coconut alcohol, propylene glycol, oleic acid, ethanolamine, ammonium hydroxide, toluene-2,5-diamine, arginine, sodium laureth sulfate, ascorbic acid, sodium sulfite, etidronic acid, 1,3-bis(2,4-diaminophenoxy)propane HCl, resorcinol, 4-chlororesorcinol, HC Blue No.7, perfume

Developer: Aqua, hydrogen peroxide, acrylates copolymer, etidronic acid, sodium laureth sulfate, 2,6-dicarboxypyridine, disodium pyrophosphate

Conditioner: Aqua, *paraffinum liquidum*, cetearyl alcohol, glucose, *cera alba*, *chamomilla recutita*, bisabolol, cetyl palmitate, cetrimonium chloride, hydroxypropyl methyl cellulose, laureth-10, alcohol denaturated, perfume, phenoxyethanol, methylparaben, propylparaben

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References

1. Sosted H, Agner T, Andersen K E, Menne T. 55 cases of allergic reactions to hair dye: a descriptive, consumer complaint-based study. *Contact Dermatitis* 2002; 47: 299–303.
2. Sosted H, Hesse U, Menne T, Andersen K E, Johansen J D. Contact dermatitis to hair dyes in an adult Danish population – an interview based study. *Br J Dermatol* 2005; 153: 132–135.
3. Katugampola R P, Statham B N, English J S et al. A multicentre review of the hairdressing allergens tested in the UK. *Contact Dermatitis* 2005; 53: 130–132.
4. Warin A P. Contact dermatitis to partner's hair dye. *Clin Exp Dermatol* 1976; 1: 283–284.
5. Seidenari S, Manzini B M, Motolese A. Contact sensitization in infants: report of 3 cases. *Contact Dermatitis* 1992; 27: 319–320.
6. Sosted H, Menne T. Allergy to 3-nitro-p-hydroxyethylaminophenol and 4-amino-3-nitrophenol in a hair dye. *Contact Dermatitis* 2005; 52: 317–319.
7. Rastogi S C. A method for the analysis of intermediates of oxidative hair dyes in cosmetics products. *J Sep Sci* 2001; 24: 173–178.
8. Basketter D A, Duangdeeden I, Gilmour N J, Kullavanijaya P, McFadden J P. Prevalence of contact allergy in an adult Thai population. *Contact Dermatitis* 2004; 50: 128–129.
9. Basketter D A, Jeffries D, Salford B J et al. The impact of exposure variables on the induction of skin sensitisation. *Contact Dermatitis* 2006: (submitted).
10. Hextall J M, Alagaratnam N J, Glendinning A K, Holloway D B, Blaikie L, Basketter D A, McFadden J P. Dose-time relationships for elicitation of contact allergy to para-phenylenediamine. *Contact Dermatitis* 2002; 47: 96–99.
11. Sosted H, Basketter D A, Estrada E, Johansen J D, Patlewicz G Y. Ranking of hair dye substances according to predicted sensitization potency – quantitative structure-activity relationships. *Contact Dermatitis* 2004; 51: 241–254.

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