CONSUMERS, COSMETICS AND SKIN SENSITIZERS

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Consumers, cosmetics and skin sensitizers
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By

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Den här är till dig mormor
SUMMARY

Background
Chemical products, cosmetics and other products handled by consumers and workers frequently contain contact allergens (skin sensitizers). Contact allergens are low-molecular substances which may induce contact allergy (Type IV allergy) after contact with skin. 15-20% of adults are allergic to one or more of the most common contact allergens, which can cause allergic contact dermatitis (eczema) on any part of the body, although the hands and face are the most common sites. Contact allergy is a lifelong condition, requiring avoidance of further skin exposure to the allergen, in order to minimize the risk of developing contact dermatitis.

Aim
The main aim of this thesis project was to contribute to a scientific basis for preventive measures to reduce contact allergy and dermatitis. Skin sensitizing hair dye substances and preservatives in products frequently used by consumers were of special concern.

Methods
In study I and II, we assessed the occurrence and use pattern of skin sensitizing hair dye substances in hair dye products available to consumers in one Northern European country and one Southern European country, based on ingredient labeling. In study III, we examined if typographical design and the order of the list of ingredients could improve the readability of product ingredient labels. This was done by comparing the readability of original product labels versus alternative formats we developed, using several quantitative and qualitative measures. Study IV was an experimental clinical study, repeated open application test (ROAT), in which we examined whether permissible concentrations of the preservative methylisothiazolinone (MI) in cosmetic rinse-off products had the potential to elicit allergic contact dermatitis.

Main findings
In study I and II we showed that many different potent skin sensitizing hair dye substances are commonly used in oxidative hair dye products on the market and the products contain a “cocktail” of these sensitizers.

In study III we showed that rather simple adjustments in the design of cosmetic product ingredient labels would improve their readability and understanding significantly.

In study IV we showed that cosmetic rinse-off products preserved with 100 ppm or 50 ppm MI elicited allergic reactions in the majority of previously sensitized individuals.
LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


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<tr>
<td>CAS number</td>
<td>Chemical Abstract Service number</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>EC3 value</td>
<td>The concentration of a substance required to elicit a three-fold increase in lymph node cell proliferative activity in exposed animals compared to the proliferation obtained in the controls, which are treated with vehicle alone.</td>
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<tr>
<td>INCI</td>
<td>International Nomenclature of Cosmetic Ingredients</td>
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<tr>
<td>LLNA</td>
<td>Local Lymph Node Assay</td>
</tr>
<tr>
<td>MCI</td>
<td>Methylchloroisothiazolinone (also called CMIT)</td>
</tr>
<tr>
<td>MI</td>
<td>Methylisothiazolinone (also called MIT)</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
</tr>
<tr>
<td>PPD</td>
<td>$p$-Phenylenediamine</td>
</tr>
<tr>
<td>QSAR</td>
<td>Quantitative structure–activity relationship</td>
</tr>
<tr>
<td>REACH</td>
<td>The EU Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals</td>
</tr>
<tr>
<td>ROAT</td>
<td>Repeated open application test</td>
</tr>
<tr>
<td>SCCNFP</td>
<td>Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers</td>
</tr>
<tr>
<td>SCCP</td>
<td>Scientific Committee on Consumer Products</td>
</tr>
<tr>
<td>SCCS</td>
<td>Scientific Committee on Consumer Safety</td>
</tr>
<tr>
<td>TDA</td>
<td>Toluene-2,5-diamine (also called PTD)</td>
</tr>
<tr>
<td>TDAS</td>
<td>Toluene-2,5-diamine sulfate</td>
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1 BACKGROUND

1.1 CONTACT ALLERGY

The field of contact allergy and allergic contact dermatitis is vast and there are countless publications on its various immunological, chemical, and clinical aspects. Here, I will just introduce the field briefly. For detailed information the reader is referred to a recent textbook (1).

Chemical products/mixtures, cosmetics and other products handled by consumers and workers frequently contain contact allergens (skin sensitizers). Contact allergens are low-molecular substances which may induce contact allergy (type IV allergy) after contact with skin. Metals are the most common cause of contact allergy, with nickel in unchallenged leading position, followed by fragrances, and preservatives (2-4). Approximately 20% of adults in the general population have contact allergy to at least one contact allergen (5), which can cause allergic contact dermatitis on the part of the body that is exposed to the sensitizing substance: the hands and face are the most common sites. Contact allergy is a lifelong condition, requiring avoidance of further skin exposure to the allergen, in order to minimize the risk of developing contact dermatitis. More than 4000 substances have been identified as contact allergens (6).

Contact allergy is a delayed type hypersensitivity (type IV hypersensitivity) which consists of two main phases, the sensitization phase and the elicitation phase (figure 1). Allergic contact dermatitis is the clinical consequence of re-exposure (the elicitation phase), which often is manifested as eczema.

**Sensitization.** In the first step of this phase the contact allergen (also referred to as hapten) penetrates into the outermost part of the skin, the epidermis, and binds to proteins to become a hapten-carrier complex. This complex is taken up by an antigen presenting cell, the Langerhans’ cell (LC), which then matures and migrates down to the regional lymph nodes and present the complex to naïve T cells. When an allergen-specific naïve T cell (i.e. a T cell that specifically recognizes this allergen) encounters the contact allergen, the cell becomes activated and starts to proliferate. Eventually, activated allergen-specific T cells leave the lymph node and begin to circulate in the blood. The individual has now become sensitized to the substance; in other words, he/she has acquired a contact allergy, which is a lifelong condition.

**Elicitation.** This phase occurs when the sensitized individual is re-exposed to the allergen. When the substance is recognized by the “patrolling” allergen-specific T cells they start to release proinflammatory mediators (cytokines and chemokines) which attract other inflammatory cells and contribute to an augmented inflammatory response in the skin. This inflammatory response leads to the clinical symptoms of allergic contact dermatitis. The dermatitis (eczema) is typically characterized by itching, erythema, edema, vesiculation, scaling, and fissuring, which occurs on sites of exposure to the substance. It can be very
troublesome for the affected individual and may result in sick-leave, change of jobs, and reduce the quality of life: the costs for the individual and society are large. Thus a sensitized individual must avoid further skin exposure to the allergen, in order to minimize the risk of developing contact dermatitis. However, avoidance can be difficult due to the widespread occurrence of many contact allergens in consumer and occupational products, and insufficient ingredient labeling.

**Figure 1.** The phases of allergic contact dermatitis.

1.1.1 Diagnosis of contact allergy (patch test)

Patch testing is the diagnostic tool for determining if a patient with a history of dermatitis has a contact allergy. The aim of this test is to expose the patient to a number of contact allergens and thus to detect an eventual contact allergy by causing an elicitation reaction on the test site. The allergens are placed in small chambers on tape strips. The most common vehicle in which the allergens are dissolved is white soft paraffin (petrolatum), but in some cases water or ethanol is more suitable. The tape strips are then applied to the patient’s skin, usually the upper back, and left for 2 days until removed. Two to 4 days after the patch test has been applied (depending on routines of the specific clinic), the patient visits the dermatologist for a patch test reading, i.e. the dermatologist investigates the eventual reactions and determines whether they are contact allergy reactions, and also grades the strength of each reaction (7). Typically, readings are performed twice (e.g. day 3 and 7) since reactions tend to develop at different time points.

The European baseline series for patch testing consists of approximately 50 of the most common contact allergens. In order to maintain its relevance, this series is regularly updated by the European Environmental Contact Dermatitis Research Group (EECDRG). The
baseline series is commercially available. In addition, there are many commercially available special series that consist of other possibly relevant contact allergens and thus complement the baseline series, for example the hair dresser series, cosmetic series, and many more.

Since patch testing is routinely performed in a large number of dermatitis patients, such epidemiological data plays a pivotal role in the continuous surveillance of allergy frequencies and temporal trends.

1.2 EU REGULATION OF COSMETICS

The main EU Chemicals regulation “REACH” aims at protecting human health and the environment by regulating the use of chemical substances, both for industrial and private use. Substances that are used as cosmetic ingredients are included (8). However, when it comes to cosmetic ingredients, REACH handles only the environmental concerns. As stated in article 14 point 5b of the regulation, risks posed to human health by cosmetic products will not be considered under REACH, but under the EU Cosmetics Regulations (previously the Cosmetics Directive).

The EU Cosmetics Directive was first published in the Official Journal of the European Union in 1976 (9). In subsequent years it has been amended numerous times to become a more comprehensive and complex legislation. In 2009 the new Cosmetics Regulation was published and applies fully since 11 July 2013 and has thus replaced its predecessor, the Cosmetics Directive (10).

1.2.1 Scope and aim of the EU Cosmetics Regulation

There are two main aims of the Cosmetics Regulation. One aim is to promote the competitiveness of the European cosmetics industry and to achieve a well-functioning internal market. This is done by harmonizing rules and reducing administration (see for example preamble points 3, 4, 24, and article 1).

The other aim is to ensure a “high level of protection of human health” (art. 1). Further, it is stated that “cosmetic products should be safe under normal or reasonably foreseeable conditions of use” (preamble point 9 and art. 3) and that “action by the Commission and Member States relating to the protection of human health should be based on the precautionary principle” (preamble point 36). There are many possibilities to achieve this aim via the regulation, for example requirements for safety assessments and good manufacturing practice; obligations for the authorities to check that products on the market comply with the regulation; possibilities to restrict certain hazardous compounds (see part 1.1.3); and requirements on labeling (part 1.1.4).

1.2.2 Definition of cosmetics

According to the regulation, a cosmetic product is “any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral
cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours” (art 2 point 1a).

It is not permissible to make medical claims about a cosmetic product, for example to say that a skin cream cures eczema or reduces muscle pain etc. Products that make such claims are medicinal and will be handled by the relevant competent authorities according to regulations for medicinal products.

1.2.3 The Cosmetics Regulation as a tool for prevention of contact allergy

The utility of the cosmetics regulation as a tool for prevention of contact allergy is mainly through annexes II-VI which, in various ways, restrict the use of substances as cosmetic ingredients. Therefore I will below give an overview.

**Annex II.** This annex lists substances that are prohibited for use in cosmetic products. This list includes mainly substances that are classified as carcinogenic, mutagenic or reproduction toxic (CMR-substances). Examples of contact allergens are musk ambrette nr 414, nickel nr 1093, peru balsam nr 1136. This annex has increased from 361 substances in 1976 to 1373 substances on 1 April 2014.

**Annex III.** This annex includes restrictions on miscellaneous cosmetic ingredients. The substances listed in this annex must only be used in accordance with the requirements specified for each of them. In the context of contact allergy, it is worth mentioning that 26 fragrance compounds must be listed on the ingredient label when their concentration exceeds 0.001% in leave-on cosmetics and 0.01% in rinse-off cosmetics (numbers 45 and 67-92 in this annex). Only a few of these 26 substances also have restrictions on concentrations in the finished product; the others can be used in as high concentrations as the manufacturer finds appropriate. The presence of other fragrance ingredients has only to be indicated by listing the term *parfum* on the label. A recent extensive review by the Scientific Committee on Consumer Safety (SCCS) concluded that many more than the 26 fragrance ingredients are established contact allergens and recommended that the consumers should be made aware also of these (11). It is likely that this recommendation (to some extent) will be followed and that more fragrance ingredients will be listed on cosmetic labels in the near future.

Importantly, this annex also sets restrictions on more than 50 skin sensitizing hair dye substances. For example *p*-phenylenediamine has a maximum allowed concentration applied to the hair of 2% and toluene-2,5-diamine has a maximum allowed concentration applied to the hair of 4%.

**Annex IV, V, and VI.** These annexes are positive lists for coloring agents (IV), preservatives (V), and UV-filters (VI), which means that only those substances that are listed here are allowed for use in cosmetics in the functions coloring, preservation and UV-filtrating respectively. They must also be used under the specific conditions stated in the annexes. A comment on Annex IV is that hair dye compounds are not yet included here, but it is the
intention that they will be, once a risk assessment of these substances has been accomplished (preamble point 28).

Annex V is of interest since preservatives are among the most frequent causes of contact allergy (after metals and fragrances) (4). It is also suitable for illustrating the large impact the cosmetics regulation can have on the prevalence of contact allergy in the population. The preservative methyl dibromo glutaronitrile (MDBGN) was introduced in the Cosmetics Directive in 1986 (under the name 1,2-dibromo-2,4-dicyanobutane), with a maximum authorized concentration of 0.1% (12). During the following years, several reports on contact allergy to cosmetic products containing MDBGN were published (13) and in 2002 a European multicenter study showed an increase in contact allergy in patch tested patients from 0.7% in 1991 to 3.5% in 2000 (14). MDBGN had now become the most frequent skin sensitizer of all preservatives. The increasing problems caused by MDBGN in cosmetics led to a restriction that MDBGN could only be used in rinse-off products from 2005 (15, 16), and later – since it was not proved to be safe in rinse-off products either – it was removed from the positive list and thus no longer permitted for use as a preservative in cosmetics from June 2008 (17-19). The regulatory interventions seem to have had the anticipated effect since a decreasing trend of contact allergy to MDBGN has since then been reported in several studies (13, 20, 21). An example of current interest is methylisothiazolinone (MI). MI was permitted as a preservative in cosmetics in 2005 (22) and during the following years there has been an alarming increase in the prevalence of contact allergy to this preservative, which will be discussed in greater depth in section 1.5.1.

Annex VI states which UV filters are allowed in cosmetics on the European market. They are often used in combination to obtain a satisfactory UV protection. Many of the frequently used filters can cause photo-allergic contact dermatitis (23-25).

### 1.2.4 Ingredient labeling of cosmetic products

Regulatory requirements on labeling of cosmetic products, including ingredient labeling, are specified in article 19 and 33 of the Cosmetics Regulation. Cosmetic products must have an ingredient label that lists all ingredients, with the exception of fragrance substances as mentioned above. The ingredients should be listed in descending order by weight down to 1%. Ingredients in concentrations lower than 1% can then be listed in any order. Coloring agents in annex IV can be listed in any order after the other ingredients and if one product series has several color shades it is permissible to use the sign “+/-” or the words “may contain” and then list all coloring agents of the whole series on all products. Note that this is not allowed for hair dye substances, which some manufacturers don’t adhere to (26, 27).

In terms of readability, it is specified that labels should be written in “indelible, easy legible, and visible lettering”. If possible, the ingredient names used on the label should be according to the International Nomenclature of Cosmetic Ingredients (INCI). The European Commission (EC) maintains a publicly available database with information on cosmetic
ingredients (CosIng), in which it is possible to find INCI names for example by searching on CAS numbers (28).

### 1.3 EU RISK ASSESSMENT OF COSMETICS

As stated previously, all cosmetic products that are placed on the market should be safe to the consumer. Therefore, cosmetic products and their ingredients must undergo a risk assessment (also referred to as safety assessment/evaluation) (figure 2).

![Safety Evaluation of Cosmetic Ingredients](image)

**Figure 2.** Risk assessment and management process for cosmetics in the EU. Reproduced from (29).

#### 1.3.1 Risk assessment by the responsible person

Before any cosmetic product is placed on the market, the responsible person (manufacturer or importer) has to ensure that the product has undergone a safety assessment and that a cosmetic product safety report (CPSR) has been created. The minimum requirements of what this CPSR shall contain are specified in annex I of the Cosmetics Regulation (10) and include: physicochemical properties of the ingredients and product; exposure assessment; toxicological profile of the ingredients including all relevant toxicological routes and endpoints; assessment conclusion on the safety of the product and the scientific reasoning leading to this conclusion etc. The risk assessment must be performed by a person who is qualified within the field of pharmacology, toxicology, medicine or a similar discipline.

#### 1.3.2 Risk assessment by the Scientific Committee of Consumer Safety (SCCS)

The SCCS provides risk assessments, so called opinions, regarding health and safety risks of consumer products (except food) such as cosmetics, household products, toys, and textiles. It
consists of a panel of independent researchers with expertise in different toxicological endpoints, including skin sensitization. The risk assessment process starts with a request from the industry or a member state to the EC on whether a substance of interest is safe to use in certain products under specified conditions. EC can then decide to give a mandate to the SCCS to perform a risk assessment regarding the substance of question. Reasons for addressing certain substances can be that member states are concerned that a substance may not be safe to the consumers under the allowed use conditions, as e.g. regarding MI (30). It can also be the case that industry is not content with the regulatory restrictions on the use of certain substances and therefore requests an assessment for an extended use, as have been the case with e.g. MDBGN and triclosan (19, 31). Furthermore, any request regarding the substances with functions that belong to annexes IV, V, and VI must be assessed by the SCCS. In order for the SCCS to perform the assessment, industry shall submit a dossier with information regarding the following: acute toxicity (if available), irritation and corrosivity (skin and eye), skin sensitization, dermal / percutaneous absorption, repeated dose toxicity, mutagenicity / genotoxicity, carcinogenicity, reproductive toxicity, toxicokinetics, photo-induced toxicity, human data.

The data shall consist of any relevant “in house” data available from industry as well as all relevant published literature (29).

The SCCS risk assessment process usually follows the generally acknowledged steps of toxicological risk assessment, which, simplified, can be described as Hazard Assessment, Exposure Assessment, and Risk Characterization. Although a quantitative risk assessment approach for the endpoint skin sensitization is under development by industry, it has yet to prove that it is a reliable tool (32) and therefore a pragmatic approach is applied, where all relevant available information is used, including also clinical data and experimental elicitation data.

1.3.3 Risk management

Based on the SCCS opinion and other aspects, the EC prepares a proposal for which actions should be taken. The member states then vote (weighted votes based on number of citizens) on whether or not this proposal shall be accepted. The EC is also responsible for the risk communication to the public.

1.3.4 Transparency

There is plenty of publicly accessible documentation on the risk assessment of cosmetics. For the SCCS there are quite detailed documents on the risk assessment procedure including requirements on the excellence, independence and transparency (29, 33). In addition, on the EC web page for the SCCS ([http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm](http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm), last accessed 4 September 2014) all opinions and similar documents are readily available, as well as a list of members including their CVs. Also, the opinions are first published as draft opinions which are open for comments during a specified period. In contrast, publicly
accessible information on the risk management process, on how it is performed and documented, is very scarce.

1.4 HAIR DYES

Hair dye products that are commonly found in the store can roughly be divided into direct hair dyes and oxidative hair dyes. Hair dyeing products are of considerable importance to the European cosmetics industry as the European hair dye market in 2004 was 2.6 billion Euros, which constituted almost one tenth of the total cosmetics market that year (34). The oxidative hair dyes are by far the most important group of products; they hold almost 80% of the total hair dye market, and the vast majority of skin sensitizing hair dye substances are used in this product category. The oxidative hair dyes (products are commonly called e.g. permanent, demi-permanent, tone-on-tone) consist of so called precursors and couplers that are low molecular substances that can penetrate the hair fiber and, with the help of hydrogen peroxide, couple together to form large dye molecules that become trapped in the hair fiber (35). The hydrogen peroxide also bleaches the natural pigment of the hair, and the alkaline condition of the products (pH 9.5 to 10.5) facilitates this as well as the penetration of precursors and couplers into the hair fiber. Since the dye compounds are actually inside the hair fibers they are resistant to wear, such as shampooing, which gives long lasting results.

Consumer use of oxidative hair dyes can cause allergic reactions, both among adults and children, sometimes so severe that hospitalization and/or sick leave is required (36, 37). Although this will not be further discussed in this thesis, it deserves to be mentioned that the hairdressers are more exposed to hair dyes and thus at greater risk of becoming sensitized compared to the typical consumer. Still, the majority of cases of hair dye allergy are caused by consumer exposure. A few examples of common hair dye substances are listed in table 1.

1.4.1 Hair dye use

Published data on hair dye use among the general population are scarce. Søsted et al. conducted an interview-based study including a representative random sample of 4000 Danish adults (38). The authors found that 75% of the women and 18% of the men had dyed their hair at some point, and 50% of the women and 5% of the men had dyed their hair within the last 12 months. The median age for hair dyeing debut was 16 years for both men and women. A recent questionnaire survey of children’s environmental health, including a random sample of 71400 Swedish children, found that 27% of girls and 9% of boys aged 12 years had dyed their hair at some point (39). Data gathered also from 16-year-olds in the county of Stockholm showed that 66% of the girls and 17% of the boys in this age group had dyed their hair at some point (40).
1.4.2 Prevalence of hair dye allergy

1.4.2.1 p-Phenylenediamine

Because p-phenylenediamine (PPD) is the only hair dye substance in the European and North American baseline series for patch testing, most epidemiological data concerns this hair dye substance only. Two recent large European multicenter studies indicate a prevalence of contact allergy to PPD of approximately 4.5% in consecutively patch tested dermatitis patients (41, 42). There seems to be a considerable variation between countries, with the lowest prevalence found in Scandinavia. Prevalence rates in the US appear to be somewhat higher than in Europe (5.5%) (43), although de Groot et al. suggested that the figures may not be perfectly comparable due to stricter selection of patients referred to patch testing in the US (44).

Assessing contact allergy in the general population is more complex than in dermatitis patients and data are scarce. Data from Germany and Denmark suggest that 1-1.5% of the general adult population is sensitized to PPD (45, 46), and a higher level of sensitization of 2.7% has been reported in a sample of adults in Bangkok, Thailand (47). Data from a Swedish population-based birth cohort showed that 1% of 2300 patch tested 16-year old adolescents were sensitized to PPD (48).

Although PPD is currently used as a screening agent for hair dye allergy it is important to stress that prevalence rates of contact allergy to PPD is not the same as prevalence rates of total hair dye allergy, which has sometimes been incorrectly insinuated (49). The use of PPD as a screener will be discussed further under section 4.1 of this thesis.

1.4.2.2 Other hair dye substances

In a European multicenter study by Søsted et al., dermatitis patients were patch tested with 5 hair dyes that are commercially available as patch test screening agents for hair dye allergy, and 22 additional hair dyes that are not yet commercially available as patch test material (41). Apart from PPD, 15 other substances elicited positive patch test reactions, 5 of these in more than 1% of the patients tested which is regarded a signal that a substance should be taken into consideration for inclusion in the baseline series (50).

Uter et al. have published data from aimed testing of hairdressers and clients with dermatitis from three time periods: 1995-2002, 2003-2006, and 2007-2012 (51-53). The term client was used for patients who were not hairdressers but for whom hair cosmetics was the suspected cause of their contact dermatitis. Several substances other than PPD gave positive reactions, with toluene-2,5-diamine (TDA) giving approximately the same number of reactions as PPD. When considering the two major sensitizers PPD and TDA, the prevalence of hair dye allergy had remained rather stable in the hairdressers. Interestingly, there was a significant increase in contact allergy in the clients, for TDA the frequency was 13.9%, 22.6%, and 26.3%, in the three respective time periods (53). The authors examined whether this rapid increase could be a selection bias due to a more selective testing and concluded that this was not the case,
which strongly indicates that allergic contact dermatitis caused by consumer use of hair dyes is a growing problem.

**Table 1.** Examples of commonly used skin sensitizing hair dye substances.

<table>
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<tr>
<th>INCI name</th>
<th>CAS No.</th>
<th>Molecular structure</th>
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<td><em>p</em>-Aminophenol</td>
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<tr>
<td>Resorcinol</td>
<td>108-46-3</td>
<td><img src="image" alt="Structure of Resorcinol" /></td>
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<tr>
<td>Toluene-2,5-diamine</td>
<td>95-70-5</td>
<td><img src="image" alt="Structure of Toluene-2,5-diamine" /></td>
</tr>
</tbody>
</table>

### 1.4.3 Risk assessment of hair dyes

An epidemiological study published in 2001 found a correlation between use of permanent hair dyes and increased risk of bladder cancer, where the risk increased with both the years of usage and the frequency of usage (54). This led to an opinion from the EC scientific committee at that time, the SCCNFP, stating that the bladder cancer risk was of concern and that the EC should take actions to assess the safety of this group of substances (55). Following this opinion the EC together with member states and stakeholders agreed on a hair dye strategy with the objective of assessing the safety of all hair dye substances in use and to create a positive list (56). The strategy set deadlines (2003, 2005, and 2007) for when industry should have submitted dossiers including all information required for the Scientific Committee to be able to conduct a proper risk assessment of the substances, as specified by the committee itself. All hair dye substances for which industry did not submit dossiers according to the timetable were banned. The legitimacy of the hair dye strategy was confirmed in 2005 when the scientific committee at that time, the SCCP, in an updated
review on hair dyes and cancer risk, once again concluded that the concerns remained (57). Industry has submitted dossiers regarding 117 hair dye substances which are currently being assessed (58).

An indirect consequence of the hair dye strategy was that much new information on the sensitizing potential of hair dyes was submitted by industry for assessment by the Scientific Committees. Some of this information has also become publicly available since the relevant studies used by the Scientific Committees are summarized in the opinions that are published. The sensitization data are derived mainly from tests in animals (LLNA, GPMT, Buehler test), which all are OECD guideline methods. The Scientific Committees have published two memorandums in which they have compiled all the sensitization data and categorized the hair dye substances into three different potency categories; extreme sensitizer, strong sensitizer, and moderate sensitizer (59, 60). Details regarding the criteria for this categorization are given in the following reference from an EC working group on sensitization (61). In September 2013 (when the most recent memorandum was published) 114 substances had been evaluated (figure 3).

![Sensitization data for hair dye substances](image_url)

**Figure 3.** Skin sensitizing potency of hair dye substances evaluated by the European Commission Scientific Committees, SCCNFP, SCCP and SCCS. Diagram based on data in the memorandum (60).

### 1.5 METHYLISOTHIAZOLINONE

Preservatives are used in products containing an aqueous phase with the function of inhibiting the growth of microorganisms. The ideal preservative has been described as colorless, odorless, water soluble, nontoxic, nonallergenic, nonirritating, with the capacity to inhibit the growth of a broad spectrum of bacteria and fungi (62). Unfortunately, a substance embracing all these characteristics has not yet been identified; for example many efficient preservatives are also important skin sensitizers.

The preservative methylisothiazolinone (MI) (table 2) has been widely used in cosmetics and other products since the mid-1970s in Europe and the early 1980s in the US (63). Until the last decade it has been used only in a combination with methylchloroisothiazolinone (MCI) in a ratio of 3:1 (MCI/MI), a well-known trade name being Kathon CG (cosmetic grade). Early on it became apparent that MCI/MI was a very potent skin sensitizer with high levels of sensitization reported in Europe and the US (64). Concerns about this development led to a
reduction of the allowed concentration of MCI/MI in cosmetics in Europe from 30 ppm (0.003%) to 15 ppm which applied from 1991 (65), and from 2015 it is banned for use in leave-on cosmetics (66). In the US, MCI/MI is allowed in 7.5 ppm in leave-on products and 15 ppm in rinse-off products, and in Japan it is allowed only in rinse-off products at 15 ppm (67). The regulatory interventions, as well as possible actions by industry, seem to have had some effect since the allergy prevalence was stabilized for a long period, although at a high level (68). MI was introduced as a preservative on its own (without MCI) in chemical products in the beginning of the 2000s (69, 70), and in 2005 it was approved in Europe as a preservative in cosmetics at a maximum level of 100 ppm (22). MI’s characteristics make it interesting to the cosmetic formulator: it acts against a broad spectrum of microorganisms; it is effective over a broad pH range; and it does not change the quality of the final product. Typically, a MI concentration of 50-100 ppm is needed for satisfactory preservation of a cosmetic product, depending on product composition. Lundov showed in a microbiological challenge test that, when combined with 0.2% phenoxyethanol, as little as 5 ppm MI was sufficient to preserve a skin cream (71).

Table 2. Basic information on MI. The CAS number for the mixture of methylchloroisothiazolinone and methylisothiazolinone (3:1 ratio) is 55965-84-9.

<table>
<thead>
<tr>
<th>INCI name</th>
<th>CAS No.</th>
<th>Molecular structure</th>
</tr>
</thead>
<tbody>
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<td>Methylisothiazolinone</td>
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<td><img src="image" alt="Methylchloroisothiazolinone" /></td>
</tr>
</tbody>
</table>

1.5.1 Frequency of use in cosmetics

In a large survey of cosmetics on the German market 2006-2009, Uter et al. found that, based on label information, the occurrence of MI had increased significantly from 0.6% in 2006 to 2.4% in 2009, with rinse-off products and sunbathing products being the types that most frequently contained MI (72). In a survey of the Swedish market in 2008, we found MI in 0.5% of the (rinse-off) cosmetics (73). MI was found in approximately 3% of the liquid soaps but not at all in shampoos or hair conditioners, where MCI/MI was common. In two market surveys in Denmark by Lundov et al. in 2010 and 2013, the occurrence of MI in cosmetics had increased, from 1.5% to 3.3% (74, 75). The vast majority of products contained between 50 and 100 ppm, according to chemical analysis (74). An increasing trend has also been reported in the US, where the number of cosmetic products preserved with MI more than doubled between 2007 and 2010 (76). MI is also used in a wide range of chemical products,
where for example paints and household detergents are common sources of exposure (73, 77, 78).

In summary, the only recent market survey of MI in cosmetics was conducted in Denmark in 2013 and it is not known to what extent this study is representative for the European market.

1.5.2 Prevalence of MI allergy

Although MI was recommended for inclusion in the European Baseline series for patch testing as late as in 2013, an increasing number of clinical epidemiological studies have reported prevalence rates of contact allergy to MI (20, 75, 79-92). The overall conclusion is that there has been a dramatic increase in MI allergy during the last decade (figure 4) and that cosmetics are one of the major sources for MI exposure (together with paints).

**Figure 4.** Selected references regarding MI sensitization levels in patch tested patients in Denmark (test concentration 0.2%) (75, 79, 91), Finland (0.05%) (80, 90), IVDK (Germany, Switzerland, Austria) (0.05%)(81, 84), UK (usually 0.2%) (89), France (usually 0.02%) (86), Belgium (usually 0.02%) (85), and Sweden (0.15% in 2006, and 0.2% from 2007) (92). Results from Denmark, Finland, and Sweden are from testing of consecutive dermatitis patients. The other reports are either from aimed testing of selected patient groups or the patient selection criteria are not clearly stated.
1.5.3 Risk assessment of MI

The SCCNFP conducted a risk assessment on MI in 2003/2004, before it was introduced in cosmetics on the European market (93, 94). They concluded that use of MI in cosmetics could not be expected to lead to many new sensitizations; this conclusion was based mainly on human sensitization tests reported by Rohm and Haas, the manufacturer that held the patent for MI. The opinion of the SCCNFP was that MI in a concentration of 100 ppm in cosmetic products did not pose a risk to the health of consumers.

In 2010, the US based Cosmetic Ingredient Review published a risk assessment which came to a similar conclusion as the SCCNFP largely based on the same references, although some additional animal studies were included (95). The fact that MI was a strong sensitizer in the local lymph node assay (LLNA) (EC3 value of 0.4%) (96) did not affect this conclusion, nor were the indications that MI allergy was a growing problem in Europe considered in this assessment (79, 97).

The above described dramatic increase of MI allergy in Europe after its approval for use in cosmetics made several member states urge the EC to conduct a new risk assessment of MI, which eventually led to an opinion by the SCCS published in 2013 (30). When epidemiological and experimental clinical studies were included, it was clear that MI was not safe in the currently permitted concentration. The SCCS concluded that no information regarding safe doses of MI in leave-on products was available, and suggested that MI would probably not cause sensitization if used in rinse-off in concentrations up to 15 ppm. It was also concluded that information on elicitation from rinse-off products was not yet available.

1.6 READABILITY

1.6.1 Measuring readability

Readability can be defined as the ease with which “the meaning of a text can be comprehended” (98), i.e. how easy it was to solve the reading task. This can be assessed using different qualitative and quantitative measures (99). Examples of objective measures are readability formulas, eye movements, and reading speed and comprehension.

A commonly used measure of readability is a combination of reading speed and comprehension. If a reader needs significantly shorter time to read format X compared to format Y and is still able to comprehend the text (usually proven by giving correct answers concerning the text), then this means that the readability was better in format X i.e. it was easier to solve the reading task.

When we read we can only see sharply in a very small portion of the visual field (6-7 letters at a time) (100), and to see these letters clearly we stop and focus on them for a short time (milliseconds). These stops when the image is stabilized on the retina are called fixations. In the reading process we then move from fixation to fixation using rapid eye movements called saccades. The saccades can go both forward and backwards. If reading requires significantly
fewer fixations and saccades and/or the fixation time is shorter for format X compared to format Y, this means that the readability is better in format X. The reader did not have to go back and forth so many times in the text and the text was clearer and thus easier to stabilize on the retina.

Readability measurements also often include subjective measures, in the form of inventories and questionnaires.

Eye movement analysis and other measures mentioned above have been used to assess readability and related questions in many different fields, for example to assess the readability of different mobile device presentation formats and to compare the efficacy of different formats of nutrition labeling etc. (99, 101, 102).

1.6.2 Readability of ingredient labels

The ability of patients with contact allergy to avoid exposure to allergens in cosmetic products has been studied using qualitative research methods (103-105). In a postal questionnaire study with patients with contact allergy to fragrances and preservatives, Noiesen and coworkers studied the patients’ ability to avoid unwanted exposure, including questions regarding reading ingredient labels. Half of the patients found it difficult or extremely difficult to read the ingredient labeling of cosmetic products and Noiesen concluded that the readability must be improved to facilitate allergen avoidance. In a questionnaire study with fragrance allergic patients by Lysdal and coworkers, a vast majority of the patients stated that clearer ingredient labelling would increase their benefit. Similar problems in avoidance of allergen due to typographical shortcomings and terminology have been seen in studies of food labeling (106-108).
2 AIM

The main aim of this thesis project was to contribute to a scientific basis for preventive measures to reduce contact allergy and dermatitis. Skin sensitizing hair dye substances and preservatives in products frequently used by consumers were of special concern.

Specific aims:

**Study I and II.** To create base-line knowledge for future follow-ups, useful in risk assessment and risk management, regarding the occurrence and use pattern of potent skin sensitizing hair dye substances in consumer available hair dye products.

**Study III.** To assess how product ingredient labels are read by consumers and to see if adjustments of the typographical design and different ordering of the ingredients on the labels could improve the readability.

**Study IV.** To examine whether cosmetic rinse-off products preserved with MI in permissible concentrations (100 ppm or 50 ppm) have the potential to elicit allergic contact dermatitis in previously sensitized individuals.
3 METHODS

This part gives an overview on the methods used. Detailed descriptions are found in the publications and the manuscript included at the end of this thesis.

3.1 SKIN SENSITIZERS IN HAIR DYE PRODUCTS (STUDY I AND II)

Two market surveys were conducted with the aim of assessing the occurrence of skin sensitizing hair dye substances in oxidative hair dye products for consumer use. One survey, representing Northern Europe, was conducted in Stockholm, Sweden (Study I), and one survey, representing Southern Europe, was conducted in Barcelona, Spain (Study II).

3.1.1 Selection of products

The aim was to include products that were expected to be common on the market and therefore used by many consumers. In order to obtain a picture of which products were common, numerous stores were visited and it was noted which hair dye brands (series) were sold and the shop staff was consulted. According to a procedure developed by us, a number of hair dye products from different brands were photographed and included in the study for further assessment of their ingredient labels. In all, 227 products were included in the studies, 122 in study I and 105 in study II.

The sampling method proved to work well for oxidative hair dye products due to the relatively limited number of products on the market compared with many other cosmetic product types. The dominance of a few international companies was obvious, although the Spanish market was somewhat more heterogeneous, with a few national/regional brands.

3.1.2 The list of potent sensitizers

A list of hair dye substances categorized as potent skin sensitizers was put together based on risk assessment reports by the SCCS and its predecessors, and on potency categorization of hair dyes based on a quantitative structure–activity relationship model (QSAR) used by Søsted et al.(59, 109, 110). The list comprised 179 substances.

3.1.2.1 Potency categorization based on in vivo model

The categorization of hair dye substances assessed by the SCCS and its predecessors is based on results from in vivo testing, largely the LLNA. Potency categorization using LLNA is based on the EC3 value, which is equal to the concentration of a substance required to elicit a three-fold increase in lymph node cell proliferative activity in exposed animals compared to the proliferation obtained in the controls, which are treated with vehicle alone. An extreme sensitizer is defined as a sensitizer with an EC3 value ≤ 0.2% (w/v); strong as EC3 > 0.2% - ≤ 2%; and moderate as EC3 value > 2% (61). Substances that elicit less than a three-fold increase in lymph node cells are not categorized as skin sensitizers in the LLNA.
Like any other predictive model the LLNA has some weaknesses. One of the main limitations is that the choice of vehicle in which the substance is dissolved may sometimes quite drastically affect the results. A study by Basketter et al. assessing the sensitizing potency of 4 preservatives in two different vehicles found that the EC3 value differed from 5-fold for MI up to 20-fold for glutaraldehyde, depending on whether acetone: olive oil (4:1) or propylene glycol was used as a vehicle (96). Other relevant limitations include the short exposure time, the animals are exposed 3 consecutive days and the proliferation is measured 3 days later, and the fact that strong irritants may give false positive results (111). Notwithstanding these limitations, the LLNA appears to correlate rather well with results in human in most cases; it is still regarded the most suitable model for potency assessments of skin sensitizers, and it is the first choice method for registration of chemicals in REACH (8, 61).

The hair dye substances that were categorized as either extreme or strong sensitizers were included in the list of potent sensitizers.

3.1.2.2 Potency categorization based on in silico model

Søsted et al. assessed the potency of 229 hair dye substances in a QSAR model, developed by Estrada et al. for assessing skin sensitizers (110, 112). The substances had been assigned by the authors to one of three categories: strong/moderate, weak, or extremely weak/non-sensitizing.

Although there are limitations in QSAR models, e.g. that there is still much unknown regarding biological and chemical processes involved in the sensitization process resulting in an inherent uncertainty of the model, the dataset generated by Søsted et al. is valuable since it contains much new information on sensitizing potency of hair dyes.

The substances categorized in this model as strong/moderate sensitizers were included in the list of potent skin sensitizers.

3.1.3 Assessment of potent sensitizers in hair dye products

The ingredient labels of the included products were scrutinized and all potent sensitizers were recorded. We calculated their frequency of use and the number of potent sensitizers in each product and the results in study I and II were compared. Also the regulatory requirements (authorized concentrations of the hair dye substances) according to the Cosmetics Directive were described (study I).

With the aim of further analyzing and visualizing the combined exposure to the potent hair dye sensitizers (study II), network diagrams were produced using the freely available software Pajek.
3.2 READABILITY OF INGREDIENT LABELS (STUDY III)

3.2.1 Study participants

Sixteen subjects (age 20-44 years, median 23, 8 female), who were recruited by advertisement, participated in the experiment. The inclusion criteria were age 18-65, ability to read newspaper text without spectacles, and no use of heavy eye makeup, since these may interfere with the eye tracking device. Contact lenses were allowed.

The study adhered to the tenets of the Declaration of Helsinki and was approved by the regional ethics review board in Stockholm, Sweden (ID: 2011/1864-31/4). All study participants gave written informed consent before taking part in the study.

3.2.2 Study design

We designed a repeated measurement experiment where each participant was instructed to find two target ingredients (cetrimonium chloride and hexyl cinnamal) in 30 cosmetic products by reading the ingredient labels presented in four different formats:

A. Original typography on real products with ingredients ordered by concentration

B. Enhanced typography with ingredients ordered by concentration

C. Enhanced typography with ingredients ordered alphabetically

D. Enhanced typography with ingredients ordered alphabetically and subheadings for fragrances and preservatives

Since the reading skills can differ largely between individuals, this type of design is more efficient than assigning subjects to four different groups that read one format each.

The original products had been purchased for a previous study where the occurrence of preservatives and fragrances in cosmetics had been investigated (73), before this study was planned. This fact, together with having 5 persons, independently from each other, select the 15 products they thought had the best readability and the 15 with the poorest readability, reduced the risk of an unbalanced sample of original products in terms of readability. The typographical design of the ingredient labels on the selected original products was analyzed and areas of improvement were identified, based on established knowledge of graphical design and readability. The alternative formats were typographically improved in the same way; the difference among them was how the ingredients were presented. In alternative B, the ingredients were ordered by concentration (as in the original products). In alternative C, the ingredients were ordered alphabetically. In alternative D, the ingredients were ordered alphabetically and also had subheadings for fragrances and preservatives.

The participants read one format at a time, with a break between each format. The presentation order of the labels within each format was randomized, and an orthogonal
latin-square design balanced the order in which the formats were read in order to assure that there was no test order effect. The experiment was conducted in a dedicated light laboratory with standardized light conditions, such that the reading conditions were the same for all participants.

3.2.3 Outcome measures

3.2.3.1 Completion time and recognition rate
Completion time (reading speed) was the time (in seconds) from the onset of reading a label to the oral response. To measure the comprehension we assessed the recognition rate. Recognition rate was the percentage of correct responses to which target ingredients the products contained.

3.2.3.2 Eye movements
The eye movement measures of main interest were the fixations (count and duration) and the saccades (count). Eye movements were recorded with the FDA approved Chronos Eye Tracking Device (C-ETD) (Chronos Vision GmbH, Germany).

3.2.3.3 Subjective measures
NASA-TLX (Task Load Index) inventory is a tool for subjective workload assessment where mental, physical, and temporal demands, as well as performance, effort and frustration levels are rated on a continuous scale of 0-100 (low-high) (113). The rating was made by the study participants themselves after reading each format.

In an additional questionnaire (subjective experience and preference inventory) the participant were asked which format they preferred and also to grade the formats from 1 (very poor) to 5 (very good) regarding readability, legibility, ease of reading, speed of reading, and organization.

3.3 METHYLISOTHIAZOLINONE IN RINSE-OFF PRODUCTS (STUDY IV)

3.3.1 Study participants
Nineteen MI-allergic study participants completed the study. They had been diagnosed by patch testing with MI (0.2% in water) at the out-patient clinics of the Centre for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden, or Gentofte Hospital, Copenhagen, Denmark between 2011 and 2013, or in 2012, respectively.

Control participants without diagnosed MI allergy were recruited by advertisement. Nineteen control participants completed the study.

The study adhered to the tenets of the Declaration of Helsinki and was approved by the regional ethics review boards in Stockholm, Sweden (ID: 2013/976-31/4) and Capital Region, Denmark (ID: H-4-2013-094). All subjects gave written informed consent before taking part in the study.
3.3.2 Study design

3.3.2.1 Confirmatory patch test

Patch testing, prior to inclusion in the study, was performed in accordance with international recommendations (7). The MI-allergic subjects were patch tested with a serial dilution of MI in water to get an indication of their patch test reactivity. The following concentrations of MI were tested: 0.2, 0.1, 0.05, 0.025, 0.01, and 0.0016%, corresponding to 60, 30, 15, 7.5, 3, and 0.48 µg MI/cm². In addition, they were tested with a vehicle control (water) and paraben mix (16% in petrolatum) (Chemotechnique, Vellinge, Sweden). The control subjects were patch tested with MI 0.2% in water and paraben mix only.

3.3.2.2 Repeated open application test (ROAT)

Test areas of 5*10 cm² were marked on the ventral side of the participants’ forearms. In a first step, 10 MI-allergic subjects and 19 control subjects applied a liquid soap with 100 ppm MI on one arm and the control soap without MI on the other arm. In a second step, 9 additional MI-allergic subjects applied the liquid soap with 50 ppm MI and the control soap without MI. The soaps were randomized to the test areas and the experiment was blinded, such that neither the subjects nor the assessor of the skin reactions knew which test area had been exposed to MI. The participants got new soap packages every week and returned the used ones to the test leader.

The participants were thoroughly instructed to apply the preparations 5 times per day, with a minimum of 2 h between each application, according to following procedure:
- moisten the test area on one arm with water
- apply to the area the amount of liquid soap extruded by one press of the soap dispenser (approximately 0.24 ml)
- distribute the soap over the entire test area with the single-use applicator (plastic spoon, convex aspect)
- rinse off 20-25 seconds after the start of application and dry gently with paper tissue
- repeat the procedure on the other arm with the other product and a new applicator
- do not use any of your own products on the test areas.

The participants were further instructed to apply a moisturizing cream (Decubal original clinic cream, Actavis Plc, Dublin, Ireland), supplied by the test leader, once daily onto both arms when they were finished with the day’s soap applications. This was done to reduce possible irritation or dryness by the intense washing.

The liquid hand soaps, produced specifically for this study (ACO Hud Nordic AB, Upplands Väsby, Sweden), reflected a simple liquid hand soap product with commonly used ingredients and concentrations. No fragrances were present in the products. The pump soap
packages dispensed approximately 0.24 ml per press, and they were color coded to reduce the risk of confusion.

3.3.3 Outcome measures

The main outcome measure was whether the use of the MI-preserved soaps would elicit an allergic contact dermatitis reaction in MI-allergic participants. A positive reaction was defined as *area of involvement > 25% of the area of application, including erythema and signs of infiltration, i.e. at least 1 papule*. If a positive reaction occurred on any of the two arms, all further applications by the participant were stopped. If no reaction, the test proceeded for a maximum of 21 days.

In addition, we compared if there was any difference in reactivity to the same dose of MI per application in the ROAT patch test (0.48 µg/cm²). Furthermore, we examined whether there was a possible correlation between MI patch test threshold and accumulated dose MI until positive ROAT reaction.
4 RESULTS AND DISCUSSION

4.1 SKIN SENSITIZERS IN HAIR DYE PRODUCTS (STUDY I AND II)

4.1.1 Main findings

- Nearly all (225 of 227) examined oxidative hair dye products contained hair dye substances categorized as potent skin sensitizers.

- A total of 35 (study I) and 25 (study II) different hair dye sensitizers were identified. The most frequently identified sensitizers are shown in figure 5.

- PPD, TDA or TDAS was identified in 96% (study I) to 99% (study II) of the products and they were never used in combination. The few products not containing any of these compounds were very light blonde shades.

- PPD was identified in 16% and 50% of the products on the Swedish and Spanish market, respectively. TDA or TDAS was identified in 80% and 49% of the products on the Swedish and Spanish market, respectively.

- The typical hair dye product contained around 5 potent sensitizers and more than 80% of the products contained 4 potent sensitizers or more.
Figure 5. Network diagram displaying hair dye substances, categorized as potent skin sensitizers, identified on the labels of ≥ 15% of examined oxidative hair dye products on the Spanish and Swedish market. Circles illustrate prevalence of substances, numbers in per cent. Lines illustrate concomitant use of substances in products. Thicker and darker lines indicate higher frequency of concomitant use of substances in products. (a) Hair dye substances in 105 products in Spain in April 2010. (b) Hair dye substances in 122 products in Sweden between August and October 2008. Reprinted from Yazar et al 2012 (Study II).
4.1.2 **Strengths and limitations**

We have only used ingredient labels in this study; no chemical analysis was performed. A clear limitation is that we did not generate any information on the concentrations of sensitizers in the products. Such information is not publically available.

An additional limitation arising from the lack of chemical analysis is that any undeclared substances would be missed. Lind et al. chemically analyzed 54 oxidative hair dye products for their content of 5 hair dye substances and compared the results with the ingredient labels (114). Five products did not have an ingredient label and would thus have been excluded from our study. All other products were correctly labeled regarding their content of the 5 substances. Søsted and coworkers analyzed 9 oxidative hair dye products for the content of 4 hair dye substances and found that all substances identified by chemical analysis was found on the label, except for one product where PPD was not labeled. However, this product was labeled to contain hydroxyethyl-PPD which could not be analyzed for and may thus have explained this result (115). In 2003, the Danish National Environmental Institute chemically analyzed 16 oxidative hair dye products for the content of 19 hair dye substances and found that all products were correctly labeled (116). It may be speculated that because of the relatively homogenous market, the complex chemistry, and the dominance of international companies, hair dye products are more accurately labeled than products of more simple composition (e.g. liquid soaps, shampoos) for which the market is more diverse and many small companies are involved.

Despite the limitations stated above, surveys of this kind generate much important information, and they are quite cost-effective. It is practically impossible to analyze such multitude of complex products for such many target ingredients; at least it would be extremely costly. Therefore a well conducted survey of a specified product group is a good complement to chemical analysis.

In the current risk assessment process, substances are assessed one by one. In reality, however, we are exposed simultaneously to multiple hazardous substances from various sources, as is certainly true for oxidative hair dye products. To the best of our knowledge ours was the first effort (study II) to analyze and visualize such cocktail exposures to skin sensitizers, and this approach has since been used by others (25, 72, 117, 118).

4.1.3 **Frequency of use of PPD**

PPD is really the “star” of hair dyes; it is an extremely potent sensitizer, has been used for many decades, and it’s the only hair dye in the European baseline series for patch testing. The majority of scientific and clinical efforts regarding hair dye allergy have been focused on PPD. We found that there were also many other potent hair dye sensitizers in the products, some of which were even more common than PPD. These results are in line with those of recent surveys in Germany (PPD in 0.3% of products, not yet published, pers. comm. Wolfgang Uter 2014) and Denmark (PPD in 29% of products) (119), and of Søsted et al. who
showed that many hair dye substances other than PPD are also used in high tonnage amounts (110).

Thyssen et al. found that the prevalence of hair dye allergy was higher in Southern Europe compared to Northern Europe, and data from North America indicate a frequency closer to that of Southern Europe (42, 43). It was proposed that the regional differences within Europe may be due to a more frequent use of dark shades in Southern Europe, and that may indeed be part of the explanation. We found that the occurrence of PPD was much higher in products in Spain compared to Sweden (with similar results in Denmark and Germany). Furthermore, a recent study from the US, that mimicked our design for the sake of comparison, found PPD in a majority of the examined products (118). It is interesting to see that the regional differences in occurrence of PPD in the products seem to correspond with the regional differences in prevalence of PPD allergy.

4.1.4 Combined exposures

The fact that oxidative hair dye products contain a combination of reactive and potent sensitizers raised our concerns regarding possible cocktail effects (additive, synergistic), which had also been suggested by others (37). It could be suspected also that the presence of hydrogen peroxide and the high pH at hair dying may be irritating and also facilitate skin absorption and sensitization.

In line with these concerns Bonefeld et al. found, in a modified LLNA, that a hair dye product containing PPD (tested as mixture of color gel and oxidizer and as color gel alone) was a much more potent sensitizer than 1% PPD in acetone/olive oil (4:1) (120). Considering the complexity of the products and in light of the findings by Bonefeld and coworkers, it was quite surprising that industry-based toxicologists recently proposed a simplified risk assessment approach for hair dyes where some assessment factors were excluded. The proposed approach involved sensitization testing only with single ingredients and not taking inter-individual variation or even vehicle effects into consideration (121).

4.1.5 PPD as a screener for hair dye allergy

Since PPD is the only hair dye substance in the European baseline series, and we have shown that in some regions it is relatively rarely present in the products, a clinically relevant question is to what extent baseline screening detects hair dye allergy in general. Although I could not find a clear answer in the literature, there are some results worth mentioning.

It appears, when compiling data from two recent large studies (41, 53) (figure 6), that PPD (currently) detects a majority of cases of hair dye allergy that would have been detected by testing with additional hair dyes that are sometimes used in patch testing, although a relatively large proportion (~24%) of TDA-positive cases are missed. However, it seems like PPD fails to detect the majority of cases of hair dye allergy when testing with patients’ own hair dye products (figure 6). In addition, Søsted et al. found that 2.2% (20 out of 914 tested) of patch tested patients had a positive reaction to p-methylaminophenol, a compound that is
not closely related to PPD. Seventy-five percent of the \( p \)-methylaminophenol positive patients were PPD negative and would thus have been missed if testing with PPD only (41).

The cross-reactivity to hair dye substances has been studied in guinea pigs (122). There was “some” cross-reactivity between PPD, TDA and \( p \)-aminophenol, in the sense that some of the animals sensitized to TDA or \( p \)-aminophenol developed a positive reaction when later exposed to PPD. However, more positive reactions were found when animals sensitized to \( p \)-aminophenol where exposed to the same substance than when they were exposed to PPD, which was also true for animals sensitized to TDA. The results of the study indicate that PPD has a rather limited potential to detect sensitization to other hair dye substances. Still, it should be interpreted with caution since the experimental setting is far from real life exposures and is thereby of limited use in a clinical perspective.

**Figure 6.** Concomitant reactions in patients diagnosed with allergy to hair dye substances. (a) Positive patch test reactions in patients that have been simultaneously tested with PPD and TDA. Based on (41) and (53) (including additional information by personal communication with Wolfgang Uter). (b) Positive patch test reactions in patients that have been simultaneously tested with PPD and the common patch test hair dye allergens \( p \)-aminophenol, \( m \)-aminophenol, pyrogallol, and hydroquinone (“other”). Based on (53) (including additional information by personal communication with Wolfgang Uter). (c) Positive patch test reactions in patients that have been simultaneously tested with PPD and their own hair dye products (“own”). Based on (123).
4.2 READABILITY OF INGREDIENT LABELS (STUDY III)

4.2.1 Main findings

- A number of areas of improvement were identified regarding the readability of the original cosmetic products' ingredient labels, and an alternative typographical design was developed and used for the alternative formats B, C, and D (table 3).

- Pairwise comparisons showed that the completion time when reading labels was statistically significantly shorter for formats B, C and D than for format A (the original product). Completion time was also significantly shorter for format C and D compared with format B. There were no significant differences in recognition rate between the formats. The rate was generally high and it improved slightly for formats B, C and D compared with format A (figure 7).

- All alternative formats were more simple to read, as shown by the statistically significantly reduced number of fixations, fixation durations, and number of saccades compared to format A. Furthermore, the number of fixations was significantly lower for format C and D compared to format B (table 4).

- The subjective measures confirmed the improved readability of the alternative labels.

**Figure 7.** Box-plot of completion time (a) and recognition rate (b) for the different formats. A = Original typography on real products with ingredients ordered by concentration; B = Enhanced typography with ingredients ordered by concentration; C = Enhanced typography with ingredients ordered alphabetically; D = Enhanced typography with ingredients ordered alphabetically and subheadings for fragrances and preservatives. The bottom of the box denotes the 25% percentile, the thick line the median, and the top of the box the 75% percentile. Whiskers extend to extreme data points that are not considered outliers. Outliers, more than 1.5 box widths from edge of box, are shown as circles. Reprinted from Yazar et al. 2014 (Study III).
Table 3. The main typographical shortcomings of the ingredient labels of a sample of cosmetic products (shampoos, hair conditioners, n=30) purchased in Sweden. The improvements that were applied when designing the alternative formats are also listed.

<table>
<thead>
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<th>Typographical shortcomings</th>
<th>Typographical improvements</th>
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<td><strong>Font size</strong></td>
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</tr>
<tr>
<td></td>
<td>6 points, corresponding to 1.5 mm upper case letters in Arial</td>
</tr>
<tr>
<td><strong>Typeface</strong></td>
<td>Typeface (i.e. bold, italic, condensed, extended) unsuitable for small font sizes. Serif typefaces not suited for the dominating printing technology (silk screen) where contours tend to lose details and smudge</td>
</tr>
<tr>
<td></td>
<td>Arial normal, bold for headings and sub headings. San-serif typeface suitable for this purpose, well known and easily accessible</td>
</tr>
<tr>
<td><strong>Alignment</strong></td>
<td>To both left and right margins (justified), or centered; uneven gaps between words</td>
</tr>
<tr>
<td></td>
<td>Left-aligned. Eliminates major composition and typographic problems</td>
</tr>
<tr>
<td><strong>Capital letters</strong></td>
<td>All letters in upper case</td>
</tr>
<tr>
<td></td>
<td>Only first letter in upper case. Guides reader between ingredient names</td>
</tr>
<tr>
<td><strong>Line spacing</strong></td>
<td>Very narrow line-spacing (leading)</td>
</tr>
<tr>
<td></td>
<td>8 points (two points larger than typeface font size). Suitable leading is dependent on line-length, typeface, font size etc.</td>
</tr>
<tr>
<td><strong>Contrast</strong></td>
<td>Poor contrast between text and background due to color combinations, glossy package material, pictures/illustrations in the background</td>
</tr>
<tr>
<td></td>
<td>Black text on white (ideally off-white) background</td>
</tr>
<tr>
<td><strong>Spacing and separation</strong></td>
<td>No or unclear markings between ingredients; uneven and/or narrow spacing</td>
</tr>
<tr>
<td></td>
<td>Significant spacing between ingredients separated by a bullet (two points larger than the typeface font size, positioned on the baseline)</td>
</tr>
<tr>
<td><strong>Splitting</strong></td>
<td>Ingredient name split onto different lines</td>
</tr>
<tr>
<td></td>
<td>The whole ingredient name on the same line</td>
</tr>
<tr>
<td><strong>Micro-typography</strong></td>
<td>Poor micro-typography: individual letters and words too tightly or too loosely/unevenly kerned; compressed and/or extended typeface cuts; wrong letter spacing (tracking)</td>
</tr>
<tr>
<td></td>
<td>Optimized micro-typography: correct letter and word spacing; no compressed and/or extended typeface cuts</td>
</tr>
</tbody>
</table>
Table 4. Key eye-tracking results for the different label formats.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Label format and eye movements mean value (standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Fixation count</td>
<td>59.69 (15.91)</td>
</tr>
<tr>
<td>Fixation duration (MS)</td>
<td>304.6 (48.96)</td>
</tr>
<tr>
<td>Saccade count</td>
<td>62.91 (17.48)</td>
</tr>
</tbody>
</table>

4.2.2 Strengths and limitations

Since the participants were recruited by advertisement they were not a random sample of the general population. The question of ingredient terminology was considered too complex to be handled within the scope of this study, and was therefore not included. Difficult ingredient terminology has been identified as an important obstacle for allergen avoidance (104, 108). We made numerous typographical improvements in the alternative formats compared to the original format, but under the experimental conditions of this study it was not possible to “isolate” these improvements one by one to see which of them had the greatest impact on the readability.

A strength of this study was the multidisciplinary approach. The project required collaboration between experts in graphical design, readability and eye-movement research, occupational and environmental dermatology, and risk assessment/legislation. Several quantitative as well as qualitative measures were used to assess the readability of the ingredient labels. In many cases the findings were supported by all or several of these measures, resulting in a high credibility of these findings and the conclusions drawn upon them.

4.2.3 Studies on readability of ingredient labels

To the best of our knowledge, this is the first study that assesses the readability of cosmetic ingredient labels, using objective and subjective measures. Much more information can be found regarding the consumers’ understanding and use of packages and food nutrition labeling. It appears that the majority of these studies used qualitative methods, but some have used eye tracking (101, 102, 124, 125). In this context, the eye-tracking measures have been used in order to compare different nutrient labeling formats’ ability to guide the consumer to important nutrients (102, 125). Furthermore, it has been used to assess which areas of the food packages attract the consumers’ attention, e.g. brand, ingredient label, nutrition label, net content, manufacturer, origin, etc.
4.2.4 Consequences for the allergic individual

As I have worked with this project and thereby mentioned it in private and work-related situations, I have understood even better that it is of great importance that ingredient labels of allergen-containing products (cosmetics, food etc.) have clear, readable and complete ingredient labels. Stories have been shared with me regarding the problems of avoiding substances to which people know they are sensitized, due to poor readability of the labels. One typical example is eczema that recurs when avoidance of the causative substances fails, and which in different ways can affect quality of life very negatively, and even result in sick leave and change of occupation. Another typical story has been the problem of avoiding food allergens. Several parents have told me about not detecting a food allergen in food given to their children, sometimes leading to dramatic (potentially life-threatening) consequences, and visits to the hospital emergency. Although the poor readability is merely one of the factors that make things difficult – terminology being another – it causes stress to many allergic individuals. These stories have support in the scientific literature, both regarding food and contact allergy (103-106, 108)

4.2.5 The regulatory situation and possible improvements

The regulatory requirements on the readability of cosmetic ingredient labels are rather minimalistic, stating that ingredient labels should be written in “indelible, easy legible and visible lettering”. Food labeling is not much better, although a new EU regulation requires that mandatory food information, such as that in the ingredient label, has a font size no smaller than 0.9 mm or 1.2 mm depending on package surface area (126). As shown in table 3, many areas of improvements were identified and suggestions were made on how they could be improved. Many of these could probably quite easily be introduced as quantitative regulatory requirements. Apart from having requirements on font size, one could also mention that printing ingredient labels in black text on white background, to assure proper contrast, would have a great impact on the readability of many ingredient labels. An idea, which to some people may seem unconventional, was to list ingredients in alphabetical order instead of in order of descending weight, as the current labeling system requires. This simple adjustment gave a good improvement in readability and should not be too easily dismissed. The main assignment of the ingredient label is to let the consumer identify ingredients that are of interest for different reasons, often for the sake of avoidance. With this in mind, the alphabetical ordering of ingredients would clearly be beneficial.
4.3 METHYLISOTHIAZOLINONE IN RINSE-OFF PRODUCTS (STUDY IV)

4.3.1 Main findings

- All MI-allergic participants had positive reactions to MI patch test doses down to 15 µg/cm² (0.05%). Three subjects reacted to the lowest dose tested, 0.48 µg/cm² (0.0016%).

- Ten out of 10 MI-allergic participants had positive reactions to the liquid soap with 100 ppm MI (0.48 µg/cm²/application) within 4-11 days.

- Seven out of 9 of the MI-allergic participants that used the liquid soap with 50 ppm MI (0.24 µg/cm²/application) had a positive reaction within 5-21 days.

- None of the 19 MI-allergic participants had a positive reaction to the control soap without MI.

- None of the 19 control participants without MI allergy had positive reactions on any arm within 21 days of usage of the soap containing 100 ppm MI or the control soap without MI.

- The difference in reactivity to MI between MI-allergic participants that had used the 100 ppm soap compared with the control participants was statistically significant (Fisher’s exact test, p < 0.001). The difference in reactivity to MI between MI-allergic participants that had used the 50 ppm soap compared with the control participants was also statistically significant (Fisher’s exact test, p < 0.001).

- The lack of positive reactions in the control participants and on the MI-allergic participants’ control arms clearly demonstrates that the positive reactions were contact allergy reactions.

Table 5. Number of participants who developed positive reactions to liquid hand soaps by rinse-off repeated open application test (ROAT). The soaps contained different concentrations of methylisothiazolinone (MI). A positive reaction was defined as: area of involvement >25 % of the area of application, including erythema and signs of infiltration, i.e. at least 1 papule.

<table>
<thead>
<tr>
<th>Liquid soap MI concentration</th>
<th>MI-allergic (pos/tested)</th>
<th>Controls (pos/tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm</td>
<td>10/10</td>
<td>0/19</td>
</tr>
<tr>
<td>50 ppm</td>
<td>7/9</td>
<td>Not tested</td>
</tr>
<tr>
<td>0 ppm</td>
<td>0/19</td>
<td>0/19</td>
</tr>
</tbody>
</table>
4.3.2 Strengths and limitations

In this study design the participants applied the soaps 5 times per day according to our instructions. This means we had to rely on good compliance by the participants in order to get reliable and comparable results, which is a limitation compared to in vivo tests such as the LLNA in mice where the allergen is applied by a technician. We took several measures to try to get as good compliance as possible. First we tried to establish a comfortable and clear communication with the participants, emphasizing the importance of following the instructions as closely as possible and also not hesitating to tell the test leader if they had made a deviation from the instructions. Clear instructions are also of great importance. During the visit before starting the ROAT we thoroughly went through the procedure and the participant were asked to apply the soaps under “supervision” by the test leader to ensure it was done correctly. The participant got information in writing and pictures as well as a link to a YouTube clip showing the application. They were offered reminders by mobile text messages at time points which they could decide themselves. Furthermore, the soap consumption was measured by weighing the packages before and after they had been used, which the participants were not aware of.

The study design involves the target organism, humans, and it also mimics realistic vehicles as well as exposure patterns such as frequency of application, application procedure etc. This design is thus relatively close to real life exposures compared to many other study designs. The participants applied the soaps 5 times per day which is more than a “standard” ROAT, which is typically using 2 daily applications. According to the SCCS guidance document on risk assessment of cosmetics (29), the number of daily exposures to the most common types of rinse-off products is approximated to: liquid hand soap (10×); shower gel (1.43×); shampoo (1×); hair conditioner (0.28×). This adds up to almost 13 exposures per day. Based on experience and on a pilot study including 5 participants, we concluded that 5 times was a good compromise between “reality” and a reasonable work load for the participants, to ensure good compliance.

4.3.3 Other ROAT studies on MI

At least three ROAT studies have studied leave-on exposure to MI (80, 127, 128). The general finding from these studies is that leave-on products preserved with 100 ppm MI elicit allergic reactions in a large proportion of individuals with MI allergy. The study by Lundov et al. (128) is most comparable with our study in terms of study design and cut-off criterion. MI-allergic subjects (n=11) and controls (n=14) were exposed to different doses of MI combined with 0.4% phenoxyethanol (in 10% ethanol in water) and a control vehicle. Sixty-four percent of the MI allergic subjects had a positive reaction to 100 and 50 ppm MI, and 18% reacted to 5 ppm MI after leave-on exposure. There was a high rate of positive reactions in our ROAT study, which may seem surprising since the soaps were rinsed-off after a short time (20-25 seconds). Several factors may be involved, as discussed below.
4.3.4 Exposure-related factors

MI-allergic participants in the present study appear to have had a somewhat higher reactivity to MI than the participants examined by Lundov and coworkers, as revealed by patch testing. This could, to some extent, have affected the results. Exposure-related factors were probably also an important part of the explanation. A number of factors coupled to the exposure situation can influence the threshold for sensitization and elicitation, for example dose/unit area, exposure duration, site of application, activation of pre- and prohaptens, frequency of exposure, and vehicle effects (129-131). Regarding the conditions of the study, the most relevant factors for further discussion are probably the relatively high frequency of exposure and vehicle effects.

4.3.4.1 Repeated low-dose exposure

The study participants were repeatedly exposed to the liquid hand soaps, which is also the case for a large part of the general population. Repeated low-dose exposure has been shown to be an important factor for elicitation as well as sensitization (132-136). In a study by Jensen et al., previously sensitized individuals applied 0.04% MDBGN once per day on one arm, and had 4 applications per day of 0.01% of MDBGN on the other arm (133). The accumulated dose required to elicit a reaction was nearly the same, indicating that the two exposure situations had a similar elicitation potential. Clearly, one explanation for the effects caused by repeated and frequent exposure is that many sensitizers can accumulate in the skin over a certain time period. Another interesting suggestion is that each exposure stimulates the immune system slightly, which eventually leads to a build-up and an immune response (134).

4.3.4.2 Vehicle effects

Combined exposures to allergens and irritants may lead to reduced elicitation and sensitization thresholds due to vehicle effects (137-139). A number of substances that are used frequently in consumer products, such as terpenes and surfactants in soap, as in our case, are efficient skin penetration enhancers (140). Furthermore, the exposure to irritants (and other allergens) can lead to augmented danger signals, i.e. exposure to the mixture induces an increased production of pro-inflammatory cytokines which leads to enhanced activation of antigen presenting cells and T cells.

4.3.5 Implications

Studies on elicitation are of great importance for the protection of individuals that have already been sensitized. For frequent sensitizers such as MI, this is particularly relevant since many MI-allergic people have probably not been diagnosed for their MI allergy and are therefore not aware of it, and those diagnosed still have trouble to avoid exposure. It is also my opinion that clinical patch test data and clinical experimental data (e.g. ROAT) should be given great weight, especially when risk assessments based on sensitization tests have failed as with for example methylidibromo glutaronitrile (141), and now also MI.
Previous risk assessments based on sensitization tests have severely underestimated the risk of MI in cosmetics and a recent re-assessment by the SCCS, primarily based on clinical data, concluded that MI should not be used in leave-on products and that information on elicitation from rinse-off products was not yet available (30). We found that levels down to 50 ppm MI (the lowest dose tested) in rinse-off products were not safe, and the elicitation threshold is likely far below the tested doses. Uter et al. found that hairdressers patch tested in 2005/2006 had a much higher prevalence of contact allergy to MDBGN compared with clients. The convincing hypothesis was that since MDBGN at the time was only used in rinse-off products such as shampoos and hair conditioners, the frequent occupational use of these product types gave them more of a “leave-on character” (52). This should be taken into consideration in the assessment of liquid hand soaps, since they are used many times per day not only by certain occupational groups but also a large part of the general population.

According to the Cosmetics Regulation cosmetic products shall be safe “under normal or reasonably foreseeable conditions of use”. Without doubt, this is currently not true regarding cosmetics that are preserved with MI. Risk managers and regulators urgently need to take proper actions to protect the consumers and workers.
5 CONCLUSIONS

The main conclusions in this thesis are as follows:

- Hair dye substances categorized as potent skin sensitizers are very common in oxidative hair dye products on the market and the products contain a “cocktail” of these sensitizers.
- Due to the frequent use of hair dye sensitizers other than PPD, there is a risk that cases of hair dye allergy are missed by patch testing with the current baseline series containing PPD only, and with the currently available hairdresser series.
- Rather simple adjustments in the design of cosmetic product ingredient labels would improve their readability and comprehensibility significantly, which would be of benefit to millions of allergic individuals and others in their everyday struggle to avoid harmful or unwanted exposures and negative health effects.
- More specific requirements on the typographical design of ingredient labels should be introduced in relevant EU regulations.
- 100 ppm and 50 ppm MI in rinse-off products elicited allergic reactions in the majority of previously sensitized individuals and the elicitation threshold is likely far below the tested doses.
- Cosmetic rinse-off products preserved with MI in the tested concentrations are not safe to the consumer. No safe level has yet been proven.

5.1 FUTURE PERSPECTIVES

Looking back at my time as a PhD student, where I have gotten the opportunity to immerse myself into the subjects covered in this thesis, I realize that I have more questions now than at the start. A number of knowledge gaps have caught my interest. One such knowledge gap of great importance concerns the effects caused by combined exposures to a “cocktail” of allergens and irritants from many different sources. This question is more of a general concern and is certainly not relevant only for allergic contact dermatitis. Also other exposure-related factors need more exploration in order to improve risk assessment and avoid future epidemics of contact allergy such as the one that has now been experienced with MI. In everyday life we are exposed not only to a combination of substances. The exposure is also repeated, often in low dose, and many times per day, possibly throughout our entire life. In this, I see another challenge in how to accurately assess the effects of this type of exposure situation, which differs so much from experimental studies/models.

The current European baseline series covers only one, and commercially available hairdresser series cover only few of the potent skin sensitizing hair dye substances present in consumer available products. It is likely that they need to be updated to better reflect the current exposure situation. One of the objectives within the EU financed scientific network the COST
Action StanDerm (Development and Implementation of European Standards on Prevention of Occupational Skin Diseases) is to assess available data on hair dye allergy, including exposure, experimental and patch test data, and to recommend a hairdresser series. I will curiously follow what will come out from this effort.

Regarding the readability of ingredient labels, it would be of great interest to develop a terminology that simplifies the labels. The INCI nomenclature is complicated and it should be possible to make improvements in this area.
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And most importantly, my amazing little family: Hille, Lea, and Signe. Jag älskar er!

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