Main conclusions, general discussion and future perspectives

By the work we described in this thesis, we aimed to gain more insight into prevalence and biological aspects of contact allergy to the potent sensitizing hair dye molecule \( p \)-phenylenediamine (PPD).

The introduction of this thesis (Chapter 1) gives a background regarding hair biology and anatomy, the invention of modern oxidative hair dyes and PPD. For PPD it describes contact allergy, metabolism, genetic aspects and European regulation.

The Chapters 2, 3 and 4 deal with the prevalence of PPD contact allergy. The case-control study presented in Chapter 2 estimates the prevalence rates of PPD sensitization in both the general and the patch-tested population of The Northern Netherlands. Furthermore, it explores possible exposure sources. In this study PPD sensitized cases from our patch test database were matched for age and gender with population-based controls, obtained from the Fragrance Study of the European Dermato-Epidemiology Network. A questionnaire was used to analyze exposure sources.

We conclude that the prevalence of PPD contact allergy is stable over the years, though high. Within the patch-tested population of the University Medical Center Groningen the prevalence of PPD sensitization was 3.3%, which is in line with rates of contact allergy to PPD in consecutive eczema patients in Europe. In the general Dutch population the prevalence of PPD sensitizations was 1.3%, which is in the range of rates reported in previous small studies in Europe. Hairdressers are more than 4 times more likely to be sensitized to PPD (\( p = 0.041 \)). The exposure to a black henna tattoo in the past increased the sensitization risk with a 2.3-fold (\( p = 0.081 \)). In individuals that had had a black henna tattoo in the past strong and extremely strong elicitation reactions were found 14 times more often than weak elicitation reactions (\( p = 0.015 \)). The latter findings can suggest that black henna tattoos are a risk factor of more severe elicitation reaction upon exposure to PPD.

The prevalence of PPD contact allergy was investigated in Chapter 3. Almost 100 000 individuals that had had a PPD patch test were included in this study. The European patch-tested population did not show any decrease in the prevalence of PPD contact allergy in the past decade. The separate patch test centres, included in this study, did show a wide variety of the number of individuals tested for PPD sensitization, with the highest number of PPD patch tests in London, Great Britain, and the lowest number in Austria, followed by Germany. We attributed this low prevalence to the exclusion of PPD from the European baseline series during some of the investigated years. The overall prevalence of PPD sensitization in Europe is about 4%. The highest overall standardized prevalence was found in Lithuania, the lowest prevalence in Slovenia. The prevalence of PPD sensitization did not decline over the investigated years. Half to three quarters of the PPD sensitizations had a clinical relevance. Regarding the reaction strength, more weak positive reactions than strong/extremely strong patch test reaction were observed. To investigate regional differences, we combined the
patch test centres based on their geographical location, creating three regions, that are North, Mid and South. Significantly more strong/extremely strong patch test reactions to PPD were found in the South region, compared to Mid and North, although the prevalence did not differ. We related this to the darker shades of hair of the population in southern Europe, as dark shades of hair dye often contain a higher concentration of PPD. From previous research it is known that the exposure to a higher concentration of an allergen can, after sensitization, more easily result in a stronger elicitation upon re-exposure.

Chapter 4 focuses on the occurrence of cross reactivity among PPD- and 2,5-toluenediamine (TDA)-sensitized individuals. Concomitant sensitizations to structurally similar molecules, (cross reactions) are a well-known event in contact allergy. They are very often observed in PPD or TDA sensitized individuals, with an obvious relation between the patch-test strength and the number of cross reactions. From 8 036 individuals, that had had at least a patch test with the European Baseline Series, 251 with a sensitization to PPD, TDA or both were included in this study. They were analyzed for the patch test strength in relation to the number of cross reactions. Furthermore, sensitization rates of allergens within the European Baseline Series (EBS) were analyzed regarding their prevalence with and without PPD or TDA sensitization. A significant difference was observed regarding the patch test strength and the number of cross reactions ($p<0.001$ for all groups). When testing separate patch test strength, this difference was not found between + and ++ strengths. PPD sensitized individuals were more likely to be sensitized to carba-mix, cobalt chloride, colophony, $p$-tertbutylfenolformaldehyderesin, parabens mix and methylisothiazolinone as well. TDA sensitized individuals were more often sensitized to carba-mix. These co-sensitizations are difficult to explain regarding exposure. We hypothesized a contribution of the skin microbiota to concomitant sensitization.

As many patients continue dyeing their hair, notwithstanding their PPD or TDA sensitization, we performed an exploratory study into this phenomenon (Chapter 5). This revealed that the behavior cannot be attributed to a decreased self-esteem or a negative image regarding grey hair. However, those that still dye their hair think their life will be worth less if they cannot dye it anymore, as they grade their life significantly lower when they would not dye their hair anymore. Apart from assigning their behaviour to a decrease in the grade of their life, individuals that still dye their hair assign this behavior to factors that lie outside their own power to influence (nicer color’ $p=0.02$, $\eta^2_p=0.10$ and ‘required for work’ $p=0.02$, $\eta^2_p=0.10$). Their own opinions and that of loved ones do not matter at all. Individuals that continue dyeing their hair, despite their hair dye allergy, might be struggling between meeting the preferred body image and living a healthy life.

$N$-acetyltransferase 1 (NAT1) is a scavenger of xenobiotics, such as unoxidized PPD, that enter the skin. Single nucleotide polymorphisms in the gene coding for the enzyme $N$-acetyltransferase 1 (NAT1) can result in the formation of slow- and fast-acetylating enzymes. Slow-acetylating enzymes are associated with a higher risk of sensitization to PPD. In Chapter
we performed a basal study into the location of the enzyme NAT1 in human skin. Using cryosections of six different healthy donors, staining for NAT1 was performed. This showed granular staining for NAT1 in the stratum corneum and perinuclear in the viable epidermis. Within the viable epidermis, no co-localisation was found with either endosomes, lysosomes, Golgi apparatus or mitochondria. Within the stratum granulosum, NAT1 was located most likely on the cell surface, as staining for corneodesmosomes localized dominant NAT1 expression. The expression in the stratum corneum is in line with the attributed function of NAT1 in human skin, which is scavenging of xenobiotics, such as PPD, that enter the skin. It was hypothesized that functions confined to nucleated keratinocytes, other than those related to lysosomes, endosomes, the Golgi apparatus or mitochondria, are the reason for the perinuclear expression of NAT1. The function of the intracellular pool remains to be determined.

In Chapter 7 the role of a pretreatment with an antioxidant on the elicitation reaction was investigated. In general, nearly all contact allergic reactions, and probably reactions elicited by PPD, are accompanied by high oxidative stress. Oxidative stress can contribute to inflammation of the skin. In this proof-of-principle study, we investigated whether pretreatment with the strongest natural appearing antioxidant, ascorbic acid (vitamin C) would reduce the elicitation reaction to a PPD containing hair dye in sensitized subjects. 12 subjects with contact allergy to PPD were included in this study. They all had a documented skin reaction to a hair-dye simulation exposure model and a history hair dye related skin complaints. In 7 out of these 12 test subjects the pretreatment with ascorbic acid emulsion had an attenuating effect on the elicitation reaction to PPD in sensitized individuals on D3 (p=0.046). No statistically significant difference was observed on D2. We concluded that the pretreatment with the antioxidant ascorbic acid was not able to prevent the elicitation reaction, though a significant reduction of the reaction strength was observed. The effect of the antioxidant on the elicitation reaction can be a result from the trapping of ROS that are released during every elicitation reaction. Another possible cause of this reduction is the interference with the auto-oxidative reaction of PPD in hair dye, preventing the formation of oxidation products that are even more toxic than PPD itself.

A case of severe persistent blepharoconjunctivitis caused by PPD in permanent eyelash dye was described in Chapter 8. This case not only emphasized the potential risk of black henna tattoos, often containing high concentrations of PPD, but also the risk of PPD-containing dye for eyelashes.

Having summarized these main findings and conclusions, two important topics deserve further discussion, which are the prevalence of PPD contact allergy and the multifactorial (or biological) cause of PPD contact sensitization, focusing on both genetics and the microbiota of the skin.
The prevalence of PPD contact allergy

PPD contact allergy has been reported since the discovery of this interesting molecule (Chapter 1). PPD contact allergy can be attributed to the use of hair dyes by consumer or occupational exposure to hair dyes by hair dressers, to black henna tattoos, to other substances such as clothing, shoes or rubber, and to cross-reacting molecules. It has been in the top ten most prevalent allergens in Europe for years.\(^1\)\(^4\) Due to the high prevalence and the potential health-related risks of sensitizations to this aromatic amine the European Union set a regulation regarding the maximum concentration of PPD in a consumer-product (Council Directive 76/768/EEC). During the following years, this regulation had been modified to some degree and had been clarified. Nowadays, as a result a maximum ‘on-head’ concentration of PPD in hair dye products of 2% is allowed (Regulation (EC) 1223/2009). In hair dye products the primary intermediates, among which PPD and TDA must be mixed in equal parts with couplers and an oxidant, usually hydrogen peroxide. The concentration of the other common hair dye molecule, TDA, was maximized as well. Other hair dye molecules, which are precursors, couplers and oxidants, are also regulated regarding their maximum allowed concentration, due to their irritant or sensitizing properties. As the prevalence of PPD contact allergy has remained stable, the desired effect of the regulation, that is the decrease of the prevalence, has not yet been achieved (Chapter 3).

The effect of regulations on the prevalence of contact allergies was already extensively investigated regarding nickel sensitization, for which a European regulation was implemented in 1991. Johansen found the prevalence in children decreased significantly from 24.8% to 9.2% between these two periods (1985/86 and 1997/98). Similar positive results of this regulation were described in various studies.\(^5\)\(^8\) Up till now, the main question, of course, is what the aim of this specific regulation regarding PPD was; that is to stabilize the prevalence of PPD sensitizations or to decrease it. If the aim was to decrease the prevalence of PPD sensitizations, the factors that contributed to this failure have to be investigated. It is a possibility, as Schnuch already hypothesized in 2008, that PPD in hair dye products only count for a part of the total prevalence of PPD contact allergy.\(^9\) Therefore, regulation of the PPD concentration in hair dye products has no great impact on the prevalence.

In Chapter 2 we reported prevalences of both the general and the patch-tested population of the northern Netherlands (Groningen and Stadskanaal). In this chapter we described the prevalence of sensitizations to contact allergens in the general population. Most prevalences are calculated or extrapolated from the patch-tested population.\(^10\) Any patch-test population is a strongly biased group of individuals, as in our own centre. Patients referred to our patch test clinic have often a history of persistent eczema, hand eczema, probably occupational-related, or a high suspicion of a contact allergy. This makes an extrapolation very difficult. Therefore, it is preferable to choose the more accurate, but far more expensive, manner to report a general population-base prevalence, that is to patch test a representative subset of the general population.\(^11\)\(^13\) In this chapter a comparison was made between our patch-tested population and our general population.
Another, rather remarkable finding is the difference in the strength of the patch test reactions in individuals in Southern European countries as compared to those in Mid- and Northern Europe. We noticed the absence of a higher prevalence of PPD sensitizations in the South (Chapter 3). In 2009, Thyssen et al. already investigated the prevalence of PPD contact allergy in Europe. They found a significant higher prevalence of PPD contact allergy in the Central and Southern European countries compared to the Northern ones, respectively, weighted averages of 53.6% and 20.3%. They imputed this to the hair colour of the individuals within the different European countries. The population of the more Southern located countries in Europe has overall darker colored hair. Since darker shades of hair dye contain higher concentrations of PPD or TDA, these individuals, when dyeing their hair, have a greater risk of exposure to higher dosages of PPD and TDA. Furthermore, grey hairs are more notable between dark hairs than between blond hairs.

PPD is more frequently used in oxidative hair dye products in Southern Europe. Yazar et al. investigated ingredients of 105 hair dye products found in stores in Spain and compared those with 122 hair dye products found in Sweden. Nearly all products (99%) they investigated contained either PPD or TDA, however, PPD and TDA were never found together in the same product. A quite remarkable finding was that in some cases the same hair dye brands contained PPD in Spain, while in Sweden the ingredients list of the same product stated TDA. Up till now, it is unclear why there is a regional difference between hair dye precursors. Although it has never been confirmed, PPD might be more suitable to create darker hair dye shades. The latter might also explain the even higher number of PPD containing hair dye products in the United Stated. The distribution of ethnic groups differs between Europe and the United States, with the latter having far more African-Americans, with very dark shades of hair (Chapter 3).

The absence of an higher prevalence in the South (Chapter 3) can indicate that the higher number of hair dye products containing PPD does actually not cause an increased sensitization rate in our investigated population. On the contrary, Thyssen et al. found a higher prevalence of PPD contact allergy in the South, although they did not investigate the patch test strength in relation to the geographical area. However, they compared the North of Europe to the South, while in Chapter 3, a comparison was made between the northern, the mid and the southern European countries. This makes it even more difficult to compare these two studies. The explanation for both findings, the higher prevalence in the South and the stronger elicitation reactions in the South, can be imputed to the higher concentration of PPD that is required to make darker shades of hair dye.

The question is if this higher concentration can be regarded as a problem. In 1966 Kligman et al. performed a study on 24 prison inmates in which he exposed their skin to PPD. The most important finding of this study was that the greater the concentration of PPD, the higher the risk of being sensitized, with a total of 100% sensitized when exposed to 10% PPD. This was also described as the dose-response reaction in contact allergy, with a strong relationship.
between the sensitizing dose of an allergen and the concentration of that allergen to which an individual will elicit a reaction. ²⁰

The risks of sensitization in relation to exposure to high PPD doses are also mentioned as the culprit in black henna tattoos. Unlike the original red henna tattoos, these black henna tattoos contain a pigment that creates the black color of the tattoo. PPD is often used as pigment for the following reasons: it is cheap, enhances the drying process and ensures a long-lasting nature of these tattoos. Black henna tattoos often contain high doses of PPD and are increasingly reported as a sensitization source in not only adults, but also in children. ²¹-²⁵ Although the sensitizing potential of black henna tattoos containing PPD underlines the risk of high concentrations of this aromatic amine, the risk of these tattoos differs greatly from that of hair dye products. Due to the presence of couplers and an oxidant, that is hydrogen peroxide, less toxic oxidation products are formed compared to auto-oxidation products formed by air-oxygen. ²⁶ Therefore, these concentrations of 'pure' PPD within the black henna tattoo, other than a hair dye product, are more harmful and are more prone to sensitize the individual. ²⁷

Many factors can contribute to the individual's risk to become sensitized. An important and problematic factor in PPD contact sensitization was already highlighted in Chapter 4. Both known and unknown sources can cause sensitization to PPD and elicit reactions upon patch testing, due to cross reactivity. This implies that an individual can either become sensitized to PPD and reacts to molecular similar substances, or an individual has never had contact with PPD, but is sensitized to e.g. the closely related TDA. Although the term cross-reactivity is commonly used, the existence of true cross reactivity is debatable, especially in hair dye allergy. Many of the hair dye ingredients have molecular structures similar to the aromatic amine PPD. When a consumer is exposed to PPD, he is simultaneously exposed to other hair dye ingredients. ²⁸-³⁰ Therefore, based on routinely recorded patch-test data, it is impossible to differentiate between true cross-reactivity and co-sensitization in hair dye allergy, at this moment.

The question remains whether the above mentioned factors indeed contribute to the stable, top ten prevalence of PPD contact allergy. We assume that to some extend the individual's genetic make-up can contribute to the sensitization risk.

The multifactorial cause of PPD contact sensitization

Genetics of (PPD) contact allergy

The role of genetics in the susceptibility of contact allergy has not yet been fully explored. Up till now, a few studies on the genetics of contact allergy have been published. For contact allergy to PPD only a few genes have been investigated, of which NAT1 and TNF were proven to influence an individual's risk of becoming sensitized. ³¹
As mentioned above, genetics, taken on their own, cannot account for the individual’s susceptibility. However, as sensitivity to certain contact allergens or to multiple contact allergens is sometimes observed in families, one could presume that genetics can account for at least part of the individual’s perceptivity for sensitization. This was studied in family studies and twin studies. However, a main problem regarding the findings in these studies is the control of confounders, such as similar exposure.

Apart from these family and twin studies, research was performed on genetic polymorphisms that appear in sensitized individuals. These polymorphisms were studied with regards to the three major different pathogenetic steps in contact allergy, that are, the amount of allergen that is available for the immune system, factors that interfere with inflammation and factors related to the immune system. The amount of allergen that is available for the immune system was proven to be of great importance in both the induction as well as the elicitation reaction. Related to the induction of a contact allergy, the concentration of the allergen the individual is exposed to is of great importance. When a genetic polymorphism results in the production of a slow-working enzyme, the amount of allergen available to the immune system can increase. Therefore, sensitization can more easily occur.

Several studies hypothesized that the impaired skin barrier was a problem in contact allergy, due to the presumed increased entry of contact allergens. In these studies, the relation between mutations in the gene coding for the skin barrier protein filaggrin and the development of contact allergy was investigated. A clear association was found regarding nickel allergy, though the results of the studies concerning contact allergy in general were inconclusive. Thyssen et al., however, found a borderline significant association between xerosis, without mutations in the filaggrin gene, and contact sensitizations other than nickel.

With respect to filaggrin mutations, an important question remains whether atopic dermatitis changes the individual’s sensitivity for the development of a contact allergy. This was investigated in various studies, but up to now this relation has not been proven. It is even claimed that atopic individuals have a lower risk of sensitization.

Other factors that can influence the amount of allergen available for the immune system are the enzymes that can detoxify the allergens by converting them into harmless substances. The most important examples of these enzymes are the N-acetyltransferases (NAT1 and NAT2). NAT1, as described in Chapter 6, is proven to play a role in PPD sensitization. Polymorphisms leading to the slow-acetylator phenotype increase the sensitization risk. In the skin, the localization of this enzyme is considered important, as air-exposed PPD almost directly oxidizes in harmful products, such as Bandrowski’s base and its precursors. It was assumed that NAT1 is located in the basal layers of the viable epidermis. In Chapter 6 we investigated the location of NAT1 in healthy human skin. We discovered that the most remarkable staining of NAT1 was in the skin layer where it may be the most effective, namely
the stratum corneum. This seems to be the most strategic place in the skin for a detoxifying enzyme such as NAT1. It is situated at that location in the skin where it has the best chance of capturing as many unoxidized PPD as possible, before the chemical is recognized by the immune system. Not only the identification of the location of this enzyme is very important, but also a more thorough investigation of the location. As we have already hypothesized, two reasons for NAT1 in the viable epidermis can be considered. The first reason is that NAT1 has been produced in the viable epidermis and will be transported to the stratum corneum. A second reason for the presence of NAT1 in the viable epidermis is that it can catch some of the unoxidized PPD that penetrated through the stratum corneum. Moreover, in order to gain insight into the expression of the enzyme related to its function, it is important to investigate the expression of this enzyme after exposure to PPD in individuals that are either sensitized to PPD or not sensitized to PPD.

NAT2 is another N-acetyltransferase, with a function and a molecular structure similar to NAT1. Polymorphisms in this enzyme resulting in the fast-acetylator phenotype also seem to carry an increased chance of becoming sensitized to para-substituted aryl compounds. They hypothesized that this can either be due to a functional problem, that is, faster acetylation can enhance sensitization, or to a genetic ‘problem’, that is, the individual’s enzyme status which can be one of many genetic markers that influence the susceptibility of sensitization to one or more contact allergens. They did not take into account that NAT2 is not expressed in the human skin. This makes the interpretation of the findings somewhat complex, as PPD, and many other para-substituted aryl amines, which are present in hair dye, are applied to the skin.

A small amount of PPD that is neither oxidized, nor acetylated in the skin, ends up in the serum. Serum PPD can be acetylated in the liver by NAT2, in a similar way as dapsone (diaminodiphenyl sulfone) or sulfamethoxazole, into mono- and di-acetylated products. However, even the acetylation in the liver does not explain the fact that fast-acetylator phenotypes were more likely to become sensitized to PPD, as acetylation detoxifies PPD and other aromatic amines. The acetylation products of PPD, monoacetyl-PPD and diacetyl-PPD, do not seem to be allergenic. Therefore, the second assumption of Schnuch et al. seems most plausible.

Mutation in genes coding for factors interfering with the immune system are also investigated in relation to PPD contact allergy. These factors include enzymes able to reduce inflammation by scavenging reactive oxygen species (ROS) and other enzymes that regulate inflammatory mediators. ROS plays a role in contact allergy in general and is assumed to be important in contact allergy to PPD in particular. ROS can be captured by the superoxide dismutases (SOD), which are present in the human skin. In the skin three different SODs are found: copper/zinc SOD present in the cytosol, copper/zinc SOD present in the extracellular space and manganese SOD present in mitochondria. In the context of PPD contact sensitizations,
the role of manganese superoxide dismutase was investigated, as SNPs in the gene coding for this enzyme can cause altered enzymatic activity or altered allocation due to differential folding of the protein. Brans et al. investigated the role of the MnSOD 47 T>C genotype, responsible for altered folding of the protein, in PPD sensitized individuals versus healthy controls. They did not find a difference between the groups regarding the MnSOD 47 T>C genotype in exon 2 (Ala-9Val), which is associated with higher MnSOD levels in the mitochondria. The reason for the absence of an effect of the higher MnSOD levels in the mitochondria due to the SNP has not been clarified up till now. Although Brans et al. did not give clear suggestions concerning the absence of an effect of this SNP, from our study on NAT1, we can assume that a possible explanation can be the location of MnSOD within the mitochondria. As we already explained in Chapter 6, the location of an enzyme is not just only related to its function, but also important for its function. Possibly PPD is not captured by MnSOD because it is located intracellular, while the enzyme function is required extracellular. The copper/zinc SOD present in the extracellular space and therefore it is more likely that this enzyme is involved.

The location of NAT1 in human skin confirms this theory, as this scavenging enzyme is localized in the stratum corneum, most likely not within the corneocytes. Therefore, we think that at least other SODs have to be investigated, regarding their role in the captivation of ROS that are formed during PPD contact allergy. This assumption is strengthened by our findings in Chapter 7, in which the pretreatment with an antioxidant, which is able to capture ROS, reduced the grade of the elicitation reaction.

The third step of the pathogenetics of contact allergy in which gene polymorphisms were studied are the factors related the immunology of a type IV hypersensitivity reaction. One of the important steps in contact sensitization to PPD is the activation of dendritic cells (DCs). These DCs are able to produce high levels of TNF that can bind to specific receptors, resulting in a delayed-type hypersensitivity reaction. Because of the role of TNF in sensitization, Blömeke et al. investigated the association between the TNFA-308 polymorphism and the sensitization to PPD. They found that the carriers of the TNFA A allele (genotypes TNFA AA and TNFA GA) were more prevalent among the PPD sensitized individuals. This genotype is associated with an increased production of TNF, resulting in an increased grade of the immunological reaction to a contact allergen, such as PPD. Many other factors, of which we do not know the effect when polymorphisms appear, are involved in the immunology of contact allergy.

The sensitization to PPD seems not only multifactorial but also multigenetic. Therefore, we are great advocates for more research into the genetics of contact allergy in general and contact allergy to PPD in particular. One approach can be the performance of a genome wide association study, into other possible gene polymorphisms which can contribute to PPD sensitization. However, another part of the mentioned multifactorial character can probably be found in the skin microbiota that is the microorganisms living on the skin.
Contact allergy to PPD and the microbiota of the skin

Nowadays, the microbiota of the skin is extensively investigated in relation to different inflammatory disorders, such as eczema and psoriasis. In Chapter 4 we already suggested a possible role of the skin microbiota on the sensitization to PPD. The microbiota of the skin comprises all microorganisms, that are bacteria, fungi and viruses living on the skin and differs between different skin areas and between individuals. It can be influenced by diseases, e.g. skin diseases such as eczema, but also by diabetes mellitus, or by drugs, such as antibiotics. The microbiota contributes to a great extent to the immune system and therefore it plays an important role in the defense against harmful influences. These commensals living on the skin can modulate T cells or bind to immune receptors, controlling homeostasis and infections. The microbiota can also contribute to the xenobiotic metabolism, by e.g. acetylating foreign substances.

The role of skin bacteria was also studied in the context of azo colorants. Azo colorants are a frequently used group of synthetic dyes to stain textile and leather, but also plastics. In fact, essentially everything can be dyed with these colorants. From the late 1990s and the early 2000s it was investigated if certain bacteria, living on the skin and in the intestines, are able to reduce these dyes to, sometimes toxic, aromatic amines. Therefore, the azo colorants releasing a detectable concentration of these toxic aromatic amines, that is above 30 ppm, were banned. The manufacturing and sale of these dyes was not allowed in the European Union any longer (EU AZO Colorants Directive 2002/61/EC). PPD however, was not included in the list of toxic aromatic amines. Therefore, azo colorants releasing PPD are still manufactured and used. It is as yet unclear whether the amount of PPD that is released from cleavage of these colorants is high enough to cause actual sensitization. However, one can presume that sensitization to PPD can be a true cross reaction or co sensitization, due to the high similarity of the azo colorants with PPD or the concomitant exposure, which has already been explained in Chapter 4, or the cleavage products of these azo colorants. Therefore, we think that the role of the microbiota in contact sensitization should be investigated in more depth.

Another remarkable finding was the four fold increased chance of sensitization in hairdressers, as described in Chapter 2. Certainly, this finding can be related to a more increased exposure to hair dye products containing PPD compared to consumers. However, one can wonder whether the microbiota of the hands of a hairdresser also contribute to these higher odds of becoming sensitized. In our opinion hairdressers are more careful regarding the exposure to hair dye products than consumers. This means that they are more aware of the proper use of gloves and the exposure time of hair dye, than most consumers are. The composition of the microbiota of the skin is highly dependent on the location. Hence, the microbiota present on the scalp can differ to a great extent from the microbiota on the hands. It can be hypothesized, that sensitization through the skin of the hands occurs more easily than sensitization through the skin of the scalp, although the concentration of PPD to which the
hands of hairdressers are exposed is much lower than the concentration an individual is exposed to when dyeing hair. Whether the body location, in particular the microbiota at a specific location can also be the culprit in the easy sensitization to PPD through exposure to a black henna tattoo, deserves further research.

### Concluding remarks

Contact allergy to PPD is prevalent not only in the Netherlands but also in Europe as a whole. In the course of decades the prevalence of contact allergy to PPD has shown a consistent and stable character. Stricter regulations have not yet made a change for the better. In this research it is shown that various factors can contribute to the stable prevalence of PPD. Obtaining an insight into the genetic make-up of sensitized individuals as well as the individual’s microbiota are important factors in determining the sensitization risk. Moreover, there is also a psychological side to the use of PPD, i.e. that many individuals, who have been sensitized, still have a strong and irresistible desire to continue dyeing their hair.

The search for strategies minimizing the skin reactions from PPD containing products should go on, in order to prevent harmful reactions in sensitized individuals. Future research should not only monitor on the prevalence of PPD contact allergy, but also focus on possible exposure sources as well as the genetic polymorphisms that make an individual more prone to become sensitized. As the bacteria residing in the human body and on the human skin are of increasing importance in various diseases, medical-biological research on this topic in relation to contact allergy in general and PPD contact allergy in particular is warranted.
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