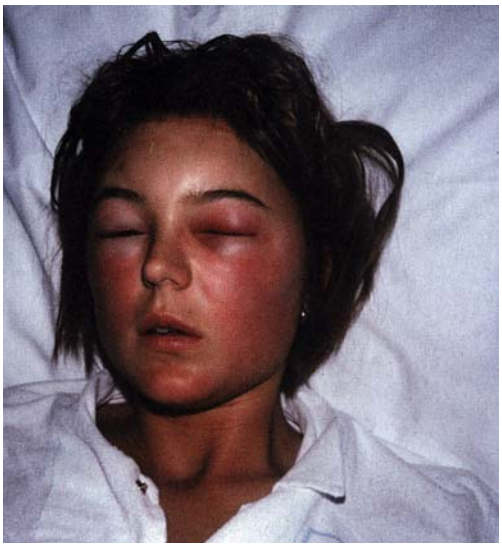
Allergic contact dermatitis to hair dye ingredients



Heidi Søsted, M.Sc. (pharm.)
National Allergy Research Centre
Gentofte Hospital. DK-Copenhagen
PhD thesis
Faculty of Health Sciences
University of Copenhagen, Denmark
Trykt i forum for Nordic dermato-venereology. Supplementum no.13. vol 12. January 2007. ISSN 1402-2915.

VIDENCENTER *for* ALLERGI

Cover photo: An 18-year-old woman with a severe, oedematous allergic reaction to hair dye (Ref. Sosted H, Agner T, Andersen KE, Menne T. 55 cases of allergic reactions to hair dye: a descriptive, consumer complaint-based study. *Contact Dermatitis* 2002; **47:** 299-303.)

ABBREVIATIONS

CAS	Chemical Abstracts Service
D	Day
CI	Confidence interval
ED	Effect dose
EU	European Union
INCI	International Nomenclature Cosmetic Ingredients
LLNA	Local Lymph Node Assay
PPD	*p*-Phenylenediamine
ppm	Parts per million
QSAR	Quantitative structure-activity relationships
SCCNFP	Scientific Committee on Cosmetic Products and Non-food products intended for Consumers
TOPS-MODE	Topological substructural molecular descriptors

Cover photo: An 18-year-old woman with a severe, oedematous allergic reaction to hair dye (Ref. Sosted H, Agner T, Andersen KE, Menne T. 55 cases of allergic reactions to hair dye: a descriptive, consumer complaint-based study. *Contact Dermatitis* 2002; **47:** 299-303.)

Preface

This study was carried out at the National Allergy Research Centre during the period 2002-2005. Aage Bang´s foundation and Ms Liv Bryhn's foundation supported the study. The data collection for the first study started in 2000 during my employment in the Danish Consumer Council.

I would especially like to express my gratitude to my three supervisors. In particular I am grateful to Jeanne for her daily support, invaluable guidance and continuing enthusiasm through the years. Torkil is especially thanked for his unique engagement in consumer protection and for his positive and courageous philosophy of open-minded research and Klaus for his valuable and constructive comments on the results. For constructive collaboration and for excellent company during my stay at Unilever Bedford I am especial thankful to Dr David Basketter, Dr Grace Patlewicz and Dr Ernesto Estrada. I thank senior scientist Ulrik Hesse from the National Institute of Public Health for scientific sparring and statistical inputs to the population study. Mette Ramm, Ania Kayser, Annette Lerche and Lone Holm Clausen, the allergy laboratory, Department of Dermatology, Gentofte Hospital are appreciated for their skilful assistance. Dr Suresh Rastogi, the National Environmental Research Institute is acknowledged for carrying out chemical analyses on hair dye ingredients. Special thanks go to my colleagues at the National Allergy Research Centre for a scientifically challenging environment and terrific social engagement. I am sincerely indebted to the patients and other volunteers who participated and made the studies possible.

Finally I wish to express my sincere thanks to my family and friends for their unfailing support and care and my daughter Josephine for sharing her happy appetite for life with me.

Heidi Søsted

July 2006

This thesis is based on the following papers/studies referred to by their roman numerals:

I Sosted H, Agner T, Andersen KE, Menné T. 55 cases of allergic reactions to hair dye: a descriptive, consumer complaint-based study. *Contact Dermatitis* 2002; **47:** 299-303.

II Sosted H, Hesse U, Menné T, Andersen KE, Johansen JD. Contact dermatitis to hair dyes in an adult Danish population - an interview based study. *Br.J.Dermatol* 2005; **153**: 132-135.

III Sosted H, Basketter DA, Estrada E, Johansen JD, Patlewicz GY. Ranking of hair dye substances according to predicted sensitization potenzy - quantitative structure-activity relationships. *Contact Dermatitis* 2004; **51:** 241-254.

IV Sosted H, Menné T, Johansen JD. Patch test dose response study of *p*-phenylenediamine: thresholds and anatomical regional differences. *Contact Dermatitis* 2006; **54**: 145-149.

CONTENTS

1 INTRODUCTION & BACKGROUND

Hair dye ingredients in a historical perspective

In ancient Egypt 4,000 years ago women used henna to colour their fingernails. The Egyptians also used other vegetable extracts and metallic compounds to change their hair colour. Today, in the 21st century people still colour their skin and hair with henna (1). Para-phenylenediamine (PPD) (fig.1) was first described in 1863 (2) and by the end of the 19th century the oxidative hair dye process had been invented. Reactions between oxidizable aromatic amines, such as PPD, toluene-2,5-diamine (fig.2), aminophenol, resorcinol and hydrogen peroxide made it possible to make a permanent colouring of hair. Since the 1960s the colouring of hair has been performed not only by professionals at hairdressing salons, but also as a popular home cosmetic procedure (1). Already in 1939 Bonnevie suggested resorcinol, PPD and aminophenol as part of a patch test standard series for diagnosing allergic contact dermatitis in patients sensitized by dyed furs, hair dyes, or through occupational exposure (3). PPD is still used for colouring of human hair and the sales of hair dye products containing aromatic amines are substantial. In 2003, retail sales of hair products (shampoos, conditioners, styling, dyes, perms, bleaches etc.) within the European Union (EU) amounted to 13,991,000,000 Euro (4).

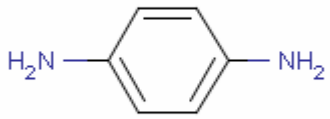


Figure 1. *p*-Phenylenediamine

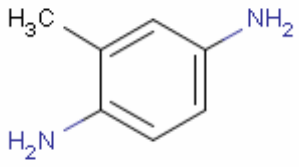


Figure 2. Toluene-2,5-diamine

Hair anatomy, growth and colour

Humans normally have 15,000 scalp hairs formed in the hair follicles (5). Hair grows in a cyclical manner approximately 1 cm per month for 3-5 years (anagen phase) and is followed by a transient stage (catagen phase) and a 2-4-month resting stage (telogen phase), during which old hair is shed. The cycle then starts again with the anagen stage and new hair starts to grow from the same follicle

(6). The growth process is independent for each follicle. 90% of follicles are in the anagen phase and 10% are in the catagen and telogen phase. The daily hair-shed is between 50 and 100 hairs (5).
The hair shaft is composed of three separate regions. The cuticle cells form a thick sheath similar to roof tiles; they are attached to the cortex that constitutes the most voluminous part. In the cortex, the fibrous proteins characteristic of hair and the keratin are located. The third zone, the medulla, is found close to the centre of the hair. The cuticle cells make a water-repellent surface and facilitate drying of hair and protect against environmental challenge (friction, tension, flexion, UV radiation, and chemical insults). They also constitute a formidable diffusion barrier, which is important when chemical modification of hair is attempted.
The large variety of natural hair colour results from the presence of variable amounts and different kinds of melanin in the hair cortex. Tyrosinase is considered as the rate-limiting enzyme of melanogenesis, resulting in the synthesis of eumelanins (black to brown pigments) and pheomelanins (yellow to red pigments). The loss of melanin content in the hair fibre is a natural manifestation of ageing leading to apparent hair whitening (7). The greying incidence occurs irrespective of sex, hair colour, and initial content of melanin (8).

Classes of hair dyes

Hair dye products are virtually the same whether they are marketed for consumers or professionals. The *oxidative dyes* consist of two components that are mixed before use. They contain precursors, which may be PPD, toluene-2,5-diamine, *p*-aminophenol and a coupling reagent (coupler) that may be resorcinol, *m*-aminophenol, 2,4-diaminophenoxyethanol or 1-naphthol. The oxidant is usually hydrogen peroxide, which oxidizes the primary intermediate and forms colourless quinone-diimines. These are rapidly polymerised in the presence of the coupler to produce dyes, which are large, intensely coloured molecules held within the hair cortex and difficult to remove. The couplers determine the final shade. Some oxidative dyes contain alkalinising agents such as ammonia, monoethanolamine or aminomethylpropanol (9,10), which promote the penetration of the dyes into the cortex of the hair straw. Hydrogen peroxide also bleaches the melanin and gives a lighter colour to the hair. The colour formed with an oxidative dye is permanent: it cannot be washed out but has to grow out. *Semi-permanent hair dyes or direct dyes* have been marketed since the 1960s (11). These products are low-molecular weight dye chemicals that penetrate the hair cuticle and partially the cortex of the hair. As a result they are somewhat resistant to shampooing. They are generally derived from nitrophenylenediamines, nitro-aminophenols or azo dyes (1,10). *Temporary dyes*

contain larger molecules and the dye does not enter the hair cuticle but stays as a layer around each hair and is normally washed out after a few washes.

Definition of a hair dye ingredient

The term hair dye ingredient is defined in the EU Cosmetic Directive as precursors, direct dyes or couplers. Hydrogen peroxide is an important ingredient in most permanent hair dyes, but it is classified as an oxidator /antimicrobial ingredient (12). In this thesis, hair dye ingredients refers to colour precursors, direct dyes or couplers, only.

A permanent hair dye product usually consists of three parts: 1) a colour gel, 2) a developer and 3) an after-treatment product. The colour gel contains precursors and couplers and often also perfume. A content of about 5 precursors and couplers is common. The developer may contain several substances; the most important is the oxidant: hydrogen peroxide, which is not an organic substance as are the dyes covered by this thesis. The last component is an 'after-treatment product' which may contain substances such as cetearyl alcohol, glycerine and preservatives. This thesis does not focus on preservatives or perfumes, since it is usually couplers, precursors and direct dyes that are the cause of hair dye product allergy, although reactions to a viscosity stabilizer and an oxidant have been recorded (13,14).

Impurities

Impurities in hair dye ingredients may contribute to the development of hair dye allergy. Only few papers have been published about impurities in hair dyes, such as *p*-toluidine and *p*-toluidine sulfonic acid as well as lead, arsenic and mercury in the hair colorant acid violet 43 (15). The EU Commission has published opinions that describe the purity of hair dyes. *p*-Toluenesulfonic acid methyl ester was found in the range 80 - 109 ppm (16) and 4-methyl-2-nitroaniline was found as an impurity in a range from 17 - 483 ppm (17). The significance of this is unknown. More information is likely to become available as the current revisions of the safety of hair dye ingredients by the EU Commission include information about impurities.

Legislation

Sensitization to PPD has, in the past, been considered so great a hazard that its use in hair dye was banned in Germany in 1906, in Sweden in 1943, in France in 1951 and in Sweden again in 1964 (18). The 6th amendment of the EU Cosmetic Directive made ingredient labelling of cosmetic products mandatory. This was a major improvement in dermatotoxicologic safety as the content of

possible contact allergens is listed on the label to the benefit of primary and secondary prevention. The labelling of chemicals must follow a standardised terminology given in the international nomenclature of cosmetic ingredients (INCI) (12). The EU Inventory of Cosmetic Ingredients is an indicative list only of cosmetic ingredients that may be used in Europe. Hair colour chemicals are in the inventory with an indication of their use as hair dyes. The EU Commission and its scientific committees are currently working on a hair dye positive list, which limits the number of hair dye chemicals permitted in the products to those with an approved scientific committee opinion documenting safety. This initiative is partly promoted by the reported potential risk for the development of bladder cancer in past users of permanent hair dyes (19,20). Today, PPD is permitted in the EU at a concentration of 6% and toluene-2,5-diamine is permitted at a concentration of 10% (12).

2 ALLERGIC CONTACT DERMATITIS

Allergic contact dermatitis is an inflammatory skin disease caused by skin contact with low-molecular-weight sensitizing substances in the environment. It is a type IV immunological reaction (21). PPD is not a sensitizer in itself as it has to be activated. A transformation to the active *p*-quinonediimine derivative can happen by air oxidation (22,23). Individuals may become sensitized from a single skin exposure to a substance or from a series of exposures over time. Once sensitized, subsequent exposure exceeding a certain threshold level will result in the development of allergic contact dermatitis. The dermatitis may be restricted to the site of allergen contact, or be widespread, and systemic reactions may occur (22).

Sensitization and elicitation

Structure activity relationship

Skin sensitizing chemicals cause allergic contact dermatitis via a number of biochemical and physiological events. Correlation between the readiness of chemicals to react with proteins to form covalently linked conjugates and their skin sensitization ability form the basis for quantitative structure activity relationship (QSAR) models (24). The biological complexities and the incomplete understanding of the processes leading to skin sensitization limit the accurateness of QSAR. Knowledge that relates chemical structure to a specific endpoint can be programmed into expert systems such as Deductive Estimation of Risk from Existing Knowledge (DEREK) (25,26). A

QSAR model developed by Benezra made it possible to rank skin sensitizers relying on available data on each substance (27). Patlewicz et al developed a QSAR method for fragrance aldehydes that relied on known local lymph node assay (LLNA) data for the tested aldehydes and compared the potency of different classes of these (28). The QSAR model applied by Estrada et al. in 2003 (24) relied on LLNA data for 93 known skin sensitizers combined with physical chemical properties for each bond between the atoms in the molecules. This model can discriminate potential allergens in three categories: strong-moderate; weak; extremely weak non-sensitizing (24). Hair dye ingredients have not been previously studied using these methods.

Sensitization studies in animals

Several animal models exist. LNNA is a predictive sensitization assay in mice studying the induction phase only. The hair dyes *p*-hydroquinone, *m*-aminophenol, *m*-phenelenediamine, *o*-aminophenol, *o*-phenylenediamine and PPD have been classified as contact sensitizers (29,30), while resorcinol was classified as a non-sensitizer in the LLNA (30). The guinea-pig maximization test (GPMT) is another sensitive animal assay (31). In this assay it is possible to study the induction phase, the elicitation phase and cross-reactions (32). The hair dye ingredients *m*-aminophenol, *p*-hydroquinone and PPD have been classified as having extreme sensitization potentials in the GPMT (29). Further, after induction with PPD, 15% of animals gave a response at challenge with 100 ppm PPD (33).

Experimental sensitization studies in humans

Marzulli and Maibach made induction studies on healthy volunteers. With repeated and occluded application of 100 ppm PPD, 7.2% of test persons were sensitized and 53% at exposure to 10,000 (1%) ppm PPD (34). Kligman used a Repeated Insult Patch Test (RIPT) procedure, where the skin was pretreated with sodium lauryl sulphate to enhance penetration of the allergen. In his studies 0.1, 1 and 10% PPD gave allergic response in seventeen, 68 and 100% of the test persons (35).

Experimental elicitation studies in humans

Elicitation with PPD in eczema patients sensitized to PPD has been reported in concentrations ranging from 100-10,000 ppm depending on dose-time relationships (36). Reactions to 3000 ppm were seen after 5 minutes, while 100 ppm elicited allergic reactions after 120 minutes (37). Lower

concentrations were not investigated. Thresholds have not been studied for other hair dye ingredients.

Clinical picture

The clinical symptoms of hair dye allergy may be severe with intense oedema of the face, particularly of the eyes, and exudation of the scalp. Erythema and swelling may extend down the neck, onto the upper chest and arms and can even become generalised (9). Less dramatic symptoms are periodic swelling of the eyes related to hair dyeing or acute eczema at the scalp margins, on the ears, sometimes extending to the neck or face (3,9). The clinical picture from hair dyes is often more severe compared to dermatitis elicited by other cosmetic products (38). Hair loss has been reported following severe scalp reactions (39,40).

Epidemiological aspects

Population-based patch test studies in Europe find a prevalence of PPD sensitization between 0.1% and 1% (41,42). In a non-clinical Thai population, 2.3% were sensitized to PPD (43). Among consecutive patch tested eczema patients, the frequency of PPD allergy is 2-5% with wide regional variations (44-46). One study from India reports a frequency among patients of 11.5% (47). A German retrospective study of female eczema patients, who had been patch tested between 1995 and 2002 and in whom hair cosmetics had been considered as being causative of their contact dermatitis, showed no changes over the period in the number sensitized to PPD. However a significant increase from 3.1% to 6.8% was found in women sensitized to toluene-2,5-diamine (48). In the same period, an increasing level of patch test sensitivity to *p*-aminophenol and toluene-2,5-diamine was also found in Finland (44).

Occupational sensitization

Hairdressing is one of the occupations most hazardous to the skin (49). Occupational contact dermatitis from PPD is common in hairdressers, and has been reported in 19% to 35% of hairdressers seen in dermatological departments (50-52). Hairdressers have a higher risk of developing allergic contact dermatitis to hair dyes compared to their clients because the duration and frequency of exposure is more intense (48,53). Professional hairdressing products follow the same statutory order as consumer products (12,54).

Cross reactions

PPD belongs to the group of para-substitued benzenes. In essence cross reactions can only be studied in animal experiments. In patients it is not possible to distinguish between simultaneous reactions and cross-reactions. The clinical experience is, however, that PPD may cross react to para-substituted hair dyes such as toluene-2,5-diamine, *p*-aminophenol, 2-nitro-PPD (55) and to disperse orange 3 (56). PPD is generally not an effective screening agent for azo dyes (57). However cross reactions or simultaneous reactions have been described, especially to disperse orange 3, *p*-aminoazobenzene and *p*-dimethylaminoazobenzene (58,59). A patient from London reacted to a PPD-containing hair dye and had cross reaction to disperse red 17 (60). PPD is also described to cross-react with N-isopropyl-N-phenylenediamine (IPPD) (54), and local anaestetics (61,62).

Primary sensitization to PPD from sources other than hair dye

Temporary black henna tattoos, in the following called temporary tattoos, may contain PPD in high concentrations and cause induction of PPD allergy (63). Typically, an eczematous reaction occurs in the original temporary tattoo weeks after the tattoo has been made as a sign of primary sensitization. In one study, 6 of 8 children with an allergic reaction to hair dye products had previously had a temporary tattoo followed by a skin reaction. In these cases the temporary tattoo is likely to have caused primary sensitization to PPD. Individuals sensitized to PPD by temporary tattoos cannot tolerate hair dyes and may experience severe clinical reactions and cross reactions to local anaesthetics and IPPD (61,64). Allergic reactions to textile azo dyes following PPD sensitization from a temporary tattoo have also been reported (65). Active sensitization through patch testing with PPD 1% is also possible (66); however, PPD-allergy is not more common among patients tested repeatedly than among patients tested only once (45,67). It has been stated that PPD sensitization is common among masons and metallurgists who wear black rubber gloves (68), but a study from 2004 found no significant risk of sensitization to PPD among male metalworkers compared with other male eczema patients OR=1.7 (0.7-3.4) (69). A case report from 1978 suggests that PPD can cross react with IPPD in black rubber and elastic (62). A case report on two pharmaceutical workers with hand and face dermatitis manufacturing paracetamol both had positive patch test reactions to PPD and *p*-aminophenol. Both workers denied previous exposure to hair dye and the primary sensitizer was suggested to be *p*-aminophenol, which can be a breakdown product from paracetamol in temperatures above 45 degrees Celsius and under humid conditions (70). The

only known significant cause of PPD sensitization except for hair dyes is temporary tattoos, which are fashionable among young people today.

Exposure to hair dye ingredients

Limited information has been published about hair dye habits in the general population. Unpublished data from 1993 report that 35-45% of American women dye their hair monthly (15). A Swedish study from 1991 showed that 45% of young women dyed their hair at least once a year (71). In a retrospective analysis of eczema patients it has been found that 71% of PPD–allergic individuals had dyed their hair (72). The literature is also limited on quantitative chemical exposure assessment. Hair dye products, which have elicited allergic reactions in single cases under normal use conditions contained 2700 ppm and 17,000 ppm PPD, respectively (73,74). In studies of allergic contact dermatitis to permanent hair dyes we found concentrations of PPD and its derivatives, in a range of 100 ppm to 39,900 ppm. 22 control products randomly collected showed a similar concentration range (73). Today, a typical dark hair dye contains 2% PPD (75), which is diluted 1:1 with developer. After hair colouring the hair is washed. Residue monomer of permanent hair dye ingredients seems to be limited after the washing (76,77).

3 OBJECTIVES

The objectives of the present study were:

I

To investigate adverse skin reactions to hair dyes compatible with an allergic genesis based on consumer complaints, the clinical picture and medical treatment.

II

To establish the frequency of hair dye induced skin reactions in a general population-based sample.

III

To collect information about all hair dye substances used in permanent or temporary hair dyes in Europe and to rank these substances according to their estimated sensitization potency. The perspective is to improve the current diagnostic work-up for hair dye allergy with new potential relevant contact allergens.

IV

To investigate the PPD elicitation threshold concentration in PPD-allergic patients and to assess possible anatomical regional differences of response.

4 MATERIALS & METHODS

4.1 Hair dye reactions based on consumer complaints (I)

4.1.1 Subjects

The principal investigator (HS) initiated the study as she had experienced many consumers contacting the Consumer Council about hair dye complaints. An advertisement was placed twice in the Danish Consumer Council's monthly magazine "Tænk + Test" in October and November 2000 published in 89200 copies for each of the two months. The magazine was distributed nationwide by post to all subscribers and was available at the Danish public libraries. It is estimated that the advertisement reached around 4% (200,000/5,000,000) of the population. The advertisement contained a two-page description of a hair dye allergy case and a call for people who had recently had an adverse reaction to a hair dye. In a box it was written (in Danish): The Danish Consumer Council is interested in hearing from people who have had burns or allergic reactions to hair dyes or bleaching agents leading to contact with the health care system. 88 persons responded within 16 months either by telephone, e-mail or letter.

4.1.2 Methods

A questionnaire was developed by the principal investigator regarding the adverse event and answered by the consumers either in writing or by telephone interview. The questions concerned the use of hair dye products in general and the causative product in particular. The questions were developed based on the previous complaints received in the Consumer Council.

This information, including medical reports if available, was examined by two dermatologists (TM/TA) who devised the inclusion criteria. These were based on clinical experience of a severe allergic reaction: oedema of the face and/or forehead, eyelids, scalp, and/or suppuration/ulceration of the scalp and/or ears related to exposure to hair dye. Suppuration and ulceration was the wording used by the consumers; in medical terms meaning, erosion and exudation (9).

4.2 Contact dermatitis to hair dyes in a general population (II)

4.2.1 Subjects

The interview survey concerned the health and morbidity of the Danes as part of a World Health Organisation (WHO) investigation and covered a representative random sample of 4,000 people from the Danish population. The sample was drawn from the Danish Civil Registration System and covered Danish citizens living in Denmark aged 18 years and above. In the total random sample of 4,000 people, 21 persons were not available for interview as they had immigrated or died. The participation rate was 65.2%.

4.2.2 Methods and statistics

The principal investigator developed questions about adverse skin reactions to hair dyes with technical assistance from the National Institute of Public Health (NIPH), because the questions should fit into a design compatible with other researchers' questions (SUSY 2003). The hair dye questions were divided in two categories: less severe symptoms and severe symptoms. Less severe symptoms were defined as redness, scaling and itching of the face, neck, ears or scalp after hair dyeing. Severe symptoms were based on the symptoms reported in study I that were compatible with a severe allergic reaction. These were defined as oedema of the face and/or forehead, eyelids, scalp, and/or suppuration/ulceration of the scalp and/or ears after hair dyeing.

Hair dyeing was described as colouring, toning, streaks or bleaching that can be done at home or at a hairdressing salon. A temporary tattoo was described as dark-black skin paints lasting about 3 weeks. The data were collected by personal interview from the end of May to the beginning of September 2003. The technical part of the data collection and the interviews were completed by the Danish National Institute of Social Research (NISR) Survey.

The questionnaire contained 168 questions: 20 general questions about social and demographic information; 18 questions about the use of health care services, including alternative treatment methods such as acupuncture, homeopathy and massage; 15 questions about smoking habits and alcohol consumption; 9 questions about hair dyes; and 106 questions about health and diseases, a joint venture with Harvard University and part of a global WHO project similar to a survey done in 2000.

The following main questions were used concerning hair dye use and hair dye side effects:

- Have you ever dyed your hair?
- What was your age the first time you dyed your hair?
- How many times have you dyed your hair in total?
- Have you dyed your hair within the last 12 months?
- Have you ever had redness, scaling or itching of the face, neck, ears or scalp after hair dyeing?
- Have you ever had oedema of the face, forehead, and scalp or around the eyes or suppuration/ulceration of the scalp and/or on your ears after hair dyeing?
- Have you been in contact with the heath care system as a consequence of a hair dye reaction? (2 questions)
- Have you been tested by a dermatologist for hair dye allergy?
- Have you ever had a temporary tattoo on your skin? *A temporary tattoo lasts about 3 weeks*

The questions were pre-tested in a total of 20 nurses and patients and were well understood. Based on the outcome, a note was made for the interviewer explained that dyeing of eyebrows and eyelashes was not included, and irrespective of the number of different colours applied on the hair it counted as only one hair dyeing. The principal investigator (HS) instructed interview leaders in how to understand the questions and clarified which products the study concerned. Each person in the test sample received an introductory letter stating that participation in the survey was voluntary and full anonymity was guaranteed. The letter of introduction did not mention hair dyeing. About 100 different interviewers conducted the interviews 2-3 days after the letter was received. If the interviewee was not available then, the interview was carried out at a later date.

Statistical analyses were performed with SPSS version 11.0 for Windows (Chicago IL. U.S.A.) and SAS version 8.2. Frequencies and 95% confidence intervals (CIs) were calculated for persons with skin symptoms. Odds ratio and 95% confidence intervals (CIs) were used to compare temporary tattooed and people with no temporary tattoos with skin symptoms (78).

4.3 Sensitization potency ranking of hair dye substances (III)

4.3.1 Design and methods

A list of hair dye ingredients was compiled from three main sources:

- The INCI list found on the European Commission's homepage (79). This list covers 261 hair dye substances.
- 61 newly regulated hair dye substances (80).
- Provisional quantitative list of 89 hair dye substances used in hair colouring products on the European market year 2002 (81).

There were differences in the chemical nomenclature between the sources. Therefore each substance name was identified by searching publicly available data sources, including ChemIDplus (82) and Chemfinder (83). Substances that could be described by an international union of pure and applied chemistry (IUPAC) chemical name, were drawn with the chemical drawing package Chemdraw Ultra (Version 6, Cambridgesoft, CA, USA) or the autonom program within Chemdraw Ultra, because the drawing programmes were most likely to be able to create a molecule structure from IUPAC names. Herbal ingredients were not included as they consist of complex mixtures of chemical molecules. The resulting list contained 315 substances that were imported into a molecular spreadsheet structure activity relationship TSAR^{TM} (Version 3.3, Accelrys). SMILES (Simplified Molecular Input Line Entry Specification), which are 1-dimensional representations of chemical structures, were generated. Then duplicate structures and salt containing ingredients (HCl, SO_4) were removed, leaving 229 unique hair dye ingredients.

Predictions for sensitization potency were made with a quantitative structure activity relationship (QSAR) model called TOPS-MODE (24). TOPS-MODE descriptors are physical chemical properties accounting for hydrophobicity, molar refractivity, polarisability, charges, polar surface area, molecular weight and van der Waals radii. The descriptors of the hair dyes were compared with descriptors and LLNA data from another set of other substances. From this comparison, the 229 hair dye substances were categorised according to their likely sensitization potency and were divided into 3 different classes: class 1: strong/moderate sensitizers; class 2: weak sensitizers; class 3: extremely weak or non-sensitizers. Further the model gave each substance a predicted sensitization potency ranking that made it possible to compare substances within the same class. The higher the ranking the more potent the allergen. A cluster analysis provided a means of grouping substances according to their chemical properties such that a representative diverse subset could be selected for further work. The algorithm applied was that of K-means, as implemented in

STATISTICA (Version 6. Statsoft Inc, USA). The algorithm was set to define 10 clusters, as 10 substances would be a reasonable number to on which to focus further patch test work. Each cluster contained other "similar" chemicals.

A literature search was subsequently conducted, which highlighted where the QSAR model performed particularly well or poorly with published experimental and clinical data.

A literature search for LLNA data and all the identified hair dye substances was done using the Medline (84) data base (1966 to July 21, 2003) using different key phrases "Local Lymph Node Assay AND hair dye(s)". 161 articles were retrieved. Another literature search was done to search for clinical human evidence using Medline database from 1966 to August 8, 2003 using "contact dermatitis AND hair dye(s)" "contact allergy AND hair dye(s)" and "sensitisation AND hair dye(s)" and "sensitization" AND hair dye(s)" as key phrases. 133 articles were retrieved. Human evidence was accepted if a positive patch test to a specific allergen was found in persons with definite or possible exposure to hair dyes.

4.4 Patch test dose-response study of *p*-phenylenediamine (IV)

4.4.1 Subjects and test materials

Candidates for testing were eczema patients 18 years or above who had had a positive patch test reaction to PPD in the period 1999-2003 at the Department of Dermatology, Gentofte Hospital, Denmark. The exclusion criterion was active eczema. 15 PPD-allergic patients were included. The protocol was approved by the local Ethics Committee for Copenhagen and informed consent was obtained from all participants before inclusion in the study. The study ran from January to July 2005.

The test material was PPD 1% in white petrolatum from Hermal, Trolab. As PPD is unstable in water/ethanol, the samples were diluted in white petrolatum. The serial dilution covered concentrations in the range from 1 to 10,000 ppm. The white petrolatum used for dilution was applied as control. To minimise the influence of oxidation, the dilutions were made weekly and stored below 5 degrees Celsius. Patch testing was performed with 8-mm Finn Chambers (Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape, Alpharma AS, Norway using 20 mg of each patch test material.

4.4.2 Methods and statistics

The Finn chambers remained in place for 2 days and on day (D)3 and D7 readings were made according to the International Contact Dermatitis Research Group (ICDRG) (85). A + reaction was

defined as homogenous redness and infiltration covering the whole test area; +? was responses less than this, as only redness or partial infiltration was present in the test area. To enable statistical calculations, patch test reactions were scored as follows: negative = 0; doubtful (+?) = 1; weakly positive (+) = 2; moderately positive (++) = 3; and strongly positive (+++) = 4. The patch tests on the back were placed with the four highest concentrations on the left side and the four lowest and the control on the right side. Patch test containing 500, 100, 50 ppm PPD and petrolatum, were also placed on the outer aspects of the upper arms and retroauricular area, in a randomised order, with two samples next to each other on each arm and behind each ear. The randomisation on the arms was identical to that behind the ears. The test preparations behind the ears were placed just below the hairline (Fig 3).

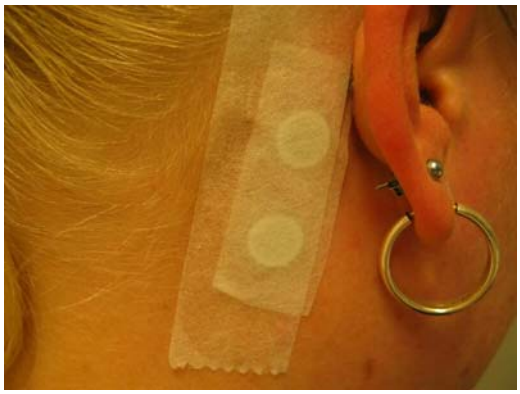


Figure 3. Patch testing with PPD on the retroauricular area.

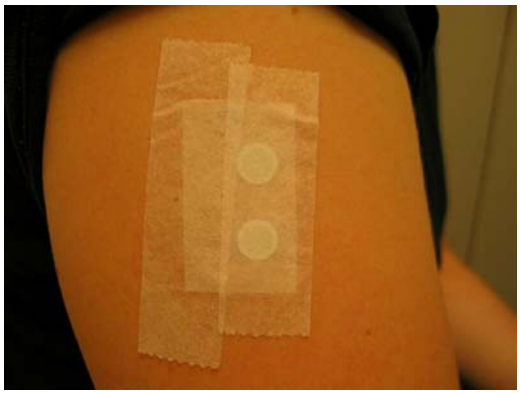


Figure 4. Patch testing with PPD on the outer aspect of the upper arm.

Data were presented as the percentage of positive patients of a total of 15 and the probability of positive response P(x) was described by the logistic regression model. The probability of positive response from a given dose x is as follows:

$$P(x) = \frac{x^{\lambda}}{ED_{50}^{\lambda} + x^{\lambda}}$$

Effect dose $(ED)_{50}$ is the concentration where 50% of patients have an allergic reaction, P=0.5; λ is a positive constant showing the steepness of the dose-response curve at ED_{50}. As it is the same 15 patients who were tested with all the doses, P(x) is a probability distribution of the threshold dose. Dose-response curves were drawn for each of the three anatomical regions. McNemar´s test and Wilcoxon test (78) were used to test for regional differences in responses between ears, arms and back.

5 RESULTS

5.1 Hair dye reactions based on consumer complaints (I)

A total of 88 persons responded to the consumer organisation. 55 persons: 52 women and 3 men, gave a history compatible with allergic hair dye dermatitis and on this background they were included in the study. 43 had dyed their hair themselves (home-treatment) and 11 had had their hair dyed at a hairdressing salon; 1 had done both. 51 had used permanent hair dyes and 3 had used a rinse; 1 did not know. 12 persons reported that the skin symptoms started less than 1 day after hair dye exposure and 23 developed symptoms later than 1 day after, while 20 did not remember. Self-reported symptoms and signs from medical records related to hair dyes are given in Table 2.

Table 2. Clinical symptoms and signs following exposure to hair dye in 55 consumers (Adopted from paper I).

Symptom/sign	**Number of persons**
Itching	29
Oedema of the face	25
Oedema of the eyelids	21
Oedema of the scalp	19
Ulceration on the scalp	17
Suppuration from the scalp	16
Eczema	15
Oedema or suppuration of the ears	12
Eczema in places other than scalp or face	11
Oedema of the forehead	10
Conjunctivitis	8
Swollen lymph nodes	7
Dizziness	4
Difficulty in breathing	4
Headache	2

13 persons forwarded a photograph of the reaction that supported/confirmed the diagnosis of allergic contact dermatitis (see cover photo). Visits to the health service are given in Table 3.

Table 3. Visits to the health care service due to adverse reaction to hair dye, (Reproduced from paper I).

Health service contacted	**Number of visits to health service**
General practitioner	37
Dermatologist	17
Duty doctor	9
Casualty	6
Admissions to hospital	5
Ophthalmologist	1
Total	**75**

These 75 visits are distributed among 48 persons. Seven persons did not contact the health service. 1 person had 4 visits to a dermatologist, and 3 persons had more than 1 visit to their general practitioner.

9 persons reported that they had made a skin test prior to the use of hair dye. They had applied the unoxidised colour to the wrist with a negative result. 14 persons visited a dermatologist. Patch tests had been carried out in 8 cases. On the initiative of the Danish Consumer Council, another 8 persons were patch tested later at either the department of dermatology at Gentofte Hospital or Odense University Hospital, Denmark. All 16 persons tested reacted positively to PPD, 7 reacted positively to toluene-2,5-diamine and 3 reacted positively to *p*-aminophenol. 3 persons spontaneously reported having had a temporary tattoo, but this question was not asked in all cases. A total of 33 persons were treated with antihistamine, and 29 were treated with (topical or systemical) corticosteroids. 7 persons were treated i.m./i.v. with antihistamine, corticosteroids or adrenaline. 1 person was suspected of having mumps. In 23 persons the dermatitis lasted for more than 3 weeks. The symptoms were itching, redness, scaling and persistent oedema. 2 persons reported hair loss. 18 persons reported sick leave due to their hair dye-related symptoms (range 1-21 days, mean 7 days).

5.2 Contact dermatitis to hair dyes in a general population (II)

It was found that 18.4% of the male respondents and 74.9% of the female respondents had ever dyed their hair. The median age of the first hair dyeing was 16 years for both men and women (range 1-80 years), see figure 5.

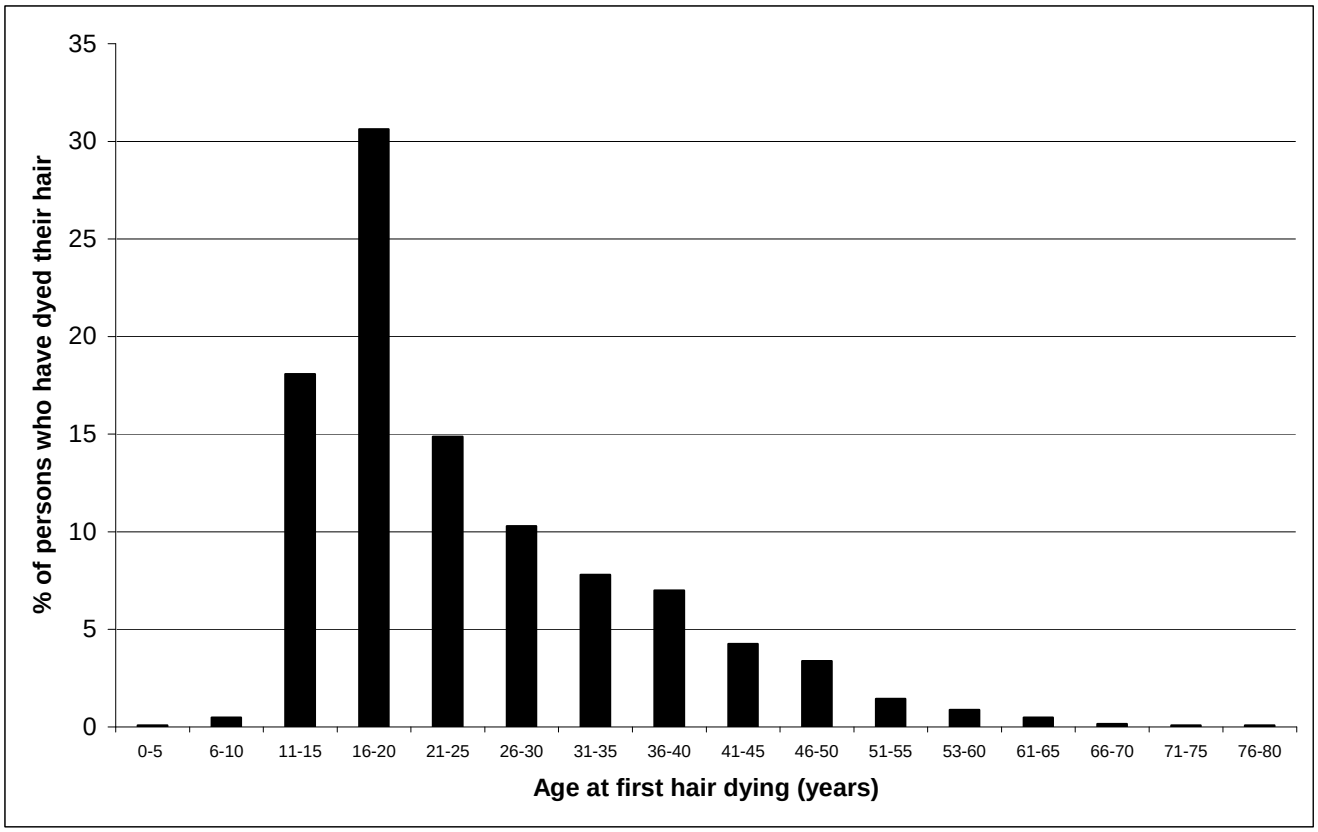


Figure 5. Age (years) at first hair dyeing in a random sample of Danish adults, both men and women. n=1244. The distribution of first use of hair dye is similar for men and women (Reproduced from paper II).

Of the 67 persons reporting skin reactions after hair colouring one person answered that he had been patch tested with a positive result. Table 4 shows frequencies of adverse reactions after hair dyeing. Of all the persons who had dyed their hair, 4.9% had ever had redness, scaling and itching following a hair dyeing. 1.4% had had oedema and /or suppuration / ulceration, 5.3% had had either one or both types of symptoms. Among the persons with symptoms, 15.6% had been in contact with health care services after the hair dye reaction.

Table 4. Life-time prevalence of skin symptoms after hair dyeing in a general adult Danish population sample with 95% confidence interval (Reproduced from paper II)

		Men		Women		Total	
		Cases	**% (CI)**	**Cases**	**% (CI)**	**Cases**	**% (CI)**
A	**Eczema symptoms**	10	4.5 (1.8-7.2)	51	5.0 (3.6 - 6.3)	61	4.9 (3.7 - 6.1)
B	**Oedema and/or suppuration/ ulceration**	3	1.3 (0-2.8)	14	1.4 (0.7-2.1)	17	1.4 (0.7-2.0)
A and or B	**Eczema and/or oedema and/or suppuration/ ulceration**	12	5.4 (2.4-8.3)	55	5.3 (4.0-6.7)	67	5.3 (4.1-6.6)

The age of individuals with symptoms had a range from 18-85 years. n=1254. Since some persons have both eczema symptoms and oedema, there is an overlap between the two groups (A and/or B). Eczema symptoms were redness, scaling, itching. CI is confidence interval.

Eleven of 17 persons (65%) with severe/oedematous symptoms reported that the skin changes started less than 1 day after hair dye exposure and 4 developed symptoms at a later time and 2 did not answer. Among the 17 persons with severe reactions, 6 (35%) had had their hair dyed at a hairdressing salon and 9 (53%) were home-dyed. Of the home-dyed, 5 were able to name the producer (all international companies) and the specific colour, 2 named the dye only, and 2 did not answer the question. Among the persons who had ever dyed their hair, 4.9% had also had a temporary tattoo. The risk of hair dye reactions was not statistically significantly correlated to former temporary tattoo applied on the skin.

5.3 Sensitization potency ranking of hair dye substances (III)

Calculations from the TOPS-MODE QSAR study showed that 75% of all the hair dyes were predicted to be strong/moderate sensitizers. PPD was predicted to be a strong/moderate sensitizer with a predicted sensitization potency value of 1.8. Other substances were predicted to be far more potent. The group of weak sensitizers included 22% of all the substances while 3% of the hair dye substances were predicted to be extremely weak or non-sensitizing. The 229 substances were divided into 10 clusters, each covering between 1 to 40 substances (See figure 6).

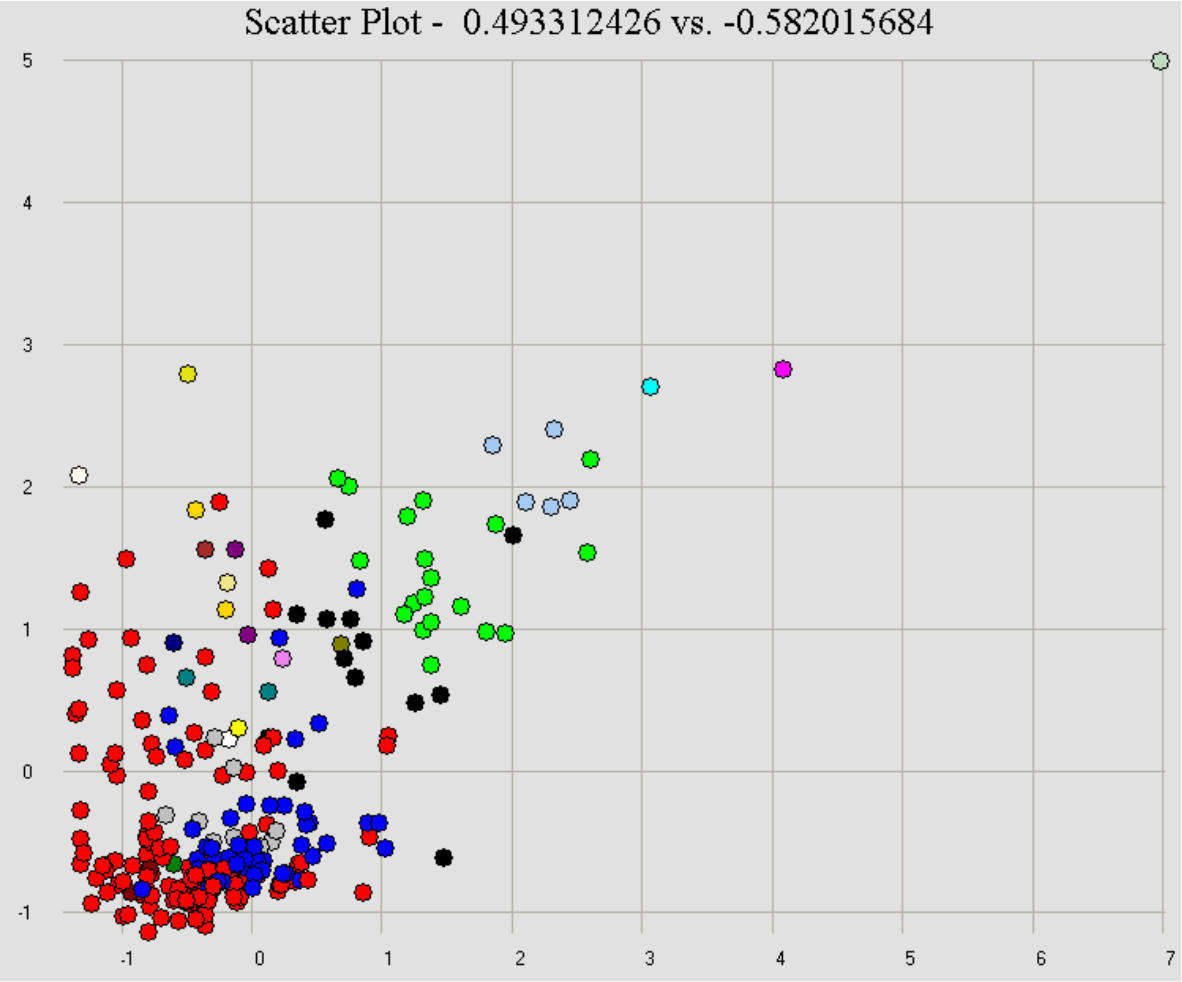


Figure 6. Cluster analysis of hair dyes grouped in 10 different clusters due to their physical-chemical properties. Chemicals with similar structural information are in the same cluster. Each dot is a molecule; each colour is a cluster. The axes are multiple.

No published data were available for most of the substances. Of the 229 substances investigated, only 21 could be identified in the literature as allergens based on human evidence and/or LLNA data.

The hair dye industry used 100 tons toluene-2,5-diamine in 2002 (81), which makes it the most used hair dye ingredient in the EU. The substance was predicted to be a moderate/strong sensitizer supported by a substantial number of clinical cases, including study I and laboratory studies (40,44,52,55,61,72,73,86-98).

Table 5 depicts 28 substances that were predicted to be moderate/strong sensitizers and which were used in hair dye products in excess of 2 tonnes per annum. These substances are listed according to their cluster numbers, such that substances from different clusters could be chosen in the development of a new patch test series. Only 4 different clusters are represented. 9 of the selected substances have been reported as clinical contact allergens. To identify commercial suppliers for the 28 substances, an Internet search was done on the homepage of Sigma-Aldrich in May 2006. 16 substances were commercially available as raw material. The purities were 98% for most of the substances and 99% for *o*-aminophenol (CAS. No: 95-55-6) and 1-naphthol (CAS. No: 90-15-3). There was no information about the nature of impurities.

Table 5. Proposed list for future investigations of patch testing for hair dye dermatitis.

INCI name	Cluster no.	Amount used (Tonnes)	CAS. No.
Acid Violet 43	1	2.1	4430-18-6
2-Amino-6-chloro-4-nitrophenol	2	5	6358-09-04
4-Amino-3-nitrophenol#¤	2	3	610-81-1
Disperse Violet 1	2	2.1	128-95-0
HC Red no. 3	2	5.0	2871-01-04
HC Blue no. 2¤	2	10	33229-34-4
Picramic acid	2	2.1	96-91-3
2-Amino-3-hydroxypyridine¤	5	4	16867-03-1
3-Nitro-p-hydroxyethylaminophenol#	5	4	65235-31-6
4-Amino-2-hydroxytoluene¤	5	70	2835-95-2
4-Amino-m-cresol¤#	5	20	2835-99-6
4-Hydroxypropylamino-3-nitrophenol	5	5	92952-81-3
m-Aminophenol#*¤	5	30	591-27-5
N.N-bis(2-hydroxyethyl)-p-phenylenediamine	5	5	54381-16-7
o-Aminophenol#¤	5	6	95-55-6
p-Aminophenol#*¤	5	50	123-30-8
1-Naphthol¤	6	6	90-15-3
2,7-Naphthalenediol#¤	6	3	582-17-2
2-Methyl-5-hydroxyethylaminophenol	6	30	55302-96-0
2-Methylresorcinol¤	6	30	608-25-3
4-Chlororesorcinol¤	6	7	95-88-5
p-Methylaminophenol	6	4	150-75-4
Resorcinol#*¤	6	90	108-46-3
1-Hydroxyethyl-4,5-diaminopyrazole sulfate	8	9	155601-30-2
2,4,5,6-Tetraaminopyrinidine	8	5	1004-74-6
2,4-Diaminophenoxyethanol HCl¤	8	9	66422-95-5
p-Phenylenediamine (PPD)#*¤	8	80	106-50-3
Toluene-2,5-diamine#*¤	8	100	95-70-5

#Reported as clinical contact allergens. *Commercially available from Chemotechnique Diagnostics, Malmö. The substances are available in 1% petrolatum. ¤Commercially available from Sigma-Aldrich 2006 www.sigmaaldrich.com.

5.4 Patch test dose-response study of *p*-phenylenediamine (IV)

15 patients aged 24-64 years participated in the study. Ten participants had experienced a previous allergic reaction to a hair dye product or a dye product for eyelashes and eyebrows. Three of the participants had never coloured their hair. No differences in the sensitivity (mean SUM score) between the group with a hair dye reaction in the past and those with no previous reaction were found. The results of 2D patch testing with different concentrations of PPD are given in table 6. For each of the three regions (back, ears and arms) dose response curves were drawn, see figure 7.

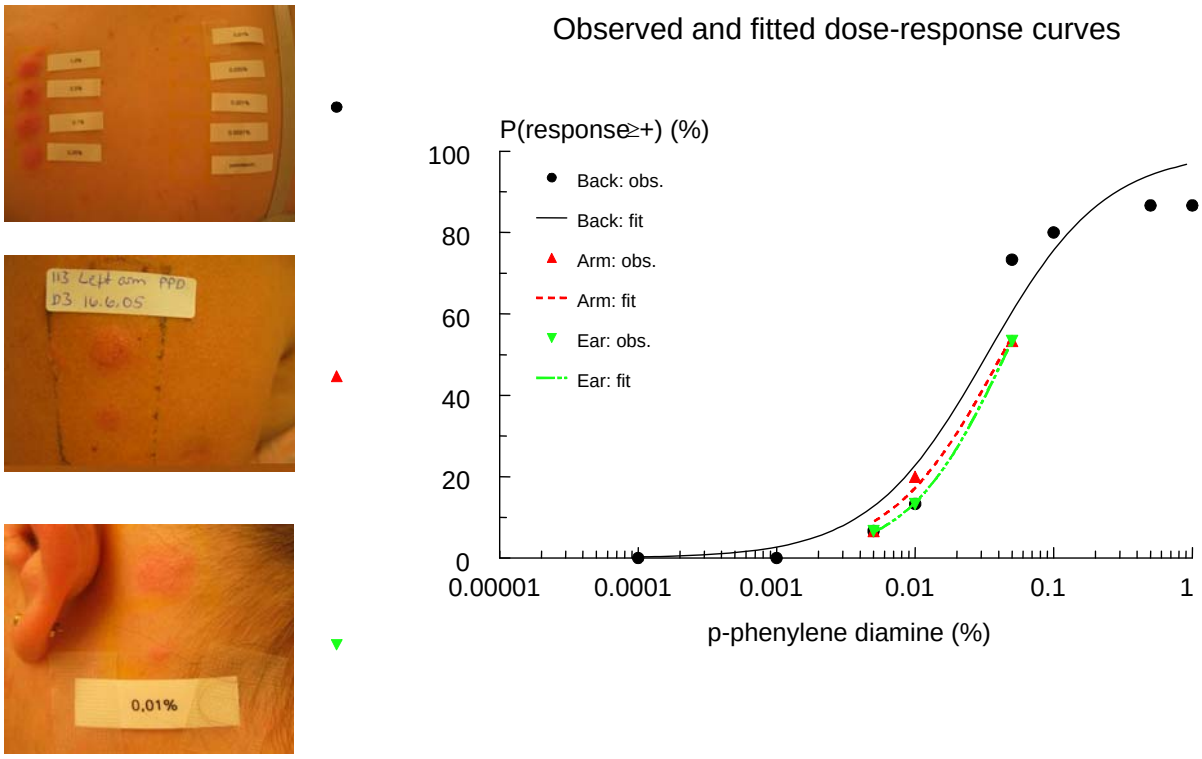


Fig. 7. Exposure dose and observed response P(response) in 15 patients. All patients had a 2D patch test read on day 2, 3 and 7. Observed responses were + or stronger. The fit was made as described in section 3.4.2.

Table 6. Results of 2D patch testing with *p*-phenylenediamine (PPD). The table shows the proportion of 15 PPD-allergic individuals who gave positive patch test reactions to treatment with doses of PPD (1-10,000 ppm) applied on different anatomical regions.

PPD Concentration (ppm)	Back Reactions (%)	Ear Reactions (%)	Arm Reactions (%)
1	0		
10	0		
50	7	7	7
100	13	13	20
500	73	53	53
1,000	80		
5,000	87		
10,000	87		

1 ppm is 0.0001%. Blank means not tested. No positive reactions were recorded to petrolatum.

The threshold value ED_{10} based on positive patch test (+ - ++ - +++) on the back was 38 ppm (CI: 4.3-100). See table 7.

Table 7. Estimated effect doses (elicitation) of PPD.

	Back		Ear		Arm	
	PPD (%)	95 % CI	PPD (%)	95 % CI	PPD (%)	95 % CI
ED_{10}	0.0038	0.00043-0.010	0.0075	0.00026-0.018	0.0056	0.00018-0.015
ED_{25}	0.011	0.0027-0.027	0.018	0.0030-0.040	0.015	0.0024-0.038
ED_{50}	0.033	0.013-0.095	0.045	0.019-0.17	0.043	0.016-0.19
ED_{75}	0.097	0.040-0.51	0.11	0.049-1.7	0.12	0.046-2.2
ED_{90}	0.28	0.098-3.4	0.27	0.095-22	0.32	0.10-35

CI: Confidence Interval. Elicitation doses for +, ++, +++ patch test reactions.

There was no statistical difference between the concentrations applied on the arms, back and behind the ears, tested with Wilcoxon test and McNemar´s test, as all P-values are above 0.05.

Table 8. Results of Wilcoxon's test analysed on 1+ reactions.

Dose (%)	Comparison	P-value
0.001	Back vs. ear	0.35
0.001	Back vs. arm	0.24
0.001	Arm vs. ear	0.89
0.01	Back vs. ear	0.22
0.01	Back vs. arm	0.69
0.01	Arm vs. ear	0.50
0.05	Back vs. ear	0.20
0.05	Back vs. arm	0.16
0.05	Arm vs. ear	0.64

6 DISCUSSION OF METHODS AND RESULTS

6.1 Hair dye reactions based on consumer complaints (I)

In the past, hair dye reactions have primary been studied retrospectively in consecutively patch tested patients and among hairdressers with occupational hand eczema. The present study was initiated by spontaneous consumer complaints concerning skin reactions to hair dyes addressed to a consumer organisation. The cases were collected by an advertisement calling for persons with adverse reactions to hair dyes The reactions from the included cases were relatively uniform, with severe oedema of the face, scalp, eyelids and ears. Other studies have described adverse reactions to hair dyes among consumers but have been somewhat unspecific (99,100). Cronin (9) described similar severe oedematous reactions after hair dyeing. Consumer complaint-based studies may add important knowledge to the clinical picture of cosmetic-related contact dermatitis. Concerning the 33 excluded consumer complaints it cannot be ruled out that some of these with chronic eczema could have had flair related to hair dye use; accordingly, the data represent a conservative estimate.

Collection of cases by advertising may introduce a selection bias. People who read a consumer magazine are a specially selected group and are probably more likely to report possible cosmetic side effects than the rest of the population. It is not possible to investigate non-responders in this type of study. At the time of the study the Danish retailers reported sales of about 10 million units of hair dye per year. This is a very gross measure with an unknown reliability. The number of exposures (units), exposure types and allergen concentration are important risk factors for adverse reactions in the individual and are prerequisites for any kind of risk assessment.
The study is descriptive and based on a selected sample of the population and the data do not allow for a frequency estimate of the occurrence of dermatitis caused by hair colouring. The main conclusion to be drawn from such a study is that the frequency of severe allergic hair dye reactions among the Danish consumers is not negligible. The data give only a qualitative impression of the problem and call for follow-up epidemiological investigations. It is known that understanding of diseases may be initiated by observations made by the layperson, e.g. Lyme disease.

14 cases had been seen by a dermatologist but only 8 had been patch tested. Antihistamines were the most used treatment. This illustrates that the health care services may not recognise or diagnose all adverse reactions to hair dyes. Eight reported a positive PPD patch test before they were

included in the study. On behalf of the Danish Consumer Council a further 8 persons who were willing to travel to the department of dermatology in Odense or Gentofte were later patch tested. All 16 persons patch tested (100%) gave a positive reaction to PPD. These patients were not selected in any way; therefore, although a classification bias is possible it is likely that a significant part of the remaining 39 would also be PPD positive if tested.

In 9 cases it was possible to obtain the used products. These were sent for chemical analysis and the content of hair dye ingredients was compared with products randomly collected. The analysis showed that there was no difference in the allergen content of the products from the patients and those randomly selected from the market (Table 9) (73). All products complied with the requirements of the Cosmetic Directive (12). The data indicate that hair dye ingredients at the present concentrations represent a health risk for the consumers.

Table 9. Chemical analysis of products used by allergic contact dermatitis patients and randomly collected products.

Oxidative hair dye precursor	**Concentration (%)**	
	Patients' products, n = 9	**Randomly collected, n=22**
toluene-2,5-diamine	0.18 - 0.98	0.01 - 3.99
m-aminophenol	0.015 - 0.38	0.01 - 0.94
p-aminophenol	0.16 - 2.1	0.31 - 1.20
p- phenylenediamine (PPD)	0.27	-

PPD was found in one hairdressers' product but not in any products for home colouring. (Reproduced from the paper by Sosted et al 2004 (73)).

6.2 Contact dermatitis to hair dyes in a general population (II)

In the present study, 75% of women and 18% of men had ever coloured their hair (range 1-80 years). In 1994 Berne et al (71) interviewed 1077 students (mean age 21 years) at a health and care science college about their use and observed side effects of cosmetics during the last five years. 54% of the female students and 10% of the male students had ever dyed their hair. Those who had dyed their hair at home were able to give both producer and brand name of the causative product. This indicates that they had understood the questions and made it possible to check if it was a permanent or a temporary dye that had caused the reaction. The higher mean age in our study population and changes in fashion in the ten years separating the two studies might explain the differences between Bernes study and the present study. In 2003, the same year as our study ran, the Norwegian consumer research institute found that 89% of women and 30% of men (N=1126) had

coloured or bleached their hair at some point in their life (100). However the response rate is not described in the report. Our data showed that the mean age of dyeing the hair for the first time was 16 years and other studies have shown that both adults and children are exposed to the same products (61) containing known potent allergens (73).

A response rate on 65.2% was obtained which is why a selection bias could be a problem. The sex and age distribution among the responders was equal to the Danish adult population, except for female responders in the age group 45-66 being overrepresented. Fewer persons from Zealand and Funen attended compared with the number of persons from Jutland. Sex, age and place of residence were known for non-responders. The hair dye questions were a minor part of a general health questionnaire study. The introductory letter, which was sent to the study population, contained information about the following topics in the interview: physical activity, importance of different diseases and the use of alternative treatments. As the letter did not mention hair dyes it is unlikely that selection bias or information bias interfered with the hair dye results. Inter-observer bias was not possible to investigate because only one interview was conducted for each interviewee. As none of the interviewers were involved in the project, it is assumed that they had no preferences for the outcome of the answers.

Our study is the first peer-reviewed interview study on cutaneous adverse effects of hair dye chemicals in the general population (101) and future studies may elucidate this issue further. Of those who had ever dyed their hair, 5.3% reported adverse reactions. The Norwegian consumer study among 1126 persons showed that 10% of those who used hair dye reported adverse effects defined as: discomfort, affliction or damage (100). The Norwegian figures are higher than ours; this could be due to a broader definition of adverse reaction and a selection bias in their study. In our study, 4.9% of those dyeing their hair reported redness, scaling and itching of the face, neck, ears or scalp after hair dyeing. It is possible that some of these reactions were irritative. The question regarding ulceration and oedematous reactions was inspired by the consumer complaints presented in Study I where these terms were used by the consumers themselves to describe the hair dye reaction. Notwithstanding that ulcerations and oedema can be present both in allergic and irritant reactions, all 16 who underwent patch testing in study I gave a positive reaction to PPD. Calling in the persons to departments of dermatology, from the present study could have given a further validation by recording their clinical history and symptoms concerning hair dye use and then patch

testing with relevant hair dye allergens. However, although theoretically possible, in reality these cases were distributed throughout the country and in practice could not be called in as this was outside the scope and philosophy of the population investigations performed by the National Institute of Public Health. Another way to validate the results could be a prospective study of new clinical cases tested with relevant hair dye ingredients to define the relative proportion of allergic versus irritative reactions caused by hair dyes among eczema patients or a new population-based questionnaire study with nested cases and controls undergoing patch testing. To limit the study, no questions were asked about persons' use of perming products, as it would be incorrect to perm hair at the same time as dyeing it. The relative acuteness of hair dye dermatitis makes it obvious to the consumer that the hair dye product is the likely cause, and it is possible that consumers solve the problem by avoiding future exposure. It is known from other studies that even people with severe adverse reactions to cosmetics do not contact the health care services (99). In this study, only 15.9% of those with a severe hair dye reaction consulted a doctor and only a minority of those will be referred to a dermatologist and eventually patch tested. It is therefore understandable that patch test data represent the tip of the iceberg based on a highly selected group.

Of our total population, including individuals who had never dyed their hair, 2.6% reported a skin reaction compatible with hair dye contact allergy. This is more frequent than expected from patch test studies (41,42) where the background population was tested with a single hair dye ingredient, PPD, showing frequencies in the range of 0.1% - 1% in Europe (41,42). In a non-clinical unselected Thai population, 2.3% were sensitized to PPD, but a large female bias may have increased the prevalence (43). Either the estimate from our study is too high or PPD is not sufficient as the only patch test material for assessing hair dye allergy. It seems that important information might be overlooked by the currently used diagnostic tests (102,103). In our study only those who answered that they ever have dyed their hair were asked if they had been patch tested by a dermatologist. It would have been valuable to know the total number who had been patch tested and the outcome. If dermatological patients only had been investigated, these estimates could not have been given.

PPD is illegally added to temporary tattoos to give deeper and faster black dye (12). In single cases temporary tattoos have been described as an important risk factor for developing hair dye dermatitis (74,104,105). Our study showed that 6.6% of the test population had had both a hair dye reaction and a temporary tattoo compared with 5.2% who had had a hair dye reaction only; however, the

difference was not statistically significant. A recent study from St John's Institute of Dermatology in London gives similar results and draws the conclusion that temporary tattoos are not the main reason for developing contact dermatitis to hair dyes (106). In children, however, a previous temporary tattoo may be a more important additional risk factor for hair dye reactions than in adults (61). For children, obviously an early exposure to hair dyes increases the lifetime risk of developing hair dye dermatitis.

6.3 Sensitization potency ranking of hair dye substances (III)

The TOPS-MODE method (24) was chosen to assess the sensitization potency of existing hair dyes, as it is a general model not restricted to one chemical class. The method was used to investigate ingredients where only the molecular structure was known. This was an advantage, as few clinical studies exist on these chemicals. As numerous chemical reactions take place in an oxidative hair dye before it is applied to the skin, the QSAR method has some limitations as effects of intermediates and the final product that come in contact with the skin are not included. Furthermore additive or synergistic allergic reactions caused by cross reactivity and multiple sensitizations are likely. Salts could not be used in the programme. This means that for some of the dyes e.g. 2,4-diaminophenoxyethanol HCL (INCI name), the calculation was made for 2,4-diaminophenoxyethanol, which might lead to an incorrect classification. This was done to make it possible to recognise the substance on the ingredients' list on the packaging of a hair dye product. Finally, a validation of the ranking of substances can be done only in a prospective clinical study including exposure information and by patch testing e.g. with the ingredients listed in table 5.

The main results are divided into three classes. Class 1 is all the hair dye substances that were predicted to be moderate/strong sensitizers. Class 2 is the hair dye substances that are predicted to be weak sensitizers. Class 3 covers substances that are predicted to be extremely weak or non-sensitizers. The values within each class are calculated on an arbitrary scale. In class 1, PPD is found among the moderate/strong sensitizers. This has a lower sensitization value than e.g. direct red 80. This means that direct red 80 is predicted to be a stronger sensitizer than PPD, but since the scales are arbitrary, it does not give information on how much stronger direct red 80 is. The values can be compared within each table, but only to establish if a substance is predicted to be more or less potent than another. Therefore it is not possible to compare values in class 1 with values from class 2, but only to establish that substances in class 1 are all predicted to be more potent than

substances in class 2 and class 3. Negative values are given in class 3 and here again the rule is that the higher the number the stronger the potency.

In 2003, Uter et al (48) described an increase in the frequency of patients with a positive reaction to toluene-2,5-diamine. This correlates well with study III, which showed that toluene-2,5-diamine was the most commonly used hair dye ingredient in Europe in 2002; this is also in agreement with exposure assessments done by chemical analysis (73).

In study III, the hair dye substances were ranked according to their predicted sensitization potential based on physical chemical properties and LLNA. This type of study has not been done before. Schlede et al ranked chemical substances according to the published sensitization data. Schlede et al. established a group of 30 experts in 1985 and 34 meetings were held between then and June 2001 (107). They divided allergenic substances into categories A, B and C: A) a significant contact allergen; B) substances with solid-based indication for contact allergenic effects; and C) substances with insignificant contact allergen or questionable contact allergenic effects. They assessed 244 substances, 11 of which were hair dyes. Comparison of the evidence-based results and the QSAR results are given in Table 10.

Table 10. A comparison of results from two classifications models (Schledes expert panel and QSAR data from study III)

Inci name (cas.nr.)	**From Schlede et al** (107)	**QSAR data** (study III)
N-Phenyl-p-phenylenediamine (101-54-2)	Significant contact allergen	Strong/moderate sensitizer
p-Aminophenol (123-30-8)	Significant contact allergen	Strong/moderate sensitizer
Nitro-p-phenylenediamine (5307-14-2)	Significant contact allergen	Strong/moderate sensitizer
p-Phenylenediamine (PPD) (106-50-3)	Significant contact allergen	Strong/moderate sensitizer
Solvent Yellow 33 (8003-22-3)	Significant contact allergen	Weak sensitizer
Toluene-2,5-diamine (95-70-5)	Significant contact allergen	Strong/moderate sensitizer
o-Aminophenol (95-55-6)	Solid-based indication for contact allergenic effects	Strong/moderate sensitizer
o-Phenylenediamine (93-54-5)	Solid-based indication for contact allergenic effects	Strong/moderate sensitizer
Resorcinol (108-46-3)	Solid-based indication for contact allergenic effects	Strong/moderate sensitizer
m-Phenylenediamine (108-45-2)	Insignificant contact allergen or questionable contact allergenic effects	Strong/moderate sensitizer
Acid Yellow 23 / tartrazine (1934-21-0)	Insignificant contact allergen or questionable contact allergenic effects	Strong/moderate sensitizer

Whether a contact allergen was listed as a significant contact allergen in the Schlede classification depended on its inherent potency, exposure conditions, concentration, duration and frequency of

exposure, as well as the size of the exposed population. Schledes classification (107) also depended on a well-functioning reporting or surveillance system. Even though the QSAR analysis (sensitization) and the evidence-based analysis (elicitation) have very different approaches, the two different methods had similar classification for 5 of 11 hair dye ingredients. The potential weakness of Schledes system was shown by the methyldibromo glutaronitrile (MDGN) being grouped as a category B substance only, even though it is reported as a major cause of contact allergy (108). MDGN is now prohibited in cosmetic stay–on products (12) based on clinical and epidemiological evidence. Furthermore, many hair dye allergens might be overlooked if they are assessed only on the background of reported cases.

A search of the literature, showed that only few of the potent hair dye allergens are evaluated by patch testing in clinical cases. PPD has been used as the major screening agent for hair dye contact dermatitis for decades without a proper prospective evaluation of whether there are sufficient cross reactivities with the other hair dye contact allergens.

Since cross reactions between some hair dye substances occur (55), a list (Table 5) of potent allergens from different clusters might be representative of substances with the same physio-chemical properties. Since the literature search for study III was done, a few papers have been published concerning rare hair dye allergens. Sosted et al published data on a 12-year-old boy with an allergic reaction to a permanent hair dye product containing 4-amino-*m*-cresol. The patient gave a ++ patch test reaction to 4-amino-*m*-cresol (CAS. No. 2835-99-6) (61). Furthermore the hair dye ingredient 3-nitro-*p*-hydroxyethylaminophenol (CAS. No. 65235-31-6), which is used in oxidative and direct dyes, was described in two clinical cases in 2005 and 2006 (103,109). Dejobert et al. reported a reaction to 2-hydroxyethylamino-5-nitroanisole (CAS. No. 50982-74-6) (109). The three allergens are predicted to be moderate to strong sensitizers and more than 2 tonnes of 4-amino-*m*-cresol and 3-nitro-*p*-hydroxyethylaminophenol were used in 2002. Although some of the allergens are available from Sigma-Aldrich, it is important to do validation studies with substances in purity grades actually used by industry.

6.4 Patch test dose-response study of *p*-phenylenediamine (IV)

In the dose-response study of PPD, the patches were placed randomly behind the ears and on the arm but were not randomised on the back. The randomisation of the patches placed behind the ears and on the arm was done because of the low number of patches (n=4), thus giving less risk of introducing errors. Optimally, randomisation should have been done also on the back. However, as figure 3 shows there is a dose response relationship both in the randomised and in the non-randomised samples. Blind reading was performed as the patches were applied by one nurse and read by another. There was no blinding of the reading of the patches on the back. Doubtful reactions (?+) to PPD were recorded but not shown, as these reactions can be irritative as well as signs of weak allergic reactions, and no control group was included. Instead + reactions was used as cut-point. The day of reading was used instead of the number of hours, to avoid giving a misleading impression of an exact time of reading, which was not always the case (85). The readings were done on D2, D3 and D7; these days of readings were chosen to make it possible for the patients to present on D4 if they missed D3. Since all the patients showed up as planned, all the readings were done on D3. The principal investigator (HS) supervised all the readings in order to inform the patients, to record the readings and to take photographs of each reading.

Fresh samples of PPD were prepared weekly to ensure that as much PPD as possible was present in the patches. The concentration was not checked, as PPD was diluted in petrolatum, which is not suitable for High Performance Liquid Chromatography (HPLC) (110). If the PPD concentrations in this study had been diluted in acetonitrile before chemical analysis, it may have been possible to analyse PPD. However, as this would have required a development of a new chemical analysis method, including stability tests and recovery tests, it was not done.

15 patients were recruited for the study. 12 had had a previous hair dye reaction. Three patients had never coloured their hair, and neither had they had a temporary tattoo. Two of the three had previously been patch tested which cannot be excluded as an explanation for PPD sensitization. For the third person there was no possible explanation for the PPD allergy. It is described that contact allergies defined by a positive patch test may be found without any clinical explanation (111).

No statistically significant difference was found between the sensitivity of the different test sites; however, the power of the study of 15 patients was limited to 35 - 50%. To achieve a power of 80%, given the data obtained, between 29 and 48 patients should have been included (Table 11). This was, however, not practically possible.

Table 11. Statistical power and number of patients.

	N=15	**Power 80%**	**Power 90%**
Back versus arm	power = 0.35	N=48	N=63
Back versus ear	power = 0.40	N=39	N=52
Back versus arm+ear	power = 0.50	N=29	N=38

The power of a test is the probability that a study of a given size would detect a statistically significant real difference of a given magnitude (78).

There is a higher inter-individual variability on the back versus arm compared to back versus ear, thus more test subjects were needed to obtain a better power.

In a controlled laboratory study using dinitrochlorobenzene (DNCB) as the model allergen, Friedmann et al showed that the dose per unit area is the key factor for induction of contact allergy concerning areas between 3 mm and 1 cm across (112), while Zachariae et al showed a clear dose-response relation in relation to the allergen concentration and elicitation of contact allergy, but not to the quantity of the allergen per unit area on the skin. Studies comparing the effect of total amount or percentage concentration on different areas are needed (113).
Study IV concerned PPD only and not a prototype hair dye containing coupler, and oxidizing agent, because a trial with such a prototype hair dye in a dilution series would require a prototype hair dye under nitrogen pressure to prevent reactions with the coupler. To patch test with a hair dye requires that the patches are applied exactly (within minutes) at the same time for each patient, as PPD will polymerise when coming in contact with a coupler, air and especially if hydrogen peroxide is added to the dye. Another unknown factor would be what substance the patient reacted to, as a prototype hair dye would contain both PPD and a coupler.

In study IV, the threshold for non-reactivity in 90% of the participants was 38 ppm PPD (CI: 4.3-100). Krasteva et al. investigated reactions to permanent hair dye products (without prior oxidation with hydrogen peroxide) containing PPD. These were applied to 34 PPD-positive hair dye allergic individuals in a single open test. 79 % reacted to 1000 ppm (compared to 80 % in study IV), 88% reacted to 5000 ppm (compared to 87% in study IV) and 97% reacted to 10,000 ppm PPD (compared to 87% in study IV) (75). The results strongly resemble those of the 2 days' patch test with PPD only carried out in the present study. Since Krasteva et al (75) did not test with

concentrations below 1000 ppm PPD, no comparisons can be made about reactions to lower concentrations in an open test.

As most of the used hair dye ingredients are predicted to be moderate/strong sensitizers (study III), substitution with other dyes is hard to do. In this study, the elicitation threshold value for 10% of the patch-tested persons (ED_{10}) on the back was 38 - 100 ppm PPD. In an earlier study we found induction or elicitation of contact allergy from products containing PPD and its derivatives in a concentration range from 100 ppm to 39,900 ppm. A group of control products randomly collected showed a similar concentration range (73). In the prevention of hair dye allergy it may be necessary to reduce the content of dye allergen to a concentration where it is tolerable for allergic patients.

In the present study, there were no statistical differences between the three regions: retroauricular area, upper arms, and back. Accordingly, PPD applied to the back may give a representative response of the sensitivity of the regions on the arm and behind the ears. This needs to be further evaluated as the power of the study was 50% or below. A final validation would be a comparison between response to a realistic use test in the scalp hair region and patch testing on the back. 10 of the 15 patients who had had a previous allergic reaction to a hair dye product did not react more strongly behind the ears than on the back and arm. Studying dose-response relationships on the back is technically easier and more cosmetically acceptable to the patient than testing in the retroauricular region.

7 CONCLUSIONS

I

The current knowledge about the frequency of adverse reactions to hair dyes is inadequate, and the consumer complaint-based data indicate that many cases may be overlooked since most of the persons had not been sufficiently diagnosed and treated in the health care system. The clinical reaction can be severe and only few cases are referred to a dermatologist and even fewer are patch tested. The frequency of allergic contact dermatitis from hair dyes is likely to be underestimated. Population studies including relevant patch testing of the cases would improve the quality of data.

II

The rate of adverse skin reactions to hair dyes was higher than expected from patch-test studies; 5.3% had adverse skin reactions among these 1.4% reported severe oedematous reactions. The reactions may be allergic as well as irritative. Another significant finding was that the typical age of starting to dye hair was 16 years, for both females and males. This means that even adolescents are exposed to hair dyes and that this procedure is not performed only to cover grey hair. Persons, even those with severe reactions, are only rarely in contact with the health care service, meaning that epidemiological figures from dermatologists refer to a highly selected minority. A previous temporary tattoo was not a significant risk factor for an adverse reaction to hair dyes.

III

229 hair dye substances were identified; the majority of these (75%) were predicted to be moderate / strong sensitizers. Among these, 28 substances were used in high tonnage and had a strong predicted sensitization potential, some of them even higher than PPD. Only PPD is already routinely used for patch testing patients with suspected hair dye allergy. The study indicates that the current main diagnostic tool, PPD, needs validation and needs to be supplemented with additional substances. An extended patch test series is suggested.

IV

The present study suggests that PPD can elicit a contact allergic reaction in a concentration (50 ppm) that is 1,200 times lower than the accepted legal limit (60,000 ppm) in hair dye products. There were no statistically significant differences in the sensitivity of the three anatomical regions. The upper back is a suitable region for patch testing patients with hair dye dermatitis. A final validation will be a comparison of patch test results and realistic exposures to hair dye allergens.

8 FUTURE STUDIES

Cases of hair dye allergy should be investigated, including patch testing with the actual used product and single ingredients in the causative products. Exposure assessment by quantitative analysis of the allergens in the used products should be carried out and for this purpose applicable analytical methods should be established for the most frequently used hair dye ingredients.

A study among patients tested in different departments of dermatology should be performed in order to clarify the relation to clinical reactions in connection to hair dyeing. The scope is to estimate the frequency of clinical hair dye reactions proved by a positive PPD patch test.

The general population study should be repeated to investigate the development in use of hair dyes and skin side effects over time, preferably supplemented by patch testing individuals reporting symptoms. Similar studies in other countries would complete the epidemiological picture.

Future work could include patch testing with the 28 substances suggested as new allergens with high use (study III). The outcome of such a study might reveal whether substances within a cluster cross react and/or their mechanisms of action are the same. The study should run on a European level to increase the number of patients for an acceptable statistical significance as well as the added community value. A prerequisite for running this study is collaboration with industry to obtain hair dye ingredients of relevant known purity.

Dose-response studies on hair dye allergens are needed to find a safe limit for use in allergenic patients. This requires a sufficient number of patients tested positive to relevant allergens other than PPD. A European study would make this possible.

9 SUMMARY

Since the end of the 19th century it has been possible to colour hair using so-called permanent hair dyes based on para-substituted aromatic amines combined with couplers and oxidizing agents. Allergic contact dermatitis caused by hair dyes has long been known as a risk for hairdressers and consumers. The frequency and severity of such reactions in the Danish population was unknown and initiated the investigation reported in the two first studies. The **first** study is a nationwide consumer complaint-based study made by advertising for persons who had experienced adverse reactions to hair dyes. Using a Danish consumer organisation, 55 persons with hair dye contact dermatitis were recruited within 16 months. The study revealed that adverse reactions to hair dyes were often severe, were frequently unreported to the health care system, and if persons were seen by a doctor, they were often misdiagnosed. The **second** study is an epidemiological investigation including a representative random sample of 4,000 persons of the Danish adult population invited to take part in an interview investigation. Adverse skin reactions to hair dye were reported in 5.3% of the respondents. 15.6% of those with symptoms had been in contact with health care services after a hair dye reaction.

For many years, *p*-phenylenediamine (PPD) has been included in the standard patch test series as the main indicator of contact allergy to hair dyes. However, many other dye ingredients are used and a **third** study was undertaken to evaluate if some of these would be relevant as a supplement to the current diagnostic testing. Furthermore such a study could indicate if less sensitizing dyes could substitute the aromatic amines. The third study covered a quantitative structure-activity relationship (QSAR) analysis based on local lymph node assay (LLNA) data and topological substructural molecular descriptors (TOPS-MODE). The objectives were to rank hair dye ingredients according to their predicted sensitization potency and to suggest a hair dye patch test series. 229 substances were identified by their chemical structure; the majority (75%) were predicted to be strong/moderate sensitizers and this group covered the 8 most used hair dye ingredients. A new patch test series consisting of 28 dye ingredients was suggested. Since most of the used hair dyes are sensitizers, it may be necessary to reduce the content of dye allergen to a concentration where it is tolerable for allergic patients. Subsequently, the **fourth** study was planned as a patch test dose response investigation of PPD thresholds and regional differences. 15 patients with a former positive patch test reaction to PPD were included. A patch test with a serial dilution of PPD in petrolatum was applied. The thresholds value for 10% of the patch-tested persons (ED_{10}) on the back was 38 ppm p-phenylenediamine. There were no statistically significant differences in responses between testing behind the ears, on the back and on the arms.

In summary, 1) among consumers, hair dyes can cause severe allergic reactions that may be misdiagnosed in the health care system, 2) in a general population-based sample, 5.3% reported adverse skin reactions to hair dyes, 3) the most used hair dye ingredients are predicted to be strong/moderate sensitizers, and 4) the threshold value for PPD in 10% of the patch tested persons (ED_{10}) is 38 ppm.

10 DANSK RESUMÉ

Siden slutningen af det 19. århundrede har det været muligt at farve sit hår med såkaldte permanente hårfarver baseret på parasubstituterede aromatiske aminer kombineret med koblere og oxidationsmidler. Kontaktallergi forårsaget af hårfarver er velkendt blandt frisører og forbrugere og formentlig hyppigst forårsaget af permanente hårfarver. Frekvensen og sværhedsgraden af sådanne reaktioner var ukendt i den danske befolkning og gav anledning til to undersøgelser. Det **første** studium var landsdækkende og baseret på forbrugerhenvendelser der stammede fra annoncering efter personer, som havde haft bivirkninger efter hårfarvning. På 16 måneder blev 55 personer med allergisk kontakteksem over for hårfarver inkluderet. Studiet afslørede at bivirkninger efter hårfarvning ofte er alvorlige og at personerne sjældent kom i kontakt med sundhedsvæsenet, og hvis de var hos lægen blev symptomerne ofte hverken diagnosticeret, udredt eller relevant behandlet. Det **andet** studium var en epidemiologisk interviewundersøgelse af en repræsentativ tilfældigt udvalgt gruppe på 4000 voksne danskere. Svarprocenten var 65,2 og 5.3 % af de respondenter, der havde farvet deres hår, havde haft hudsymptomer forenelige med kontakteksem. 15.6 % af personer med symptomer havde været i kontakt med sundhedsvæsenet på grund af deres hårfarve reaktion.

p-Phenylenediamine (PPD), har været i den europæiske standard lappetest serie i mange år, kendt som hovedindikatoren for kontaktallergi over for hårfarveingredienser selvom der kan benyttes mange forskellige farvestoffer i hårfarver. På denne baggrund blev det **tredje** studium igangsat med henblik på at evaluere om nogle af disse ingredienser kunne være relevante, som supplement til den nuværende diagnostiske test. Ydermere ønskedes det undersøgt om andre mindre sensibiliserende ingredienser ville kunne substituere de aromatiske aminer. Det tredje studium omfattede en analyse baseret på kemisk struktur (QSAR TOPS-MODE) opbygget på basis af dyredata (LLNA). Formålet var at rangordne hårfarveingredienser ud fra deres prædikterede sensibiliserings potens og at foreslå en hårfarve lappetest serie. 229 ingredienser blev identificeret ved deres kemiske struktur; hovedparten af dem (75 %) blev forudsagt til at være stærkt/moderate sensibiliserende og denne gruppe indeholdt de 8 mest anvendte hårfarveingredienser. En ny lappetest serie blev foreslået bestående af 28 farveingredienser. Siden de mest anvendte ingredienser blev prædikteret til at være stærkt til moderat sensibiliserende, er substitution næppe mulig og det kan blive nødvendigt at reducere indholdet af farveallergener til et niveau, hvor de kan tåles af allergikere. På den baggrund blev et **fjerde** studium planlagt som en dosis response undersøgelse af *p*-phenylenediamine m.h.p. at bestemme grænseværdier og anatomiske regionale forskelle i allergisk respons. 15 patienter med en tidligere påvist PPD kontaktallergi blev inkluderet og fik lagt en seriefortynding af PPD i petrolatum på ryggen, bag øret og på overarmen. Tærskelværdien på ryggen for de 10 % mest følsomme personer (ED_{10}) var 38 ppm *p*-phenylenediamine. Der var ikke signifikant forskel i responset mellem de tre forskellige anatomiske regioner.
Vi fandt således at hårfarver kan fremkalde alvorlige allergiske reaktioner blandt forbrugere, der ofte hverken bliver diagnosticeret, udredt eller relevant behandlet i sundhedsvæsenet, 2) 5.3 % af den generelle befolkning rapporterede bivirkninger efter hårfarvning forenelig med kontakteksem, 3) de hårfarvestoffer, der blev anvendt mest, blev også prædikteret som stærkt til moderat allergifremkaldende og 4) 10 % af de PPD allergiske patienter reagerede på 38 ppm PPD ved lappetest.

11 REFERENCES

1. Corbett JF. Hair coloring. *Clin.Dermatol.* 1988; **6:** 93-101.

2. Hofmann AW. Organische Basen. *Jahresberichte ueber die fortschritte der chemie* 1863; **3:** 422.

3. Bonnevie P. Aetiologie und pathogenese der Ekzemkrankheiten. Klinische Studien über die Ursachen der Ekzeme unter besonderer Berücksichtigung des Diagnostischen Wertes der Ekzemproben. 1939. Busch, Copenhagen / Barth, Leipzig.

4. The European Cosmetic Toiletry and Perfumery Association (Colipa). The European cosmetic, toiletry & perfumery Market 2003. 1-26. 2004.

5. Wolfram LJ. Human hair: a unique physicochemical composite. *J Am.Acad.Dermatol.* 2003; **48:** S106-S114.

6. Courtois M, Loussouarn G, Hourseau C, Grollier JF. Ageing and hair cycles. *Br.J Dermatol.* 1995; **132:** 86-93.

7. Lloyd T, Garry FL, Manders EK, Marks JG, Jr. The effect of age and hair colour on human hairbulb tyrosinase activity. *Br.J Dermatol.* 1987; **116:** 485-489.

8. Keogh EV, Walsh RJ. Rate of greying of human hair. *Nature* 1965; **207:** 877-878.

9. Cronin E. Hair preparations. *Contact Dermatitis.* Edingburg, London, New York: Churchill Livingstone, 1980: 115-126.

10. Nohynek GJ, Fautz R, Benech-Kieffer F, Toutain H. Toxicity and human health risk of hair dyes. *Food Chem.Toxicol.* 2004; **42:** 517-543.

11. Bouillon C, Wilkinson Je. *The science of hair care*. Boca Raton , FL: Taylor & Francis, 2005.

12.Council directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the member states relating to cosmetic products, amended. European Communities off.Journal L262 27.9. 1976.

13. Bowling JC, Scarisbrick J, Warin AP, Downs AM. Allergic contact dermatitis from trideceth-2-carboxamide monoethanolamine (MEA) in a hair dye. *Contact Dermatitis* 2002; **47:** 116-117.

14. Le Coz CJ, Schneider GA. Contact dermatitis from tertiary-butylhydroquinone in a hair dye, with cross-sensitivity to BHA and BHT. *Contact Dermatitis* 1998; **39:** 39-40.

15. Fiume MZ. Final report on the safety assessment of Acid Violet 43. *Int.J Toxicol.* 2001; **20:** 1-6.

16. European Commission. Opinion on Quinolinium, 4-formyl-1-methyl-,salt with 4-methylbenzenesulfonic acid (1:). SCCP/0923/05, 1-20. 2005.

17. European Commission. Opinion on hydroxyethyl-2-nitro-p-toluidine. SCCP/0924/05, 1-25. 2006.

18. Fregert S. [Chemical demonstration of paraphenylene diamine in hair dyes]. *Hautarzt* 1972; **23:** 393-394.

19. European Commission SCCNFP. Opinion of use of Permanent Hair Dyes and Bladder Cancer Risk. SCCNFP/0143/01. 2001.

20. European Commission SCCNFP. Use of permenent hair dyes and bladder cancer. SCCNFP/0797/04. 2004.

21. Sieben S, Kawakubo Y, Al Masaoudi T, Merk HF, Blomeke B. Delayed-type hypersensitivity reaction to paraphenylenediamine is mediated by 2 different pathways of antigen recognition by specific alphabeta human T-cell clones. *J.Allergy Clin.Immunol.* 2002; **109:** 1005-1011.

22. Frosch PJ, Menne T, Lepoittevin JP. *Contact Dermatitis 4th Edition*. Springer-Verlag Berlin Heidelberg Germany, 2006.

23. Lepoittevin JP. Metabolism versus chemical transformation or pro- versus prehaptens? *Contact Dermatitis.* 2006; **54:** 73-74.

24. Estrada E, Patlewicz G, Chamberlain M, Basketter D, Larbey S. Computer-aided knowledge generation for understanding skin sensitization mechanisms: the TOPS-MODE approach.

Chem.Res.Toxicol. 2003; **16:** 1226-1235.

25. http://www.chem.leeds.ac.uk/LUK/derek/index.html. 2003.

26. Sanderson DM, Earnshaw CG. Computer prediction of possible toxic action from chemical structure; the DEREK system. *Hum.Exp.Toxicol.* 1991; **10:** 261-273.

27. Benezra C, Sigman CC, Bagheri D, Helmes CT, Maibach HI. A systematic search for structure-activity relationships of skin sensitizers. II. Para-phenylenediamines. *Semin.Dermatol.* 1989; **8:** 88-93.

28. Patlewicz GY, Wright ZM, Basketter DA, Pease CK, Lepoittevin JP, Arnau EG. Structure-activity relationships for selected fragrance allergens. *Contact Dermatitis* 2002; **47:** 219-226.

29. Basketter DA, Scholes EW. Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. *Food Chem.Toxicol.* 1992; **30:** 65-69.

30. Gerberick GF, Robinson MK, Ryan CA, Dearman RJ, Kimber I, Basketter DA, Wright Z, Marks JG. Contact allergenic potency: correlation of human and local lymph node assay data. *Am.J.Contact Dermat.* 2001; **12:** 156-161.

31. Magnusson B, Kligman AM. The identification of contact allergens by animal assay. The guinea pig maximization test. *J Invest Dermatol.* 1969; **52:** 268-276.

32. Weibel H, Hansen J, Andersen KE. Cross-sensitization patterns in guinea pigs between cinnamaldehyde, cinnamyl alcohol and cinnamic acid. *Acta Derm.Venereol.* 1989; **69:** 302-307.

33. Bronaugh RL, Roberts CD, McCoy JL. Dose-response relationship in skin sensitization. *Food Chem.Toxicol.* 1994; **32:** 113-117.

34. Marzulli FN, Maibach HI. The use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man. *Food Cosmet.Toxicol.* 1974; **12:** 219-227.

35. Kligman AM. The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. *J.Invest Dermatol.* 1966; **47:**

375-392.

36. McFadden JP, Wakelin SH, Holloway DB, Basketter DA. The effect of patch duration on the elicitation of para-phenylenediamine contact allergy. *Contact Dermatitis* 1998; **39:** 79-81.

37. Hextall JM, Alagaratnam NJ, Glendinning AK, Holloway DB, Blaikie L, Basketter DA, McFadden JP. Dose-time relationships for elicitation of contact allergy to para-phenylenediamine. *Contact Dermatitis* 2002; **47:** 96-99.

38. Berne B, Lundin A. [Adverse effects of cosmetics--an unembellished report. Every 10th consumer has complaints--few consult physician]. *Lakartidningen* 1994; **91:** 2143-2144.

39. Aguirre A, Zabala R, Sanz de Galdeano C, Landa N, Diaz-Perez JL. Positive patch tests to hydrogen peroxide in 2 cases. *Contact Dermatitis* 1994; **30:** 113.

40. Tosti A, Piraccini BM, van Neste DJ. Telogen effluvium after allergic contact dermatitis of the scalp. *Arch.Dermatol.* 2001; **137:** 187-190.

41. Nielsen NH, Linneberg A, Menne T, Madsen F, Frolund L, Dirksen A, Jorgensen T. Allergic contact sensitization in an adult Danish population: two cross-sectional surveys eight years apart (the Copenhagen Allergy Study). *Acta Derm.Venereol.* 2001; **81:** 31-34.

42. Schafer T, Bohler E, Ruhdorfer S, Weigl L, Wessner D, Filipiak B, Wichmann HE, Ring J. Epidemiology of contact allergy in adults. *Allergy* 2001; **56:** 1192-1196.

43. Basketter DA, Duangdeeden I, Gilmour NJ, Kullavanijaya P, McFadden JP. Prevalence of contact allergy in an adult Thai population. Contact Dermatitis 50[3], 128-129. 2004. Ref Type: Abstract

44. Hasan T, Rantanen T, Alanko K, Harvima RJ, Jolanki R, Kalimo K, Lahti A, Lammintausta K, Lauerma AI, Laukkanen A, Luukkaala T, Riekki R, Turjanmaa K, Varjonen E, Vuorela AM. Patch test reactions to cosmetic allergens in 1995-1997 and 2000-2002 in Finland - a multicentre study. *Contact Dermatitis* 2005; **53:** 40-45.

45. Dawe SA, White IR, Rycroft RJ, Basketter DA, McFadden JP. Active sensitization to para-phenylenediamine and its relevance: a 10-year review. *Contact Dermatitis* 2004; **51:** 96-97.

46. Schnuch A, Geier J, Uter W, Frosch PJ, Lehmacher W, Aberer W, Agathos M, Arnold R, Fuchs T, Laubstein B, Lischka G, Pietrzyk PM, Rakoski J, Richter G, Rueff F. National

rates and regional differences in sensitization to allergens of the standard series. Population-adjusted frequencies of sensitization (PAFS) in 40,000 patients from a multicenter study (IVDK). *Contact Dermatitis* 1997; **37:** 200-209.

47. Sharma VK, Chakrabarti A. Common contact sensitizers in Chandigarh, India. A study of 200 patients with the European standard series. *Contact Dermatitis* 1998; **38:** 127-131.

48. Uter W, Lessmann H, Geier J, Schnuch A. Contact allergy to ingredients of hair cosmetics in female hairdressers and clients - an 8-year analysis of IVDK data. *Contact Dermatitis* 2003; **49:** 236-240.

49. Dickel H, Kuss O, Blesius CR, Schmidt A, Diepgen TL. Occupational skin diseases in Northern Bavaria between 1990 and 1999: a population-based study. *Br.J Dermatol.* 2001; **145:** 453-462.

50. Armstrong DK, Jones AB, Smith HR, Ross JS, White IR, Rycroft RJ, McFadden JP. Occupational sensitization to p-phenylenediamine: a 17-year review. *Contact Dermatitis* 1999; **41:** 348-349.

51. Lodi A, Mancini LL, Ambonati M, Coassini A, Ravanelli G, Crosti C. Epidemiology of occupational contact dermatitis in a North Italian population. *Eur.J.Dermatol.* 2000; **10:** 128-132.

52. Shah M, Lewis FM, Gawkrodger DJ. Occupational dermatitis in hairdressers. *Contact Dermatitis* 1996; **35:** 364-365.

53. Lind ML, Boman A, Sollenberg J, Johnsson S, Hagelthorn G, Meding B. Occupational dermal exposure to permanent hair dyes among hairdressers. *Ann.Occup.Hyg.* 2005; **49:** 473-480.

54. Herve-Bazin B, Gradiski D, Duprat P, Marignac B, Foussereau J, Cavelier C, Bieber P. Occupational eczema from N-isopropyl -N'-phenylparaphenylenediamine (IPPD) and N-dimethy-1,3 butyl-N'-phenylparaphenylenediamine (DMPPD) in tyres. *Contact Dermatitis* 1977; **3:** 1-15.

55. Fautz R, Fuchs A, van der Walle HB, Henny V, Smits L. Hair dye-sensitized hairdressers: the cross-reaction pattern with new generation hair dyes. *Contact Dermatitis* 2002; **46:** 319-324.

56. Goon AT, Gilmour NJ, Basketter DA, White IR, Rycroft RJ, McFadden JP. High frequency of simultaneous sensitivity to Disperse Orange 3 in patients with positive patch tests to para-phenylenediamine. *Contact Dermatitis* 2003; **48:** 248-250.

57. Hausen BM. Contact allergy to disperse blue 106 and blue 124 in black "velvet" clothes. *Contact Dermatitis.* 1993; **28:** 169-173.

58. Seidenari S, Giusti F, Massone F, Mantovani L. Sensitization to disperse dyes in a patch test population over a five-year period. *Am.J Contact Dermat.* 2002; **13:** 101-107.

59. Seidenari S, Mantovani L, Manzini BM, Pignatti M. Cross-sensitizations between azo dyes and para-amino compound. A study of 236 azo-dye-sensitive subjects. *Contact Dermatitis* 1997; **36:** 91-96.

60. Ho SG, White IR, Rycroft RJ, McFadden JP. Allergic contact dermatitis from para-phenylenediamine in Bigen powder hair dye. *Contact Dermatitis* 2004; **51:** 93-94.

61. Sosted H, Johansen JD, Andersen KE, Menne T. Severe allergic hair dye reactions in 8 children. *Contact Dermatitis* 2006; **54:** 87-91.

62. Shmunes E. Purpuric allergic contact dermatitis to paraphenylenediamine. *Contact Dermatitis* 1978; **4:** 225-229.

63. Avnstorp C, Rastogi SC, Menne T. Acute fingertip dermatitis from temporary tattoo and quantitative chemical analysis of the product. *Contact Dermatitis* 2002; **47:** 119-120.

64. Marcoux D, Couture-Trudel PM, Riboulet-Delmas G, Sasseville D. Sensitization to para-phenylenediamine from a streetside temporary tattoo. *Pediatr.Dermatol.* 2002; **19:** 498-502.

65. Saunders H, O'Brien T, Nixon R. Textile dye allergic contact dermatitis following paraphenylenediamine sensitization from a temporary tattoo. *Australas.J.Dermatol.* 2004; **45:** 229-231.

66. Hillen U, Jappe U, Frosch PJ, Becker D, Brasch J, Lilie M, Fuchs T, Kreft B, Pirker C, Geier J. Late reactions to the patch-test preparations para-phenylenediamine and epoxy resin: a prospective multicentre investigation of the German Contact Dermatitis Research Group. *Br.J Dermatol.* 2006; **154:** 665-670.

67. Becker D, Mahler V, Szliska C, Loffler H, Brasch J, Hillen U, Schnuch A, Elsner P. The concentration of para-phenylenediamine (PPD) for routine patch testing in a standard series needs to be redefined. *Contact Dermatitis.* 2005; **53:** 186-187.

68. Camarasa JM. First epidemiological study of contact dermatitis in Spain - 1977. Spanish Contact Dermatitis Research Group. *Acta Derm.Venereol.Suppl (Stockh).* 1979; **59:** 33-37.

69. Geier J, Lessmann H, Schnuch A, Uter W. Contact sensitizations in metalworkers with occupational dermatitis exposed to water-based metalworking fluids: results of the research project "FaSt". *Int.Arch.Occup.Environ.Health.* 2004; **77:** 543-551.

70. Walker SL, Ead RD, Shackleton DB, Beck MH. Two cases of occupational allergic contact dermatitis to p-aminophenol in pharmaceutical workers manufacturing paracetamol. *Contact Dermatitis* 2005; **52:** 290-291.

71. Berne B, Lundin A, Malmros PE. Side effects of cosmetics and toiletries in relation to use. A retrospective study in a Swedish population. *Eur.J.Dermatol.* 1994; **4:** 189-193.

72. Koopmans AK, Bruynzeel DP. Is PPD a useful screening agent? *Contact Dermatitis* 2003; **48:** 89-92.

73. Sosted H, Rastogi SC, Andersen KE, Johansen JD, Menne T. Hair dye contact allergy: quantitative exposure assessment of selected products and clinical cases. *Contact Dermatitis* 2004; **50:** 344-348.

74. Brancaccio RR, Brown LH, Chang YT, Fogelman JP, Mafong EA, Cohen DE. Identification and quantification of para-phenylenediamine in a temporary black henna tattoo. *Am.J.Contact Dermat.* 2002; **13:** 15-18.

75. Krasteva M, Cottin M, Cristaudo A, Laine G, Nohynek G, Orton D, Toutain H, Severino V, Wilkinson J. Sensitivity and specificity of the consumer open skin allergy test as a method of prediction of contact dermatitis to hair dyes. *Eur.J.Dermatol.* 2005; **15:** 18-25.

76. European Commission. Opinion on Exposure to reactants and reaction products of oxidative hair dye formulations. SCCP/0941/05. 2005.

77. Rastogi SC, Sosted H, Johansen JD, Menne T, Bossi R. Unconsumed precursors and couplers after formation of oxidative hair dyes. Contact Dermatitis. 2006. In Press

78. Altman DG. *Practical Statistics for Medical Research*. London: Chapman & Hall, 1999.

79. European Commission. The Inventory list of Cosmetics Ingredients http://pharmacos.eudra.org/F3/home.html. 2003.

80. European Commission. Twenty-sixth Commission Directive 2002/34/EC adapting to technical progress Annexes II, III and VII to Council Directive 76/768/EEC. 2002. Official Journal of the European Communities.

81. The European Cosmetic Toiletry and Perfumery Association (Colipa). List of hairdyes used in haircolouring products in 2002. 2003.
Ref Type: Personal Communication

82. http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp. 2003.

83. http://www.chemfinder.com. 2003.

84. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi. 2003.

85. Wilkinson DS, Fregert S, Magnusson B, Bandmann HJ, Calnan CD, Cronin E, Hjorth N, Maibach HJ, Malten KE, Meneghini CL, Pirila V. Terminology of contact dermatitis. *Acta Derm.Venereol.* 1970; **50:** 287-292.

86. Broeckx W, Blondeel A, Dooms-Goossens A, Achten G. Cosmetic intolerance. *Contact Dermatitis* 1987; **16:** 189-194.

87. Gottlober P, Gall H, Bezold G, Peter RU. [Allergic contact dermatitis in beauty parlor clients]. *Hautarzt* 2001; **52:** 401-404.

88. Guerra L, Bardazzi F, Tosti A. Contact dermatitis in hairdressers' clients. *Contact Dermatitis* 1992; **26:** 108-111.

89. Guerra L, Tosti A, Bardazzi F, Pigatto P, Lisi P, Santucci B, Valsecchi R, Schena D, Angelini G, Sertoli A, . Contact dermatitis in hairdressers: the Italian experience. Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali. *Contact Dermatitis* 1992; **26:** 101-107.

90. Hansson C, Thorneby-Andersson K. Allergic contact dermatitis from 2-chloro-p-phenylenediamine in a cream dye for eyelashes and eyebrows. *Contact Dermatitis* 2001; **45:**

235-236.

91. Hsu TS, Davis MD, el Azhary R, Corbett JF, Gibson LE. Beard dermatitis due to para-phenylenediamine use in Arabic men. *J.Am.Acad.Dermatol.* 2001; **44:** 867-869.

92. Ibsen HH, Andersen KE. [Picture of the month: contact allergy]. *Ugeskr.Laeger* 2000; **162:** 6858.

93. Krasteva M, Cristaudo A, Hall B, Orton D, Rudzki E, Santucci B, Toutain H, Wilkinson J. Contact sensitivity to hair dyes can be detected by the consumer open test. *Eur.J.Dermatol.* 2002; **12:** 322-326.

94. Mahendran R, Stables GI, Wilkinson SM. Contact leukoderma secondary to occupational toluenediamine sulfate exposure. *Contact Dermatitis* 2002; **47:** 117-118.

95. Matsunaga K, Hosokawa K, Suzuki M, Arima Y, Hayakawa R. Occupational allergic contact dermatitis in beauticians. *Contact Dermatitis* 1988; **18:** 94-96.

96. Nethercott JR, MacPherson M, Choi BC, Nixon P. Contact dermatitis in hairdressers. *Contact Dermatitis* 1986; **14:** 73-79.

97. Tosti A, Bardazzi F, Valeri F, Toni F. Erythema multiforme with contact dermatitis to hair dyes. *Contact Dermatitis* 1987; **17:** 321-322.

98. van der Walle HB, Brunsveld VM. Dermatitis in hairdressers. (I). The experience of the past 4 years. *Contact Dermatitis* 1994; **30:** 217-221.

99. de Groot AC. Adverse reactions to cosmetics. Thesis, State University of Groningen, The Netherlands. 1988.

100. Berg L. Adverse effects from cosmetic products. 1, 1-60. 2004. Oslo, Statens institut for forbruksforskning.

101. Khumalo NP, Jessop S, Ehrlich R. Prevalence of cutaneous adverse effects of hairdressing: a systematic review. *Arch.Dermatol.* 2006; **142:** 377-383.

102. Le Coz CJ, Kuhne S, Engel F. Hair dye allergy due to 3-nitro-p-hydroxyethyl-aminophenol. *Contact Dermatitis* 2003; **49:** 103.

103. 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.

104. Chung WH, Chang YC, Yang LJ, Hung SI, Wong WR, Lin JY, Chan HL. Clinicopathologic features of skin reactions to temporary tattoos and analysis of possible causes. *Arch.Dermatol.* 2002; **138:** 88-92.

105. Le Coz CJ, Lefebvre C, Keller F, Grosshans E. Allergic contact dermatitis caused by skin painting (pseudotattooing) with black henna, a mixture of henna and p-phenylenediamine and its derivatives. *Arch.Dermatol.* 2000; **136:** 1515-1517.

106. Ho SG, Basketter DA, Jefferies D, Rycroft RJ, White IR, McFadden JP. Analysis of para-phenylenediamine allergic patients in relation to strength of patch test reaction. *Br.J.Dermatol.* 2005; **153:** 364-367.

107. Schlede E, Aberer W, Fuchs T, Gerner I, Lessmann H, Maurer T, Rossbacher R, Stropp G, Wagner E, Kayser D. Chemical substances and contact allergy--244 substances ranked according to allergenic potency. *Toxicology* 2003; **193:** 219-259.

108. Jensen CD. Contact allergy to the preservative methyldibromoglutaronitrile. 1-30. 2005. Dep. Dermatol., Odense University Hospital, Denmark. 2005.

109. Dejobert Y, Piette F, Thomas P. Contact dermatitis to 2-hydroxyethylamino-5-nitroanisole and 3-nitro-p-hydroxyethyl aminophenol in a hair dye. *Contact Dermatitis.* 2006; **54:** 217-218.

110. Rastogi SC. A method for the analysis of intermediates of oxidatve hair dyes in cosmetics products. J.Sep.Sci. 24, 173-178. 2001.
(Full)

111. Jensen CD, Andersen KE. Course of contact allergy in consecutive eczema patients patch tested with TRUE Test panels 1 and 2 at least twice over a 12-year period. *Contact Dermatitis.* 2005; **52:** 242-246.

112. Friedmann PS. The immunology of allergic contact dermatitis: the DNCB story. *Adv.Dermatol.* 1990; **5:** 175-195.

113. Zachariae C, Hall B, Cottin M, Cupferman S, Andersen KE, Menné T. Experimental elicitation of contact allergy from a diazolidinyl urea-preserved cream in relation to anatomical region, exposure time and concentration. *Contact Dermatitis* 2005; **53:** 268-277.