Scientific Committee on Consumer Safety

SCCS

OPINION ON

Allergy Alert Test (AAT) as a proof-of-concept study

The SCCS adopted the final Opinion by written procedure on 10 September 2019

ACKNOWLEDGMENTS

SCCS members listed below are acknowledged for their valuable contribution to the finalisation of this Opinion.

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This Opinion has been subject to a commenting period of a minimum eight weeks after its initial publication (from 13 May 2019 until 12 July 2019).
Comments received during this time period were considered by the SCCS. For this Opinion, comments received resulted in the following main changes: SCCS comments in sections 3.2.4, 3.3.2.1. and 3.3.2.5. These changes are not impacting the discussion and conclusion parts that remain unchanged.

1. ABSTRACT

The SCCS concludes the following:

(1)*In light of the study provided and the SCCP/1104/07 opinion, does the SCCS consider the new harmonized Allergy Alert Test (AAT), with the conditions listed above, a suitable test to provide a signal indicative of an allergic reaction to hair dyes when used by laypersons?*

The SCCS acknowledges the standardisation of the allergy test offered by the proposed AAT as a prerequisite for possible future use of such a test by consumers. Within the precision limits of the study, it has been demonstrated that the rating of the test results by a well-informed layperson corresponds well with that of a dermatology expert. However, as the study participants may have constituted a selective, more educated, or motivated subset of the general user population, the effectiveness of the test in terms of general applicability to all consumers still needs to be demonstrated.

The diagnostic performance indicates a very good specificity, but a potentially moderate sensitivity, leaving room for the possibility of false-negative AAT reactions in sensitised consumers who may then experience allergic contact dermatitis to a subsequently applied product.

In addition, the results provided by the study can only partly enable assessment of the benefit and risk of the AAT for the consumers, and the SCCS cannot, at this stage, endorse the use of the AAT as an "alert" for contact allergy to hair dyes in consumers.

(2)*Does the SCCS have any further scientific concerns regarding the AAT?*

The SCCS reiterates the concern expressed in the previous Opinion (SCCP/1104/07) that the AAT carries the same basic risk of sensitisation as the use of a hair dye product by a consumer. If the AAT is used on a regular basis before hair dyeing, it will inevitably increase the number of exposures, and this may increase the risk of sensitisation.

The SCCS would also like to reiterate from the previous Opinion SCCP/1104/07 to point out that the use of hair dye products on the skin and for *in vivo* diagnostic purposes is not covered by the Cosmetics Regulation. In this regard, the development and scientific evaluation of other screening methods for hair dye sensitisation (e.g. through a suitable, validated questionnaire) should be considered.

Keywords: SCCS, scientific opinion, Allergy Alert Test, AAT, proof-of-concept study, Regulation 1223/2009, SCCS/1607/19

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TABLE OF CONTENTS

2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Some hair dyeing products placed on the EU market contain the advice to assess skin sensitisation by performing a user test before dyeing the hair ("self-tests"). The exact protocol depends on the producer. In principle, the user applies the product to a small area of their skin and observes any signs of abnormal effects in the next 48 hours. Should they notice any such effects, they are advised to avoid using the hair dye and consult a medical professional.
In its opinion, SCCP/1104/07 - "Sensitivity to hair dyes-consumer self-testing", the SCCP concluded the following:
☐ *"There is a risk that "self tests" with hair dye products and with separate kits lead to misleading and false-negative results, thus giving individuals who are allergic to hair dye substances the false impression that they are not allergic or not at risk of developing an allergic reaction by dyeing their hair.*
☐ *There is potential risk that "self tests" result in induction of skin sensitisation to hair dye substances.*
☐ *Self-testing may offer protection to those individuals who perform the recommended test and develop a positive reaction. However, the proportion of hair dye chemical allergic individuals who do produce a positive reaction from this in vivo diagnostic test is unknown."*

According to the Applicant, so far no standardised approach to this type of test is in place. There is variation in the dose, site and duration of exposure. In addition, the consumer is expected to be able to perform the test and understand the alert signs.
The aim of the study in the current submission is to assess whether a defined self-test, referred to as Allergy Alert Test (AAT), can elicit a self-noticeable alert signal to a hair dye when performed by a layperson. Based on the study, the Applicant recommends the following conditions for the AAT:
☐ Site of application: forearm
☐ Product: hair colouring product mixed with the developer is equal volumes (1:1 ratio), whenever relevant
☐ Amount of product applied: pea-size, spread evenly in a thin layer across a 2x2 cm skin area
☐ Application condition: open (not occluded)
☐ Duration of application: 45 minutes, followed by rinsing
☐ Self-evaluation period: 2 days (48 hours)

The Applicant does not intend the AAT to be used for diagnosis of contact allergy.

Terms of reference

(1) *In light of the study provided and the SCCP/1104/07 opinion, does the SCCS consider the new harmonized Allergy Alert Test (AAT), with the conditions listed above, a suitable test to provide a signal indicative of an allergic reaction to hair dyes when used by laypersons?*

(2) *Does the SCCS have any further scientific concerns regarding the AAT?*

3. OPINION

The Applicant provided the SCCS with a study report ("FinalReport17.01.2018 PoC") that describes the details of the proof of concept study performed (1). This Final Report was used in developing the present Opinion. Part of the results have already been published (2). In this Opinion, the SCCS has included all the elements from the Final Report (1) that were deemed essential for the evaluation of the protocol proposed by the Applicant. Additional information from the Final Report is listed in the Annex "Additional information".

3.1 Scope and aim of AAT (Study Objectives)

From the Final Report on Allergy Alert Test Proof of Concept Study
The first objective of the study was to assess whether an Allergy Alert Test (AAT) can elicit a self-noticeable signal indicative of an allergic reaction to an ingredient in a hair dye product when applied by a potential hair dye-allergic consumer. By definition, an elicitation signal can only be generated in individuals that have previously been sensitised. Since PPD (*p*-phenylenediamine) is a relevant sensitiser among hair dye consumers and is most commonly used for diagnosis, it has been chosen as indicator hair dye for this study (3,4). Therefore, PPD allergic individuals with a history of hair-dye related contact dermatitis were selected to analyse the efficacy of an AAT. The control group consisted in PPD-negative hair dye consumers with no history of hair-dye related reactions.

The second objective was to analyse if elicitation reactions following AAT exposure (45 min simulating hair dye use conditions) were noticeable by the subjects themselves any time after application. It was assessed if consumer self-evaluation is feasible to indicate an alert for an allergic reaction to the hair dye product by comparing it with the dermatologist's evaluation. Dermatological evaluation was performed at day 2 (48 hours). In addition, the AAT response was also studied at day 4 (96 hours) or later to assess possible late reactions. Previously recommended application sites for an AAT (behind the ear; on the forearm) were comparatively assessed.

Application conditions were chosen to simulate hair dye use conditions, that is open application of hair colouring formula mixed with developer at typical in-use ratio for up to 45 min followed by wash off In addition, this was considered to avoid unnecessary exposure in terms of duration and concentration while maintaining reasonable sensitivity.

The third objective was to assess the AAT on subjects with different levels of reactivity to PPD as defined by patch test grades from weak (+) to extreme (+++). The aim was to study if subjects with stronger reactivity (indicating a higher risk of severe reactions to hair dyeing) are adequately alerted.

Furthermore, the AAT was assessed under conditions mimicking varying exposure scenarios of consumers in real life when exposed to different hair colour shades from light to medium to dark, corresponding to increasing PPD concentrations in the hair colour products. Therefore, PPD allergic subjects underwent AAT testing with experimental hair dye products containing increasing concentrations of PPD representative of average light (0.05%), medium (0.25%) and dark (0.75%) shades, respectively, as well as the highest allowed use concentration in the EU (2%). This was done to estimate the lowest experimental PPD concentration capable of eliciting an AAT reaction. When compared to the hair colour shade level declared to have caused the subject's contact dermatitis symptoms in the past, information was generated about which PPD concentration experimentally applied in the AAT corresponds to the colour shade that was thought to have caused past allergy symptoms.

3.2 INVESTIGATIONAL PLAN (Methods)

3.2.1 Overall study design and plan

From the Final Report on Allergy Alert Test Proof of Concept Study
The study is open, non-randomised and controlled. The protocol is similar to the protocol used in a previous study (5). Subjects with a history of allergic reactions to hair dyes and a proven allergy to PPD carry out the AAT with experimental products containing different concentrations of PPD corresponding to light, medium and dark shades of hair colourants. Positive results to the AAT by dermatological evaluation or self-evaluation is validated against a defined "gold standard" for hair dye allergy: the elicitation of clinical manifestations of allergic contact dermatitis by the application of a particular hair dye product in real life conditions. Test site evaluations by dermatologists and by study subjects carried out independently are compared.

The rationale of the study is to demonstrate that an AAT can provide a signal indicative of an allergic reaction in an individual likely to react to a hair colouring product. This is achieved by the comparison in each consumer of two eliciting concentrations:

- The estimated eliciting concentration in real life conditions (A). For this, the triggering hair colouring products are classified as belonging to light, medium and dark shades. The consumer recognises the eliciting group of shades using colour charts. These are declarative data.
- The estimation of the minimal concentration of PPD (B) able to elicit a reaction in the study conditions; this is achieved by the consecutive testing of hair colouring test products representative of the groups of shades from the lightest to the darkest. They contain increasing concentrations of PPD. These are experimental data.

At the end, the two eliciting concentrations are compared. The success criterion is met if the experimental eliciting concentration is lower or equal to the declared real-life eliciting concentration ($B \leq A$). In this case, it is assumed that that the AAT can give a signal indicative of allergy.

In parallel to the PPD-positive subjects, PPD-negative control subjects are tested with the AAT.

The study design is different for PPD-positive and PPD-negative subjects (see Figure below): In PPD-positive subjects, consecutive applications of the lightest to the darkest experimental product and of the control product are carried out until a clear positive reaction appears on day 2, as observed by both dermatological and self-evaluation. A doubtful reaction by dermatological grading is considered as an objectified reaction only if a clear positive reaction on day 2 is elicited by the next experimental product containing a higher concentration of PPD. Each PPD-negative subject is tested with only one experimental product and the control product. A diagnostic patch test to PPD is performed in PPD-positive subjects with a negative or doubtful reaction to Product D. A use test on a mini zone (10 cm^2) is carried out whenever possible in subjects negative to the group of shades declared as eliciting a reaction in a real-life situation. Number of subjects: It was planned to recruit 60 PPD-positive and 60 PPD-negative subjects, and to end with 40 – 50 valid subjects in each group. The sample size calculation was based on PASS Power Analysis and Sample Size Software 2008.

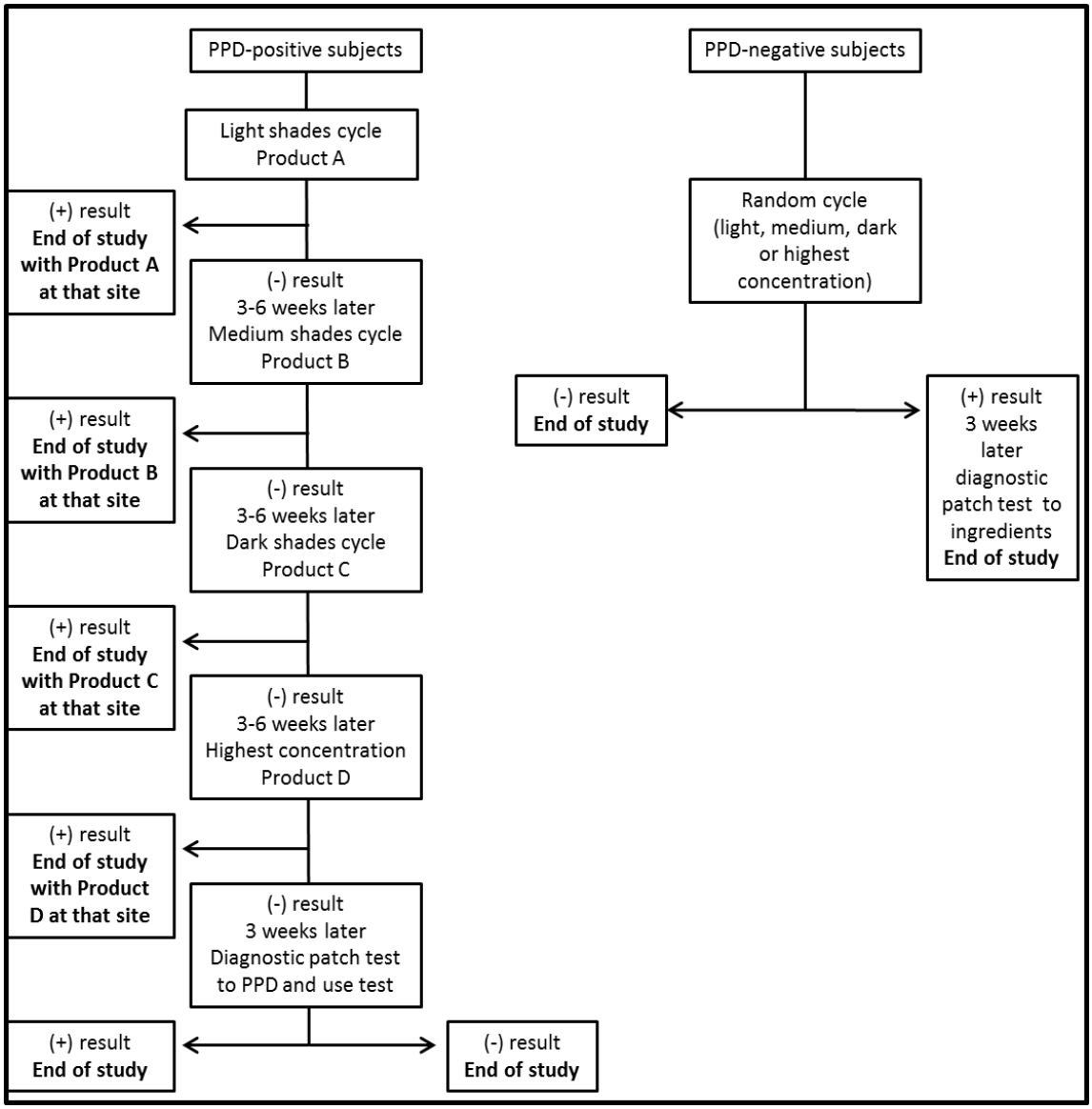


Figure 3.2.1 - 1: Study design and follow-up

Test products

For the purpose of the study, hair colouring products have been divided into 3 groups:

- Lighter shades: number 7-10 (blond, light blond, very light blond and lightest blond): mean PPD concentration 0.05% after mixing with developer.
- Medium shades: number 4-6 (brown, light brown and dark blond): mean PPD concentration 0.25% after mixing with developer.
- Darker shades: number 1-3 (black, darkest brown and dark brown): mean PPD concentration 0.75% after mixing with developer.

The test products, representative of the three groups of shades are formulated according to current hair dye technology. In addition to PPD all test products contain several other hair dye molecules commonly used in hair dye formulation.

- **Product A** is representative of the lighter shades.
- **Product B** is representative of the medium shades.
- **Product C** is representative of the darker shades.
- **Product D** corresponds to the maximum allowed concentration of PPD in Europe: 2% after mixing with the developer.

Control test material
Product Y: A hair colourant formulation without hair dye molecules, otherwise similar to products A, B, C and D. It will be applied simultaneously to these products after mixing with developer.

A marketed developer containing 6% hydrogen peroxide will be used for all formulations.

3.2.2 Discussion of study design

From the Final Report on Allergy Alert Test Proof of Concept Study
The study was carried out using PPD as a model allergen because there is a better possibility to recruit subjects allergic to PPD than subjects allergic to any other colourant. Indeed, PPD is tested in the standard series on all patients suspected to have contact sensitisation. It is a marker for sensitisation to para-substituted colourants. PTD (toluene-2,5-diamine) and a few other colourants are contained in the hairdressers' series and are used less frequently, therefore the possibility to recruit experimental subjects is lower. It should be noted that even when using PPD as a model allergen, the recruitment of a sufficient number of subjects in one single centre was not possible, hence the design of a multicentre study.

This was not a blinded study, as the application site of the experimental products A, B, C and D could be easily determined by the slight skin discolouration caused by the colourants, while the control product did not contain colourants and thus did not induce skin discolouration. The protocol used in the study excludes randomisation, as products are applied sequentially.

All experimental subjects underwent the applications with Product A through Product D until a clear-cut reaction (self-perceived by subjects and objectified by the dermatologist). The control group of subjects was approximately the same size as the experimental group. A criterion for assigning control subjects to the different experimental products was to match them in age (within 5 years) and sex to the subjects who had reacted to the products A, B, C and D. Each study centre had to apply the experimental products to approximately the same number of control subjects. There was no randomisation in the control group.

Wash out time: 3 – 6 weeks between two consecutive applications in PPD-positive subjects. This time is required to ensure that the skin on which the next experimental product will be applied is free from any alterations possibly induced by the previous product.

The purpose of the use of a control product not containing PPD in PPD-positive subjects was to ascertain that the observed reactions to the experimental products were allergen-specific, i.e. true allergic reactions and not false positive irritant reactions. The control group of PPD-negative subjects was used to verify the specificity of the test: indeed, an irritant experimental product may induce irritant reactions in control subjects that are not allergen-specific.

Follow-up:
- Patch testing with PPD of PPD patch test-positive subjects who did not react to Product D was planned to confirm that the subject is still sensitised to PPD. Indeed, the positive patch test to PPD prior to the inclusion in the study may have been carried out up to 5 years before the beginning of the study; the results may have been false positive (irritant reaction) or the sensitivity of the subject may have changed.
- Use test on mini-zones: The estimation of the real-life shade level in each subject relied on the cross reactions between PPD and other colourants. Indeed, the exact hair colouring product could not be determined in each subject. Since cross sensitivity

between PPD and PTD is important but is less than 100%, a confirmation step (use test) was planned to be carried out in subjects which did not fulfil the success criterion (the experimental eliciting concentration is lower or equal to the declared real life eliciting concentration). This step was also useful in the cases subjects did not remember exactly the shade level to which they had reacted in real life situation.

Different types of data were collected during the study:

- Group of shades recognised by subject: declarative data. The exact eliciting concentration and the primary intermediate (e.g. PPD or PTD) are unknown. Since the declared real-life eliciting concentration may be significantly higher than the experimental product representing the group of shades (products A, B and C) and since cross reactivity between PPD and PTD is not 100%, a use test is highly recommended in subjects in which success criteria are not met
- Severity of the clinical reaction: declarative data
- Diagnostic patch test results to PPD before and after the application of experimental products: experimental data
- Minimal eliciting concentration in AAT (4 concentrations representative of groups of shades): experimental data
- Use test with experimental product representative of the group of shades declared as eliciting in real life conditions: experimental data

The highest weighting was given to experimental data, as well as to the self-evaluation of subjects which are declarative data.

3.2.3 Selection of study population

See 3.2.7

3.2.4 Treatments

From the Final Report on Allergy Alert Test Proof of Concept Study

Table 3.2.4 – 1: PPD concentration:

	Product A	Product B	Product C	Product D
PPD in hair colouring formula (%)	0.1	0.5	1.5	4
PPD after mixing with developer (%)	0.05	0.25	0.75	2
Experimental product representative of	Light shades	Medium shades	Dark shades	Max allowed concentration in EU

Besides PPD all test products contain 2 secondary intermediates commonly used in hair dye formulations (resorcinol and 2-Methyl-5-hydroxyethylaminophenol):

Table 3.2.4 – 2: Secondary intermediates

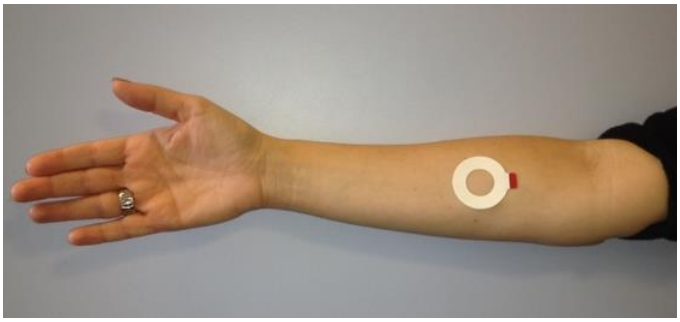
	Product A (%)	Product B (%)	Product C (%)	Product D (%)
PPD	0.1	0.5	1.5	4.0
Resorcinol	0.061	0.31	0.92	2.45
2-Methyl-5-hydroxyethylaminophenol	0.061	0.31	0.92	2.45

Control test material:
Product Y: A hair colourant formulation without hair dye molecules, otherwise similar to products A, B, C and D. It will be applied simultaneously to these products after mixing with developer. A marketed **developer** containing 6% hydrogen peroxide is used with all products. It is mixed extemporaneously with Products A, B, C, D and Y in a ratio 1:1.

Product application:
The experimental test product and the corresponding control product are applied openly behind the ears and on volar aspects of forearms according to a scheme shown below. The hair colouring formula is mixed extemporaneously with the developer.

A circular adhesive device (Monaderm) is used to delimit the test areas. 0.150 ml of each product is applied to the skin within the adhesive device using the supplied multipipette and combitips. Excess product is wiped off the combitip before product application. The surface of the treated skin is about 3.8 cm^2. The products are spread manually, using a vinyl glove to protect the hand. The adhesive devices are removed 45 minutes later. The test sites are marked with a Chemotechnique Diagnostics or a similar skin marker before removing the adhesive devices. Products are wiped off with a paper towel rinsed with water. Finally, the skin is wiped gently, without rubbing.

Applications sites:
Each experimental test product (A, B, C and D) and the corresponding control product Y are applied according to the scheme below. The location of each product and the type of application are reported for each subject. Volunteers with long hair are advised to wear hairpins and to take care not to wipe off the product.



	Left	Right
Forearm	Experimental products A➔B➔C➔D	Control product Y
Retro-auricular area	Experimental products A➔B➔C➔D	Control product Y

Figure 3.2.4.1 – 1: Experimental set-up

SCCS comment

The SCCS was informed by the Applicant that hair dye products would not include a Monaderm adhesive device for standardisation.

3.2.5 Efficacy

From the Final Report on Allergy Alert Test Proof of Concept Study
The efficacy of the AAT was evaluated from the following perspectives:

A) Consumer perspective
Positive AAT/reactivity: AAT **self-perceived by subject**, *reaction present 6 hours post-application and/or later*
Successful AAT/performance: Positive AAT to an experimental product with a concentration equivalent or lower than the mean concentration of declared real-life eliciting group of shades.
Probably successful: Negative AAT to experimental products with concentrations equivalent or lower than the mean concentration of declared real-life eliciting group of shades but subject not available for use test.

B) Broader perspective (dermatologist + subject)
Positive AAT/reactivity: AAT **self-perceived by subject***, reaction present 6 hours post-application and/or later* **and objectified by dermatologist** as a doubtful or positive reaction *at Day 2 and/or Day 4.*
Successful AAT/performance: Positive AAT to an experimental product with a concentration equivalent or lower than the mean concentration of declared real-life eliciting group of shades.
Probably successful: Negative AAT to experimental products with concentrations equivalent or lower than the mean concentration of declared real-life eliciting group of shades but subject not available for use test.

Self-perception by subjects was confirmed using a diary (auto-evaluation). Subjects performed auto-evaluation on all test sites once daily from Day 0 (15 minutes after product removal) to Day 5 and later if needed (see Annex).

Follow-up

- Whenever possible, a use test should be carried out on subjects who do not develop a positive alert test to the group of shades they have declared they have reacted to in real-life conditions. Hair on a limited surface of the scalp (10 cm^2) will be coloured with the experimental product and the control product will be applied on the contralateral side in the same conditions.
- A diagnostic patch test to PPD should be performed 3 weeks after a negative or a doubtful reaction to product D in PPD-positive subjects. It will be preceded whenever possible by a use test.
- A diagnostic patch test to PPD and to the other ingredients of the hair dye formulations should be carried out 3 weeks after a positive reaction to product A, B, C or D in control subjects. The ingredients will be tested at their usual diagnostic concentration or at product concentration.
- A diagnostic patch test to the ingredients of the control product should be carried out after 3 weeks in the case of a positive reaction to the control product in test subjects and in control subjects.

SCCS comment

The terms used for the first two outcomes "Positive AAT/reactivity" and "Successful AAT/performance" are plausible and relevant to assess the study objectives. However, "Probably successful" as defined in "successful AAT/performance" may have comprised an unknown share of false-negative participants. Hence, the SCCS does not agree to the interpretation that these subjects had a "probable" successful AAT.

3.2.6 Safety

From the Final Report on Allergy Alert Test Proof of Concept Study

All adverse events were collected through an adverse event form and subject withdrawals were described in a subject withdrawal form included in the protocol.

3.2.7 STUDY SUBJECTS

From the Final Report on Allergy Alert Test Proof of Concept Study

It was planned to recruit 60 PPD-positive and 60 PPD-negative subjects, to end with 40 – 50 valid subjects in each group. 50 PPD-positive subjects and 50 PPD-negative subjects were recruited. 46 PPD-positive and 48 PPD-negative subjects completed the Allergy alert test applications and readings.

The flowchart of PPD positive subjects from the recruitment to the end analysis is shown below. For discontinued subjects and subjects not taken into consideration in the efficacy analysis see listings in other Chapters.

The flowchart of PPD-positive subjects from the recruitment to the end analysis is shown below:

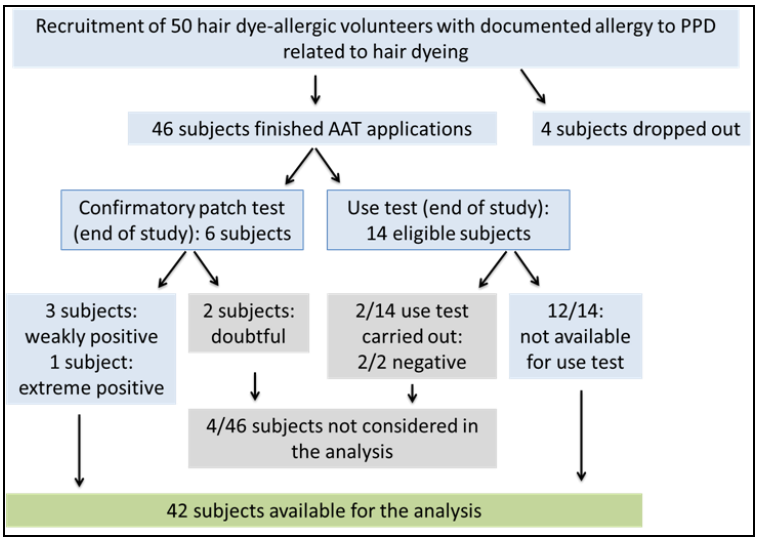


Figure 3.2.7 – 1: Flowchart of study subjects

SCCS comment
The SCCS has noted that most of the subjects (12/14) who were eligible were not available for the use test. The results of the AAT in these 12 subjects is therefore equivocal and this adds to the uncertainty of the study.

3.3 EFFICACY EVALUATION (Results with AAT)

From the Final Report on Allergy Alert Test Proof of Concept Study
The following were not taken into consideration in the efficacy analysis:

- 2 subjects who did not react to a confirmatory diagnostic patch test to PPD with a positive reaction. This confirmatory patch test was carried out on subjects who did not develop a positive AAT to Product D. The two subjects would not satisfy the recruitment criteria.
- 2 subjects who had a negative use test with the declared real-life eliciting shade level.

The remaining 42 subjects were included in the analysis (see Flowchart above)

Among the PPD-negative subjects, no subject was excluded from the analysis.

3.3.1 Demographic and other baseline characteristics

From the Final Report on Allergy Alert Test Proof of Concept Study
Demographic data of PPD-positive and PPD-negative subjects who completed AAT tests and readings are shown in an Appendix.

The declared group of eliciting shades for the PPD-positive subjects (see Annex, Table 3.3.1 – 1) were:

- Light shades: 5/46
- Medium shades: 19/46
- Dark shades: 22/46

Grade of positive patch test reactions to PPD on entering the study:

- (+): 8/46

- (++): 19/46
- (+++): 19/46

Severity of declared clinical reactions:

- Mild (reaction on scalp, possibly neighbouring skin): 9/46
- Moderate (involvement of scalp and oedema of neighbouring skin): 22/46
- Severe (with facial oedema): 10/46
- Very severe (with hospitalisation): 5/46

3.3.2 Efficacy results

3.3.2.1 Efficacy results of PPD-positive subjects with history of allergic reactions to hair dyes

From the Final Report on Allergy Alert Test Proof of Concept Study
Eliciting concentrations (Day 2) in 46 subjects. Reaction self-perceived by the subjects and objectified by a dermatologist.

Table 3.3.2.1 – 1: Eliciting concentrations

No	Real life eliciting product			Eliciting concentration behind the ear (%)	Eliciting concentration on forearm (%)
	Group of shades	Mean concentration of group of shades (%)	Concentration range of group of shades(%)		
IT08	Light	0.05	0.02 - 0.2	0.05	0.05
UK08	Light	0.05	0.02 - 0.2	0.05	0.05
DE02	Light	0.05	0.02 - 0.2	0.05	0.25
NL04	Light	0.05	0.02 - 0.2	0.05	0.25
NL15	Light	0.05	0.02 - 0.2	0.75	2
AU02	Medium	0.25	0.07 - 0.48	0.05	0.05
AU10	Medium	0.25	0.07 - 0.48	0.05	0.05
IT03	Medium	0.25	0.07 - 0.48	0.05	0.05
NL12	Medium	0.25	0.07 - 0.48	0.05	*0.05*
DE04	Medium	0.25	0.07 - 0.48	*0.05*	0.05
IT09	Medium	0.25	0.07 - 0.48	0.05	0.05
IT10	Medium	0.25	0.07 - 0.48	0.05	0.05
NL11	Medium	0.25	0.07 - 0.48	0.05	0.25
UK05	Medium	0.25	0.07 - 0.48	0.05	0.75
NL09	Medium	0.25	0.07 - 0.48	0.25	*0.05*
NL05	Medium	0.25	0.07 - 0.48	0.25	0.25
IT02	Medium	0.25	0.07 - 0.48	0.25	0.25
AU13	Medium	0.25	0.07 - 0.48	2	No reaction
NL06	Medium	0.25	0.07 - 0.48	0.25	0.75
AU06	Medium	0.25	0.07 - 0.48	0.75	2
AU05	Medium	0.25	0.07 - 0.48	2	2
UK01	Medium	0.25	0.07 - 0.48	2	2
NL14	Medium	0.25	0.07 - 0.48	No reaction	No reaction
UK02	Medium	0.25	0.07 - 0.48	No reaction	No reaction
DE03	Dark	0.75	0.37 - 2	0.05	0.05
DE05	Dark	0.75	0.37 - 2	0.05	0.05
IT01	Dark	0.75	0.37 - 2	0.05	0.05
AU14	Dark	0.75	0.37 - 2	0.05	0.05
AU15	Dark	0.75	0.37 - 2	0.05	0.25
AU11	Dark	0.75	0.37 - 2	0.05	0.25
UK07	Dark	0.75	0.37 - 2	0.25	0.25
NL02	Dark	0.75	0.37 - 2	0.25	*0.25*
AU08	Dark	0.75	0.37 - 2	0.25	*0.25*
NL08	Dark	0.75	0.37 - 2	0.25	0.25
AU12	Dark	0.75	0.37 - 2	0.25	0.75
UK03	Dark	0.75	0.37 - 2	*0.25*	*2*
AU01	Dark	0.75	0.37 - 2	0.75	0.75
AU07	Dark	0.75	0.37 - 2	0.75	0.75
AU09	Dark	0.75	0.37 - 2	0.25	0.25
NL01	Dark	0.75	0.37 - 2	*0.75*	2
AU03	Dark	0.75	0.37 - 2	0.75	2
AU04	Dark	0.75	0.37 - 2	2	No reaction
UK09	Dark	0.75	0.37 - 2	No reaction	No reaction
NL13	Dark	0.75	0.37 - 2	No reaction	No reaction
NL07	Dark	0.75	0.37 - 2	No reaction	No reaction
UK06	Dark	0.75	0.37 - 2	No reaction	No reaction

Table 3.3.2.1 – 2: Follow-up of subjects who did not react to declared real-life eliciting product (by use test) and/or to Product D (by diagnostic patch test to PPD)
Patch Tests

Subject number	Sex	Age	Severity of real life reaction	Patch test to PPD prior to inclusion in study	Real life eliciting shade	Concentration of eliciting group of shades (%)	Patch test to PPD (end of study)		Use test to declared real-life eliciting shade	Continues colouring with oxidation hair dyes
							1st reading	2nd reading		
NL07*	F	71	Moderate	+	Dark	0.75 (0.37 - 2)	Doubtful (Day 2)	Doubtful (Day 4)	Not done	Yes, with no or minor reactions
UK02	F	54	Mild	+	Medium	0.25 (0.07 - 0.48)	Negative (Day 2)	Weakly positive (Day 4)	Not done	Yes, with no or minor reactions
NL14	F	66	Moderate	+	Medium	0.25 (0.07 - 0.48)	Extreme positive (Day 3)	Strongly positive (Day 7)	Not done	Yes, with no or minor reactions
UK06	F	37	Moderate	++	Dark	0.75 (0.37 - 2)	Negative (Day 2)	Weakly positive (Day 4)	Not done	Yes, with a moderate reaction
NL13**	F	48	Mild	++	Dark	0.75 (0.37 - 2)	Weakly positive (Day 3)	Weakly positive (Day 7)	Negative (Product B 0.25% PPD)	Yes, with no or minor reactions (highlights only)
UK09*	F	69	Mild	+	Dark	0.75 (0.37 - 2)	Negative (Day 2)	Doubtful (Day 4)	Not done	Yes, with no or minor reactions
AU13**	F	18	Mild	+	Medium	0.25 (0.07 - 0.48)	Not done		Negative (Product C 0.75% PPD)	Yes, with no or minor reactions

* NL07 and UK09 patch tests would not satisfy the recruitment criteria; therefore the 2 subjects were not taken into consideration in the analysis.
** NL13 and AU13 had a negative use test with the declared real-life eliciting levels of shades and were not taken into consideration in the analysis.
Therefore 42 subjects are left available for the analysis.

SCCS comment
Of the 46 participants, 4 were removed for reasons stated above, based on the follow-up by confirmative tests. Two of the volunteers (NL07 and UK09), who were weakly sensitised prior to the study, showed doubtful reactions on day 4. The SCCS does consider these two participants (with negative AAT reactions) as weakly sensitised to PPD. Instead of excluding them from the study, they should have been considered as false-negatives in the AAT.

It is not explained why AU04 with a negative AAT on the forearm (like A13) had not been followed-up. Also, according to Table 3.3.2.1 – 2, in NL13 the "mini zones use test" had been performed with product B (0.25% PPD) and not product C (0.75% PPD) despite historic elicitation by a "dark" shade (0.75% mean PPD). In contrast, in Table 3.3.2.2 – 1, a use test for NL13 with an appropriate product (C) is mentioned, which was also negative. As clarified by the Applicant, subject NL13 was tested in use test with product C.

3.3.2.2 **Use tests on mini zones**

From the Final Report on Allergy Alert Test Proof of Concept Study
Follow-up of subjects in which the AAT was not successful with the product representative of the eliciting group of shades (positive or doubtful AAT by objective scores + self-perceived reaction by subject)

- Product corresponding to declared real-life eliciting group of shades applied on the hair of 10 cm^2 area to the left of the scalp; control product applied to the right.
- Contact time: 45 minutes
- Reading at D2 and D4 using the same scales as for AAT

Table 3.3.2.2 – 1: Use test in minizones results

Subject number	Sex	Age	Severity of real life reaction	Pt to PPD prior to inclusion in study	Pt to PPD end of study	Real life eliciting shade	Concentration of eliciting group of shades (%)	Positive AAT		Use test	
								Behind the ear	On forearm	Product tested	Result
AU13	F	18	Mild	+	Not done	Medium	0.25 (0.07 - 0.48)	Product D (2% PPD)	No reaction	B (0.25% PPD)	Negative
NL13	F	48	Mild	++	+	Dark	0.75 (0.37 - 2)	No reaction	No reaction	C (0.75% PPD)	Negative

3.3.2.3 **Overall results**

From the Final Report on Allergy Alert Test Proof of Concept Study
In 42 subjects available for the analysis:

- 38 subjects (90.5%) reacted on Day 2 on both test sites to one of the experimental products with a reaction which was both
  - Perceived by the subject
  - Objectified by dermatologist
- 1 subject (2.4%) reacted only on one test site (behind ear) with a perceived and objectified reaction
- 3 subjects (7.1%) did not react to any of the experimental products with a perceived and objectified reaction; they were followed up with a diagnostic patch test to PPD:
  - 2 subjects were negative on D2 and weakly positive on D4
  - 1 subject had a (+++) positive reaction on D3, (++) on D7

SCCS comment
The Applicant has proposed using the forearm as the site of application in the AAT, although the retroauricular skin seems to be slightly more sensitive. For instance, the results indicate that 39/42 subjects showed a positive AAT at the retroauricular site, i.e. 92.9% (95% CI: 80.5– 98.5%), compared to 38/42 subjects at the forearm, i.e. 90.5% (95% CI: 77.4– 97.3%). Also, the eliciting concentration is often lower at the retroauricular site. It needs to be clarified why the Applicant selected the forearm for the AAT.

3.3.2.4 **AAT reactivity/efficacy**

From the Final Report on Allergy Alert Test Proof of Concept Study
The AAT reactivity on the two test sites was compared; no relevant difference was found. The AAT reactivity on the two test sites was analysed in subjects with different grades of patch test reactions. AAT reactivity increases on both test sites with the increase of the patch test grade. All subjects with extreme positive patch test reactions (+++) to PPD reacted to some level of PPD in the experimental products. This means that most if not all strongly sensitized consumers will be alerted by the AAT.

Concentration dependency of positive AAT
The concentration dependency of AAT from consumer perspective and from a broader perspective is represented in another Chapter, for both sites. There small differences between the reactivity of the two sites which are shown with arrows. Most of the subjects react to Product A (0.05% PPD) and those who have not reacted to Product A react to products containing higher concentrations of PPD. Overall about 90% react on both sites, from consumer and from broader perspective.

SCCS comment
The SCCS does not agree with the Applicant that there were no differences in AAT reactivity on the two test sites. Table 3.3.2.1 – 1 clearly shows that in many volunteers the eliciting concentrations are lower where the AAT is performed on the retroauricular skin compared to the forearm. In 5 subjects (DE02, NL04, NL15, UK05, AU06), the eliciting shade in the AAT

was darker than the historically eliciting shade, based on results obtained on the forearm. It is also of note that the "success criterion" has not been met in 5 of 19 patients with +++ reactions. (The pre-study patch test reactions are shown in Table 3.3.1 – 1 in the annex)

3.3.2.5 **AAT performance**

From the Final Report on Allergy Alert Test Proof of Concept Study
The AAT performance was evaluated by comparing the estimated eliciting concentration in real life situation to the minimal eliciting experimental concentration. This was done from consumer perspective (AAT self-perceived) and from a broader perspective (AAT self-perceived and objectified).

From consumer perspective (reaction self-perceived), the AAT gave a signal indicative of an allergic reaction at the estimated use shade level or below in 83% of subjects behind the ear and in 74% of subjects on the forearm. From a broader perspective (reaction self-perceived by subject and objectified by dermatologist, the AAT performance was similar: 83% behind the ear and 71% on the forearm.

The AAT performance on the two sites was compared from consumer's perspective, from a broader perspective (self-perceived and objectified by a dermatologist) and from dermatologist's perspective only.

Comparisons between AAT performance behind the ear versus the forearm analysed by means of 2x2 tables shows that the rate of reactivity was statistically higher behind the ear only from dermatologist's perspective (McNemar's test, binominal). There was no statistically significant difference from consumer's perspective and from a broader perspective.

SCCS comment
The fact that the differences between retroauricular and forearm skin areas had only been noted, to a significant extent, by dermatologists may substantiate the notion of the retroauricular region being less suitable for the AAT. The self-perceived reaction on the forearm to a concentration that is equal to or lower than that in previously eliciting hair dye products is one primary outcome. The noted reactions in 31/42 participants would indicate a sensitivity of 73.8% (95% CI: 58.0– 86.1%), compared to reactions in 35/42 behind the ears, yielding a sensitivity of 83.3% (95% CI: 68.6– 93.0%).

However, out of all 46 participants (Table 3.3.2.1-1), only 27 had a self-perceived (and dermatologically confirmed) positive AAT on the forearm to a concentration less or equal to the mean concentration previously considered eliciting. As all self-perceived reactions on the forearm were verified by a dermatologist, this is equivalent to just considering "self-perceived" reactions. This reduces the sensitivity of this "success criterion" to just 27/42, i.e. 64.3% (95% CI: 48.0–78.4%). The Applicant should explain how a "success rate" of 31/42 concerning the forearm application was derived.

The PPD concentration in previously used eliciting products of a certain shade may have been in the upper range, i.e., above the "mean" concentration employed in the experimental product. Hence, a negative AAT reaction to that shade might not necessarily represent a false-negative result. Such potential "mismatch" of PPD concentrations adds uncertainty to the second outcome (fourth objective), putting all derivations and conclusions based on this outcome into question.

Based on results obtained in the retroauricular region (following dermatologists' rating), the proportion is 34/42, i.e. 81% (95% CI: 65.9– 91.4%). Thus, if the dermatologist's evaluation is considered, and not self-perceived AAT reactivity to any shade, sensitivity in

the retroauricular region is significantly higher (p=0.016, exact McNemar test). Clarification is therefore needed why the Applicant selected the forearm as the preferred skin site for the AAT.

3.3.2.6 **AAT performance in subjects with different patch test grades**

From the Final Report on Allergy Alert Test Proof of Concept Study
AAT performance was further analysed taking into consideration the grade of the patch test reaction to PPD of the subjects. As for the reactivity, the AAT performance was highest for the subjects with stronger patch test reactions to PPD. A further analysis was carried out taking into consideration the requirement of a confirmation step (use test) in subjects which did not fulfil the success criterion (the experimental eliciting concentration is lower or equal to the declared real-life eliciting concentration).

14 subjects should have undergone this confirmatory use test. Only two subjects (AU13 and NL13) accepted to participate in this part of the study and the use test was negative in both of them. This means that the experimental product that was applied in the AAT could not elicit allergic contact dermatitis of the scalp at the time of the study, therefore the negative AAT successfully predicted the absence of risk of an allergic reaction to the product to be used.

For the remaining 12 subjects who were not available for the use test, it was considered that there is a high likelihood that the results would have been the same as in subject AU13 and NL13, ie negative. These AATs termed "probably successful" are shown in another Chapter. It is noteworthy that a definitely unsuccessful AAT was not shown in any of the subjects.

SCCS comment
The SCCS considers it inconsistent to exclude AU13 and NL13 due to a negative verification (use) test, but quote this as a supporting result for a good negative predictive value. The Applicant's conclusion that a "definitely unsuccessful AAT" was not shown in any of the subjects is not supported by the results. It is possible that the AAT would have been unsuccessful in the 12 subjects for whom a use test is lacking. The SCCS does not agree that these cases can be considered "probably successful" and regards them as potential false-negatives in the AAT.

3.3.2.7 **Comparison of judgment of the AAT reaction by a dermatologist and by the subject**

From the Final Report on Allergy Alert Test Proof of Concept Study
The judgement of the AAT reactivity and performance on the two test sites by a dermatologist and by the subjects was compared. Comparisons between dermatologist's and consumer's judgement analysed by means of 2X2 tables did not show any statistically significant difference regarding AAT reactivity and AAT performance when the two test sites were analysed separately.

Efficacy results of PPD-negative subjects with no history of allergic reactions to hair dyes: All control subjects evaluated the AAT to the experimental products as negative. One subject had a transient itch on both test sites while the product was on, which settled before the Day 0 reading. Another subject had a weak spotty erythema at Day 0 reading that remained unnoticed by him.

Tabulation of individual response data: Individual response data of PPD-positive and PPD-negative subjects are provided in an Appendix.

3.3.3 Efficacy conclusions

From the Final Report on Allergy Alert Test Proof of Concept Study
SUMMARY
In 42 PPD-positive subjects available for the analysis:

- 38 subjects (90.5%) reacted on Day 2 on both test sites to one of the experimental products with a reaction which was both
  - Perceived by the subject
  - Objectified by a dermatologist
- 1 subject (2.4%) reacted only on one test site (behind ear) with a perceived and objectified reaction.
- 3 subjects (7.1%) did not react to any of the experimental products with a perceived and objectified reaction; they were followed up with a diagnostic patch test to PPD:
  - 2 subjects were negative on D2 and weakly positive on D4.
  - 1 subject had a (+++) positive reaction on D3 and (++) on D7.

All control subjects evaluated the AAT to the experimental products as negative.

No adverse events related to the application of the experimental or control products were reported.

(Additional presentations of results can be found in the Annex)

Table 3.3.3 – 1: Comparison of judgement 'dermatologist vs. self-perceived' on the forearm

a) AAT reactivity (positive AAT to any product at Day 2)

		Dermatologist reading (positive or doubtful)	
		+	-
Self-perceived	+	38	0
	-	0	4

b) Meets success criteria

		Dermatologist reading (positive or doubtful)	
		+	-
Self-perceived	+	30	1
	-	0	11

Comparisons between dermatologist's and consumer's judgement analysed by means of 2X2 tables did not show any statistically significant difference regarding AAT reactivity and AAT performance when the two test sites were analysed separately.

CONCLUSIONS:

- Open testing under realistic hair dye use conditions (AAT design) was efficient to cause a reaction noticeable by the majority of study subjects (39/42 subjects available for analysis) within 48 hours. This was objectified by dermatological evaluation.
- The dermatological evaluation did not find significant differences between Day 2 and Day 4. Therefore, a self-evaluation period of 2 days is feasible.
- Comparison of the two test sites (response rate of 90% on forearm and 93% behind the ear) did not reveal statistically significant differences, both by self-assessment and when combined with a dermatological assessment.

- All subjects (19/19) with the highest reactivity to PPD (+++) already showed a reaction to PPD concentrations between 0.05 and 0.75% in the AAT, indicating that they would be adequately alerted to avoid hair dyeing with the product tested.
- The evaluation of the "performance" of the AAT (eliciting PPD concentration in experimental product corresponds to color shade level declared causative for past allergy symptoms) is mainly dependent on the declarative data. The AAT performance was highest for the subjects with stronger patch test reactions to PPD. Based on the performance criteria, the AAT was considered "predictive" in 35/42 subjects behind the ear and in 31/42 subjects on the forearm. There was no statistically significant difference in the AAT performance behind the ear and on the forearm from the consumer's perspective and when confirmed by a dermatologist's evaluation.

SCCS comment
If the outcome of self-perceived reaction as verified by a dermatologist on the forearm is considered, the number of positive AAT reactions was 38/42 (see 3.3.2.3). The equivalence of readings on day 2 and 4 has not been illustrated in the results, e.g. by a cross-tabulation of day 2 vs. day 4 results of self-perceived and dermatologist-evaluated reactions, respectively. The concordance between dermatologists and participants rating of a "success criterion" based on forearm results is, according to Cohen's kappa 0.94 (95% CI: 0.82–1.0), i.e. almost perfect (6).

All participants with an extreme positive (+++) patch test reaction to PPD (shown in Table 3.3.1 -1 [Annex]) had a positive reaction to the AAT both on the forearm and in the retroauricular region, confirming their sensitisation status. However, 5 of these 19 participants did not meet the "successful AAT/performance" criterion based on forearm results. If retroauricular results (Table 3.3.2.1-1) were considered, the number of failures with regard to the "successful AAT/performance" criterion would be reduced to 2/19 (NL15 and AU06). In view of a higher sensitivity in the retroauricular region, there is a need to explain why the forearm region has been chosen for the intended real-life application.

3.4 SAFETY EVALUATION

From the Final Report on Allergy Alert Test Proof of Concept Study
No adverse events related to the application of experimental Products A, B, C and D or to the control Product Y were observed. No serious adverse events were observed. No safety issues related to the AAT procedure, experimental and control products were observed in PPD-positive subjects with a history of allergic reactions to hair colouring products and in control PPD-negative subjects.

(see also Annex)

SCCS comment
The possibility that the controls who were previously not sensitised to PPD may have been sensitised by the experimental application (AAT) has not been addressed in this study. In the small control group, it would probably be unlikely that this would happen following a single exposure. In real life, however, such a safety concern would exist where the use of hair dyes would be regular and repeated, and if consumers were regularly performing self-testing. Therefore, this study cannot provide sufficient scientific grounds to make conclusions on the potential risk of induction of skin sensitisation to hair dye substances by the AAT itself.

3.5 General Discussion

The Applicant has provided a full description of the highly standardised study protocol used for a specific, well-defined approach to the AAT. In view of a multitude of hitherto existing variations of the AAT (7)(8), standardisation efforts are welcome.

The study had four objectives. The first objective of the study was to assess whether an AAT can elicit a self-noticeable signal indicative of an allergic reaction to an ingredient in a hair dye product when applied by a potential hair dye-allergic consumer. The results show that the AAT can elicit a positive reaction in participants allergic to PPD. Sensitivity, in terms of the proportion of positive AATs among those sensitised, is good. The retroauricular area seems to be more sensitive than the volar area on the forearm. A clarification is needed why the forearm is selected for the AAT by the Applicant.

The second objective was to analyse if elicitation reactions following AAT exposure noticeable by the subjects themselves could be verified ("objectified") by a dermatologist. The results demonstrate a very good concordance between self- and expert-evaluated allergic skin reactions. Hence, the participants were able to recognize a positive AAT reaction.

The third objective was to assess the AAT on subjects with different levels of reactivity to PPD as defined by patch test grades from weak (+) to extreme (+++). The aim was to study if subjects with stronger reactivity (indicating a higher risk of severe reactions to hair dyeing) are adequately alerted. For this objective, the sensitivity is less satisfactory.

The fourth objective was to assess the AAT under conditions mimicking varying exposure scenarios of consumers in real life when exposed to different hair colour shades from light to medium to dark, corresponding to increasing PPD concentrations in the hair colour products. To this end, a "successful AAT/performance" criterion had been defined, indicating "success" if the shade eliciting in the AAT was the same as or a lighter shade as the one eliciting dermatitis after previous hair dye product application. Of note, based on forearm results, such-defined success was achieved in 27/42, i.e. 64.3% (95% CI: 48.0–78.4%).

An important aspect for the appraisal of results obtained in a study sample, such as the present AAT study, is the precision of results. To this end, the SCCS has calculated interval estimates (confidence limits or confidence intervals [CIs]) to the proportions observed in the study. Depending on the respective outcome, the CIs illustrate that despite a very reasonable sample size for a study of this type there is some imprecision regarding the true sensitivity. This is illustrated by the observation of the "success criterion" (concentration in historical shade ≤ concentration in AAT) having been met by only 27 of the 42 allergic volunteers, ie, 64.3%, 95% CI: 48.0 – 78.4, see above. Even if the calculation of a success in 31 of 42 "forearm AAT positive" instead of 29 would indicate a sensitivity of 73.8% (95% CI: 58.0 – 86.1%): the confidence interval includes the possibility that almost every second participant will have a false-negative AAT result.

Furthermore, it is unclear to which extent the high level of standardisation achieved in the study presented can be transferred to the intended use in consumers. This aspect of effectiveness of the AAT (in contrast to the efficacy proven under study conditions) should address the reliability of the description of the AAT for consumers. Study participants should ideally include different countries/languages and different social classes. The present sample may constitute a selected subgroup of these, potentially being more educated and motivated – and better instructed in a study context than by leaflets in products or other means of promoting AAT instructions in a real-world setting. For instance, a circular adhesive device had been used to delineate the study area on the skin – how would a reliable description of the area of application be achieved in a real-world context? Moreover, the degree of practical adherence to the suggested procedure should be examined, for

instance, whether consumers actually wait for 2 days for possible skin reactions to appear before they continue to dye their hair. Furthermore, studies addressing effectiveness e.g. by following-up consumers using the AAT with regard to not developing allergic contact dermatitis of the scalp/head by application of the corresponding product (presumably usually after a negative AAT) could be conducted. These should ideally include prior information on known sensitisation or previous hair dye product reactions from the consumer/participant, and possibly also a head-to-head comparison of the effectiveness of the AAT with a history-based questionnaire screening approach; the latter possibly similar to the questionnaire items used in the present study to derive information on the inclusion of "positive" subjects. Such a study would supplement the present results, which focussed on sensitivity and specificity, by providing an important estimate of the predictive values of the AAT (see below) under real-life conditions. Indeed, the predictive values of the AAT cannot be estimated in a "case control" setting, where the prevalence of contact allergy is fixed by design.

Finally, the SCCS reiterates the concern expressed in the previous Opinion (SCCP/1104/07) that the AAT carries basically the same risk of sensitisation as when using an oxidative hair dye product. If the AAT is used on a regular basis before hair dyeing, the number of exposures is increased. Moreover, as written in SCCP/1104/07, the SCCS wishes to point out that the use of hair dye products on the skin and for *in vivo* diagnostic purposes is not covered by the current Cosmetics Regulation, even if it is called an "alert" instead of "diagnostic test". The development and scientific evaluation of alternative screening methods for hair dye sensitisation (e.g. by use of a suitable, validated questionnaire) is encouraged.

4. CONCLUSION

1. *In light of the study provided and the SCCP/1104/07 opinion, does the SCCS consider the new harmonized Allergy Alert Test (AAT), with the conditions listed above, a suitable test to provide a signal indicative of an allergic reaction to hair dyes when used by laypersons?*

The SCCS acknowledges the standardisation of the allergy test offered by the proposed AAT as a prerequisite for possible future use of such a test by consumers. Within the precision limits of the study, it has been demonstrated that the rating of the test results by a well-informed layperson corresponds well with that of a dermatology expert. However, as the study participants may have constituted a selective, more educated, or motivated subset of the general user population, the effectiveness of the test in terms of general applicability to all consumers still needs to be demonstrated.

The diagnostic performance indicates a very good specificity, but a potentially moderate sensitivity, leaving room for the possibility of false-negative AAT reactions in sensitised consumers who may then experience allergic contact dermatitis to a subsequently applied product.

In addition, the results provided by the study can only partly enable assessment of the benefit and risk of the AAT for the consumers, and the SCCS cannot, at this stage, endorse the use of the AAT as an "alert" for contact allergy to hair dyes in consumers.

2. *Does the SCCS have any further scientific concerns regarding the AAT?*

The SCCS reiterates the concern expressed in the previous Opinion (SCCP/1104/07) that the AAT carries the same basic risk of sensitisation as the use of a hair dye product by a consumer. If the AAT is used on a regular basis before hair dyeing, it will inevitably increase the number of exposures, and this may increase the risk of sensitisation.

The SCCS would also like to reiterate from the previous Opinion SCCP/1104/07 to point out that the use of hair dye products on the skin and for *in vivo* diagnostic purposes is not covered by the Cosmetics Regulation. In this regard, the development and scientific evaluation of other screening methods for hair dye sensitisation (e.g. through a suitable, validated questionnaire) should be considered.

5. MINORITY OPINION

/

ANNEX: additional information

For convenience, the numbering of this Annex follows the order from the text of the Opinion.

Additional information not essential for the appraisal of the study methods and results have been submitted by the Applicant, which shall not be repeated and commented for the purpose of the present opinion.

3.2.3 Selection of study population

3.2.3.1 Inclusion criteria

From the Final Report on Allergy Alert Test Proof of Concept Study
60 PPD-positive and 60 PPD-negative subjects were planned to be recruited to end with 40 – 50 valid subjects in each group. 46 PPD-positive and 48 PPD-negative subjects completed the Allergy alert test applications.

Inclusion criteria for PPD-positive subjects:

- Consecutively patch tested patients, males and females, 18-72 years of age, who have shown positive reactions to PPD (+, ++ and +++) within the last 5 years included in the Department's database; possibility to also recruit from neighbouring dermatological departments.
- Clinical relevance of the positive patch test reaction: self-declared past exposure to oxidation hair dyes and clinical manifestations compatible with contact sensitivity to hair dyes (documented in CRF). All subjects were required to provide information on the approximate shade (light, medium or dark) responsible for their reaction. Colour charts were provided to help them. Severity of hair colourant-associated allergic contact dermatitis was quantified.
- Healthy skin on the test site for at least 3 months on entering the study;
- Completed written informed consent form. A copy of the informed consent form and the subject information sheet was submitted to the Ethics committee.

Inclusion criteria for PPD-negative subjects:

- Consecutively patch tested patients, males and females, oxidative hair dye consumers, 18-72 years of age, who have shown negative reactions to PPD and to all other tested allergens (tests carried out 12 months to 3 weeks before inclusion into the study);
- Sex and age-matched (within 5 years), whenever possible, to test subjects;
- No history of adverse reactions to hair colouring products;
- Healthy skin on the test site for at least 3 months;
- Completed written informed consent form. A copy of the informed consent form and the subject information sheet was submitted to the Ethics committee.

3.2.3.2 Exclusion criteria for PPD-positive and PPD-negative subjects

- Hairdressers;
- Current acute or widespread eczema at any site, any eczema on the test sites within the last 3 months before study;
- Significant past medical history which in the opinion of the Investigator can interfere with the study;
- Febrile illness lasting more than 24 hours in the six days prior to each patch application;

- Past or concomitant medication likely to affect the response to the test articles or confuse the results of the study (systemic treatment: corticosteroid or immunosuppressive 1 month prior and during the study);
- Recent vaccination (less than 3 weeks prior to patch application);
- Insulin-dependent diabetes;
- Recent history of extensive sun exposure;
- Deliberate exposure of the test sites to natural sunlight or artificial sources of UV light in the two weeks preceding the study, during the study, and during the two weeks following the study;
- Participation in a diagnostic patch test during the six preceding weeks for PPD-positive subjects and during the three preceding weeks for PPD-negative subjects;
- Pregnancy/wish or breast feeding.

3.2.3.3 Removal of subjects from study

From the Final Report on Allergy Alert Test Proof of Concept Study
The participation of the subjects in this study may be discontinued for any of the following reasons:

- the subject wishes to withdraw,
- intercurrent illness,
- violation of the prohibitions and restrictions, described in the Patient Information Sheet, non-respect of the inclusion and exclusion criteria, development of any condition considered in the non-inclusion criteria, serious adverse event.

Subjects are free to withdraw without prejudice at any time and do not need to give a reason. In the case of premature withdrawal of a subject, the Investigator will fill in a Subject Withdrawal Form which will be restituted to the sponsor at the end of the study.

3.2.4.2 Identity of investigational products

From the Final Report on Allergy Alert Test Proof of Concept Study

Table 3.2.4.2 – 1: Internal product and batch codes

	Product A	Product B	Product C	Product D	Product Y	Developer
Formula No	1086947	1086948	1086949	1086950	1086946	178914
Batches	RAL20031 07 February 2014	RAL20032 07 February 2014	RAL20033 10 February 2014	RAL20034 10 February 2014	RAL20030 6 February 2014	RAL20019
	RAM20035 27 January 2015	RAM10065 22 january 2015	RAM20036 23 January 2015	RAM20034 22 January 2015	RAM10064 22 January 2015	RAM40015

Stability of the investigational products (pH, viscosity, general aspect and organoleptic features) as well as the concentrations of the three colourants, were verified by the labs before shipment to the study centers and at regular intervals thereafter. All parameters were found stable throughout the study. PPD-positive experimental subjects treated with the different batches are listed in an Appendix.

3.2.4.3 Method of assigning subjects to treatment groups

From the Final Report on Allergy Alert Test Proof of Concept Study
No specific method of assignment was used. The study was not blinded, as the application site of the experimental products A, B, C and D could be easily determined by the slight skin discolouration caused by the colourants, while the control product did not contain colourants

and thus did not induce skin discolouration. All experimental subjects underwent the applications with Product A through Product D until there was a clear-cut reaction using the same protocol, therefore there was no need of randomisation. The controls group of subjects had approximately the same size as the experimental group. A criterion for assigning control subjects to the different experimental products was to match them in age (within 5 years) and sex to the subjects that had reacted to the products A, B, C and D. Each study centre had to apply the experimental products to approximately the same number of control subjects. There was no randomisation in the control group and experimental products were allocated within sites for the control subjects. Treatment assigned to the PPD-negative subjects are shown in Appendix

3.2.4.4 Selection of concentrations in the study

From the Final Report on Allergy Alert Test Proof of Concept Study
For the purpose of the study, hair colouring products have been divided into 3 groups:

- Lighter shades: number 7-10 (blond, light blond, very light blond and lightest blond): mean PPD concentration 0.05% after mixing with developer.
- Medium shades: number 4-6 (brown, light brown and dark blond): mean PPD concentration 0.25% after mixing with developer.
- Darker shades: number 1-3 (black, darkest brown and dark brown): mean PPD concentration 0.75% after mixing with developer.

- **Product A** is representative of the lighter shades.
- **Product B** is representative of the medium shades.
- **Product C** is representative of the darker shades.
- **Product D** corresponds to the maximum allowed concentration of PPD in Europe: 2% after mixing with the developer.

Table 3.2.4.4 – 1: Concentration of PPD in products A to D

	Product A	**Product B**	**Product C**	**Product D**
PPD in hair colouring formula (%)	0.1	0.5	1.5	4
PPD after mixing with developer (%)	0.05	0.25	0.75	2
Experimental product representative of	Light shades	Medium shades	Dark shades	Max allowed concentration in EU

3.2.4.5 Blinding

The study was not blinded (see above)

3.2.4.6 Prior and concomitant therapy

From the Final Report on Allergy Alert Test Proof of Concept Study
Prior therapies (30 days preceding the study) and concomitant therapies were listed in the "Prior and concomitant medications" form (Appendix) at baseline and during the following visits.

3.2.4.7 Treatment compliance

From the Final Report on Allergy Alert Test Proof of Concept Study

The treatments were administered in the study centres and did not require subject compliance. The diaries were checked for self-evaluation at the required time points.

3.2.5 Efficacy

Self-perception by subjects was confirmed using a diary (auto-evaluation). Subjects performed self-evaluation on all test sites once daily from Day 0 (15 minutes after product removal) to Day 5 and later if needed (see Annex).

The diary is reviewed by the investigator and the chief findings are transferred in the case report form. The reading scale for the dermatological evaluation is based on the scoring method proposed by Johansen et al. (9) and used in previous open test validation studies (3,5) (See diagram for test reading). This method comprises: i) The dermatologist's clinical evaluation of the severity of the reaction, "overall clinical impression". The overall clinical impression is recorded using a six-point grading scale: negative, doubtful, weakly positive, moderately positive, strongly positive and irritant reaction; ii) An objective evaluation using a set of objective parameters, graded individually (erythema, infiltration, vesicles). Sensory manifestations reported by the subjects are also taken into account.

Diagram for test reading.

I. Overall clinical impression	Negative	Doubtful	Weakly positive	Moderately positive	Strongly positive
II. Involved area of application	0 (0)	1-24% (1)	25-49% (2)	50-89% (3)	90-100% (4)
III. Erythema	Involvement	None (0)	Spotty (1)	Homogeneous (2)	
	Strength	Weak (1)	Medium (2)	Strong (3)	
IV. Papules / infiltration	None (0)	Few : one or two (1)	Some : three or four (2)	Many : five or more (3)	Homogeneous infiltration (4)
V. Vesicles	None (0)	Few : one or two (1)	Some : three or four (2)	Many : five or more (3)	Confluent (4)
VI. Sensory manifestations	Burning/stinging (Yes/No)		Itching (1)		
VII. Total score					

3.2.5.1 Efficacy

Table 3.2.5.1 – 1: Diagram for self-evaluation

Overal test evaluation	Positive ☐			Negative ☐	
Did you notice any reaction developing at test site	Yes ☐	No ☐	If yes, how long after test application did it appear	.....hours	.....days
Itching	Yes ☐	No ☐	If yes, how long after test application did it appear	.....hours	.....days
Redness	Yes ☐	No ☐	If yes, how long after test application did it appear	.....hours	.....days
Swelling	Yes ☐	No ☐	If yes, how long after test application did it appear	.....hours	.....days
Oozing	Yes ☐	No ☐	If yes, how long after test application did it appear	.....hours	.....days
Did you notice any other manifestation (please describe)	Yes ☐	No ☐	If yes, please describe…… …… ……		

Table 3.2.5.1 – 2: The visits for PPD-positive subjects are shown on the following Table:

		Visit No	
	Screen	1	
Products A and Y	Day 0	2	Application
	Day 2	3	Reading
	Day 4	4	Reading
Products B and Y	Day 0	5	Application
	Day 2	6	Reading
	Day 4	7	Reading
Products C and Y	Day 0	8	Application
	Day 2	9	Reading
	Day 4	10	Reading
Products D and Y	Day 0	11	Application
	Day 2	12	Reading
	Day 4	13	Reading
Follow-up if required (Patch test/use test)	Day 0	14	Application
	Day 2	15	Reading
	Day 4	16	Reading

Table 3.2.5.1 – 3: The visits for PPD-negative subjects

		Visit No	
	Screen	1	
Products A, B, C or D and Y	Day 0	2	Application
	Day 2	3	Reading
	Day 4	4	Reading
Follow-up if required (Patch test/use test)	Day 0	5	Application
	Day 2	6	Reading
	Day 4	7	Reading

3.2.6 Data quality assurance

From the Final Report on Allergy Alert Test Proof of Concept Study
Prior to the start of the study, a meeting with all investigators and their assistants was organised. During this meeting, there was a presentation and training of the application protocol (pipette use etc.) was provided. A presentation and training was also provided on how the different forms (CRF, etc.) should be filled in and communicated. In addition, training and presentations covering the aims and goals of the study were again provided during the first visit of all centres. These steps were taken to prepare investigators and to standardise performances. Furthermore, each centre was visited during the study phase to reassure quality of data assessment.

The monitor verified during every single visit that the materials were adequately stored in order to maintain the quality of all test materials and fresh test material was provided through the sponsor. Data storage and storage of the original data sheets were evaluated during the monitor's visits. Further, data collection and reporting of adverse reactions were evaluated throughout the study. The investigators were advised that all serious adverse events (SAEs) must be reported within 24 hours to the sponsor as specified in the study protocol. No SAEs were recorded during the study.

Furthermore, specific safety surveillance measures of non-SAEs were in place and monitored. Overall low complaints were recorded, indicative of the good safety profile. Monitoring reports are provided in an Appendix. Inter-center standardisation methods and quality assurance procedures are provided in another Chapter.

3.2.7 Statistical methods planned in the protocol and determination of sample size

3.2.7.1 Determination of sample size

From the Final Report on Allergy Alert Test Proof of Concept Study
Originally it was intended to enroll 60 PPD negative control subjects and 60 PPD-positive subjects. Group sample size was set at a maximum of 4, achieving 80% power to detect an odds ratio of 0.002 in a design with 3 repeated measurements when the proportion from the control group is 0.917, the correlation between observations on the same subject is in the range of 0.10000 and 0.95, and the alpha level is 0.05. The proportion is based on the assumption that one subject at most (1.7%) in the control group will react positive to the skin self-test and that the majority in the PPD positive group (91.7%) will react, resulting in a proportion of 0.02.

Due to the multilevel technique for repeated measures, we intended to include at least 50 subjects (10–12). The sample size calculation is based on PASS Power Analysis and Sample Size Software 2008. An additional pre-study sample size calculation was performed based on the assumption that 45 participants could be enrolled in each group (45 PPD positives and 45 PPD negative controls), whereby only 1 subject in the PPD group would not react, and 5 would react in the control group. With alpha 0.05 and power 0.8 the OR would be 0.003. The calculated 95% confidence interval of the OR shows that a sample size of 45 versus 45 would be large enough to demonstate a significant difference between the two groups.

3.2.7.2 Changes in the conduct of the study or planned analyses

From the Final Report on Allergy Alert Test Proof of Concept Study
An interim analysis was performed when 30 PPD-positive participants were enrolled and finished with their test-cycles, in order to check whether the planned sample size of 45 in each group would still be valid. In this group of 30 PPD-positive (who had also had a reaction after hair-dying) there were 2 who did not show a positive test reaction. An

assumption was made that in the control group, 2 participants would show a positive reaction.
The calculated OR would be 0.005. Calculation with SPSS SamplePower (test: Chi²) would yield a sufficient power of almost 100% to reject the null-hypothesis that that there are no differences between the two groups in reactivity to the test procedure. Based on the interim-analysis, it was decided to stop the enrolment of new participants when there were 40 in each group. The analyses would then be entirely performed within the group of PPD positive subjects with a history of reactions to a hair-dying procedure.

The analyses would be descriptive and include:

- Comparison of reactivity on forearm with reactivity behind ear
- Comparison of judgment of the test reaction by a dermatologist and judgment by the participant
- Comparison of declared reaction to hair-dye with the reactivity to tested colour shade
- Comparison of the strength of the original PPD patch-test and the reactivity to the tested colour shade

3.2.8 Study subjects

The recruitment in the different study centres and the participation in the different test procedures are shown in the following table:

Table 3.2.8 – 1: Recruitment in the different study centers and the participation in the different test procedures

Study centers	Number of PPD-positive subjects					Number of PPD-negative subjects		
	Having entered the study	Having finished the AAT tests	Drop-outs	Patch test to PPD at the end of the study	Use test at the end of the study	Having entered the study	Drop-outs	Having finished the AAT tests
Groningen	15	13	2	3	1	17	0	17
Heidelberg	5	4	1	0		2	1	1
Sheffield	9	8	1	3		10	0	10
Graz	15	15	0	0	1	15	1	14
Rome	6	6	0	0		6	0	6
Total	50	46	4	6		50	2	48

3.3.1 Demographic and other baseline characteristics

Table 3.3.1 – 1: PPD-positive subjects who completed AAT tests and readings (N=46)

No	Sex	Age	Severity of real life reaction	Pt to PPD prior inclusion in study	Real life eliciting product		
					Group of shades	Mean concentration of group of shades (%)	Concentration range of group of shades(%)
IT08	F	66	Moderate	+++	Light	0.05	0.02 - 0.2
UK08	F	37	Very severe	+++	Light	0.05	0.02 - 0.2
DE02	F	58	Mild	+++	Light	0.05	0.02 - 0.2
NL04	F	39	Moderate	+++	Light	0.05	0.02 - 0.2
NL15	F	55	Severe	+++	Light	0.05	0.02 - 0.2
AU02	F	49	Severe	++	Medium	0.25	0.07 - 0.48
AU10	F	27	Moderate	++	Medium	0.25	0.07 - 0.48
IT03	F	55	Moderate	+++	Medium	0.25	0.07 - 0.48
NL12	F	17	Moderate	++	Medium	0.25	0.07 - 0.48
DE04	F	44	Severe	+++	Medium	0.25	0.07 - 0.48
IT09	F	38	Moderate	+++	Medium	0.25	0.07 - 0.48
IT10	F	33	Moderate	+++	Medium	0.25	0.07 - 0.48
NL11	F	51	Moderate	++	Medium	0.25	0.07 - 0.48
UK05	F	58	Moderate	+++	Medium	0.25	0.07 - 0.48
NL09	F	30	Moderate	+++	Medium	0.25	0.07 - 0.48
NL05	M	68	Moderate	++	Medium	0.25	0.07 - 0.48
IT02	F	42	Moderate	+++	Medium	0.25	0.07 - 0.48
AU13	F	18	Mild	+	Medium	0.25	0.07 - 0.48
NL06	F	52	Moderate	++	Medium	0.25	0.07 - 0.48
AU06	F	24	Moderate	+++	Medium	0.25	0.07 - 0.48
AU05	F	46	Severe	+	Medium	0.25	0.07 - 0.48
UK01	F	53	Mild	++	Medium	0.25	0.07 - 0.48
NL14	F	66	Moderate	+	Medium	0.25	0.07 - 0.48
UK02	F	54	Mild	+	Medium	0.25	0.07 - 0.48
DE03	F	20	Very severe	+++	Dark	0.75	0.37 - 2
DE05	F	25	Severe	+++	Dark	0.75	0.37 - 2
IT01	F	24	Severe	++	Dark	0.75	0.37 - 2
AU14	F	27	Severe	+++	Dark	0.75	0.37 - 2
AU15	M	37	Very severe	++	Dark	0.75	0.37 - 2
AU11	F	22	Very severe	++	Dark	0.75	0.37 - 2
UK07	F	40	Moderate	+++	Dark	0.75	0.37 - 2
NL02	F	52	Moderate	+++	Dark	0.75	0.37 - 2
AU08	F	23	Severe	+	Dark	0.75	0.37 - 2
NL08	F	53	Moderate	+++	Dark	0.75	0.37 - 2
AU12	F	42	Moderate	++	Dark	0.75	0.37 - 2
UK03	F	61	Moderate	++	Dark	0.75	0.37 - 2
AU01	M	21	Mild	+	Dark	0.75	0.37 - 2
AU07	F	44	Severe	++	Dark	0.75	0.37 - 2
AU09	F	58	Severe	++	Dark	0.75	0.37 - 2
NL01	F	52	Mild	++	Dark	0.75	0.37 - 2
AU03	F	24	Mild	++	Dark	0.75	0.37 - 2
AU04	F	54	Very severe	++	Dark	0.75	0.37 - 2
UK09	F	69	Mild	+	Dark	0.75	0.37 - 2
NL13	F	48	Mild	++	Dark	0.75	0.37 - 2
NL07	F	71	Moderate	+	Dark	0.75	0.37 - 2
UK06	F	37	Moderate	++	Dark	0.75	0.37 - 2

Table 3.3.1 – 2: Comparison of age in PPD-positive and PPD-negative groups

	PPD-positive subjects reacting behind the ear		PPD-positive subjects reacting on the forearm		Altogether		Controls	
	Number	Mean age	Number	Mean age	Number	Mean age	Number	Mean age
Product A	19	38.2	13	35.5			12	43.8
Product B	11	47.4	12	45.3			11	42
Product C	6	36.7	6	41.2			12	39.9
Product D	4	42.7	7	45			13	37.7
All products	40	41	38	41	46	43	48	40.8

Table 3.3.1 – 3: Comparison of sex variables in PPD-positive and PPD-negative groups

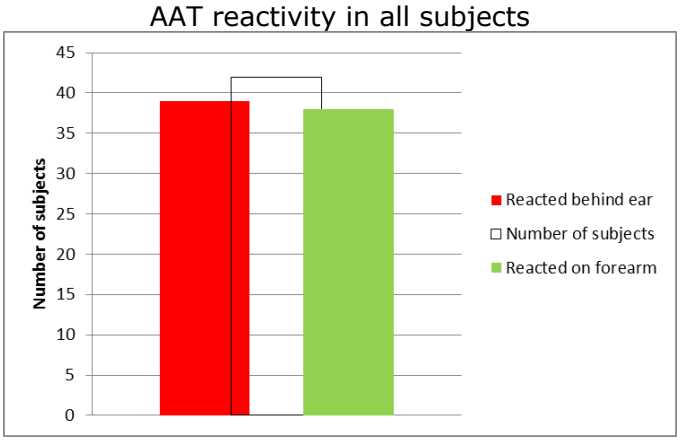
	Males (number)	Females (number)	Total
PPD-positive subjects	3	43	46
PPD-negative subjects	2	46	48

Table 3.3.1 – 4: Declared shade levels in PPD-positive and PPD-negative subjects

	Declared eliciting shades in PPD-positive subjects	Declared used shades in PPD-negative subjects
Light	5	17
Medium	19	11
Dark	22	12
Light and dark	-	1
Light and medium	-	2
Medium and dark	-	2
All shaders	-	3
Total	46	48

The following results presentation is taken from the "Efficacy results" section of (1)

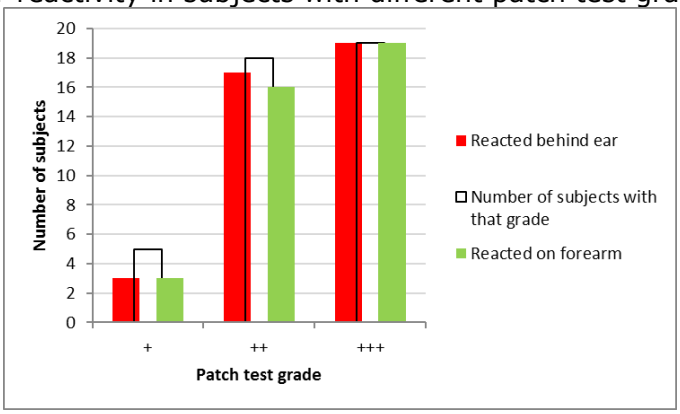
Reaction **self-perceived** by the subject + **positive or doubtful AAT** (objective score) at Day 2 (N=42 subjects available for analysis).



Analysis of AAT reactivity on the two test sites (AAT self-perceived and objectified).

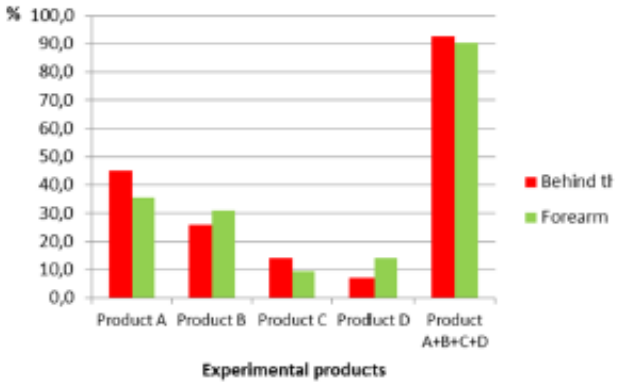
		Behind the ear	
		+	-
On forearm	+	38	0
	-	1	3

AAT reactivity in subjects with different patch test grades



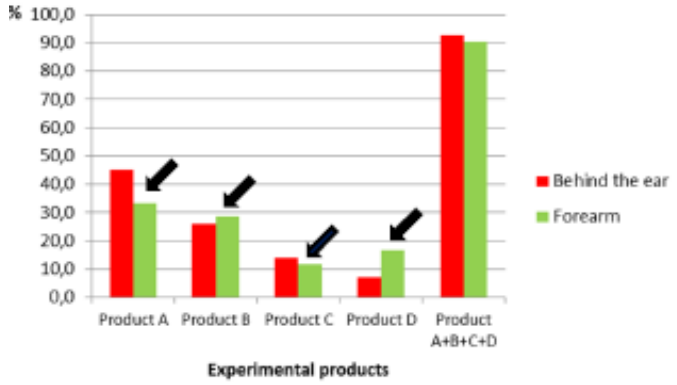
Concentration dependency of positive AAT in 42 subjects available for analysis.
Concentration dependency of self-perceived reactions (Day 2)

	Subjects reacting with **self-perceived** reaction			
	Behind ear		On forearm	
	N	%	N	%
Product A (0.05%)	19	45.2	15	35.7
Product B (0.25%)	11	26.2	13	31.0
Product C (0.75%)	6	14.3	4	9.5
Product D (2%)	3	7.1	6	14.3
Product A+B+C+D	*39*	*92.9*	*38*	*90.5*
No reaction	3	7.1	4	9.5



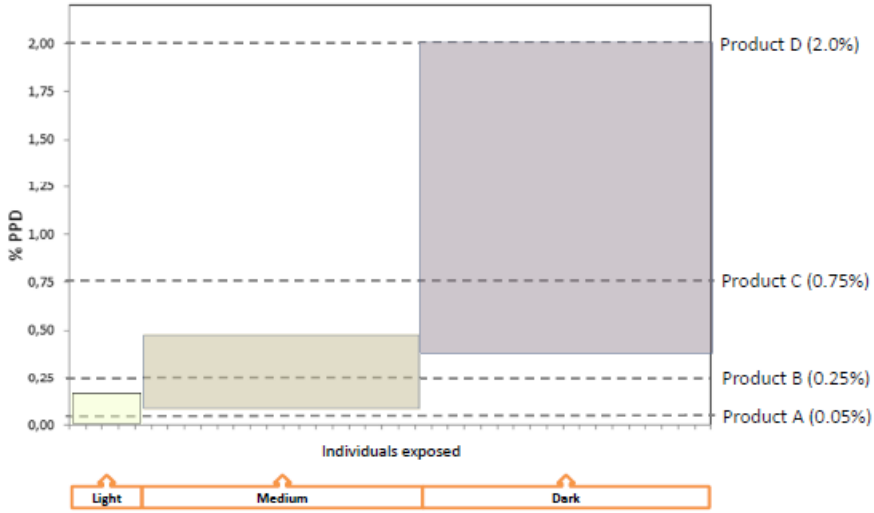
Concentration dependency of self-perceived and objectified reactions (Day 2)

	Subjects reacting with **self-perceived** and *objectified* reaction			
	Behind ear		On forearm	
	N	%	N	%
Product A (0.05%)	19	45.2	14	33.3
Product B (0.25%)	11	26.2	12	28.6
Product C (0.75%)	6	14.3	5	11.9
Product D (2%)	3	7.1	7	16.7
Product A+B+C+D	*39*	*92.9*	*38*	*90.5*
No reaction	3	7.1	4	9.5

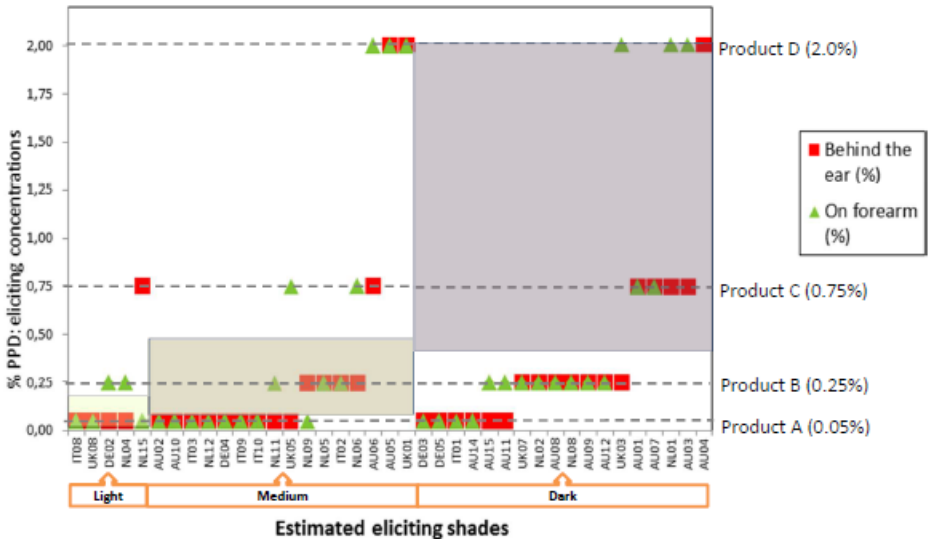


AAT performance
PPD concentrations in groups of shades and in experimental products: Evaluation based on estimated real-life eliciting PPD concentrations

PPD concentrations in groups of shades:
Light: 0.02 - 0.2%
Medium: 0.07 – 0.48%
Dark: 0.37 - 2%



Comparison of AAT eliciting concentrations and declared hair colour shade level used: self-perceived reactions in 39/42 subjects at Day 2.

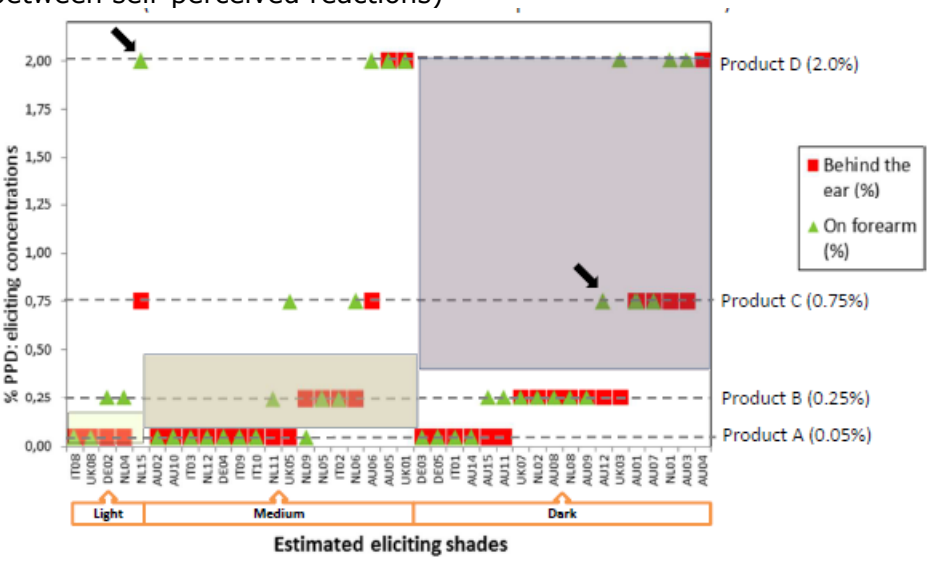


Positive AAT responses in 39/42 test subjects:
39 behind the ear; 83% (35) reacted to estimated use concentration or below.
38 on forearm; 74% (31) reacted to estimated use concentration or below.

Analysis of AAT performance (meets success criteria): <u>self-perceived</u>

		Behind the ear	
		+	-
On forearm	+	30	1
	-	5	6

Comparison of AAT eliciting concentrations and declared hair colour shade level used: self-perceived and objectified reaction in 39/42 subjects at Day 2 (arrows show differences between self-perceived reactions)



Positive AAT responses in 39/42 test subjects
39 behind the ear; 83% (35) reacted to estimated use concentration or below.
38 on forearm; 71% (30) reacted to estimated use concentration or below.

Analysis of AAT performance (meets success criteria): self-perceived and objectified

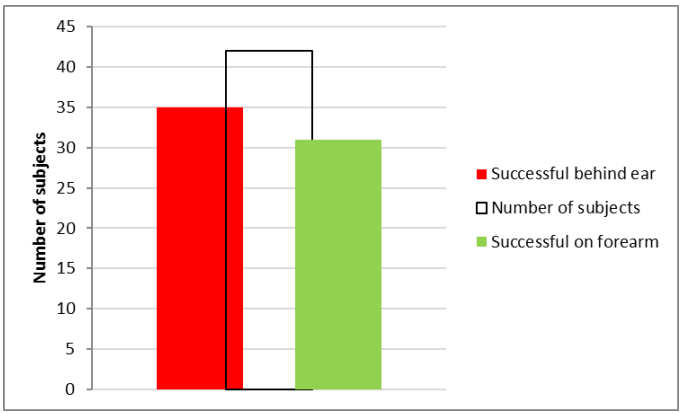
		Behind the ear	
		+	-
On forearm	+	30	0
	-	5	7

AAT performance (meets success criteria): objectified

		Behind the ear	
		+	-
On forearm	+	30	0
	-	6	6

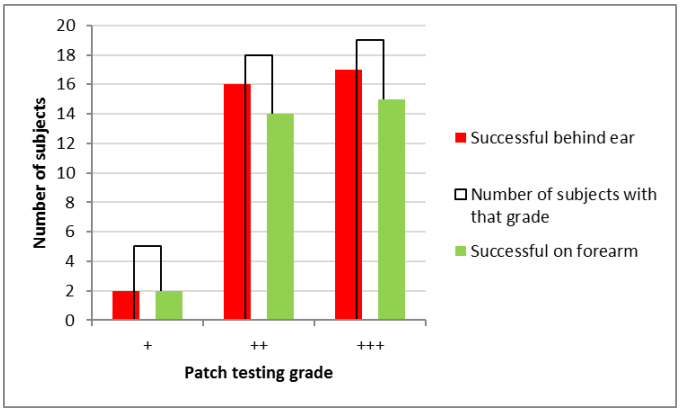
Comparisons between AAT performance behind the ear versus the forearm analysed by means of 2x2 tables shows that the rate of reactivity was statistically higher behind the ear only from dermatologist's perspective (McNemar's test, binominal). There was no statistically significant difference from the consumer's perspective and from a broader perspective.

AAT performance in subjects with different patch test grades
AAT successful
Success criterion: Self-perceived reaction by subject to an experimental product with a concentration equivalent or lower than the real-life eliciting concentration at Day 2 (N=42 subjects available for analysis)
In all subjects



Analysis of AAT performance (meets success criteria): self-perceived

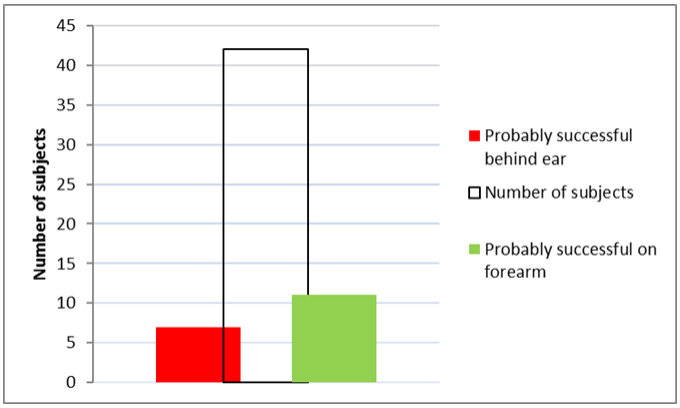
In subjects with different patch test grades



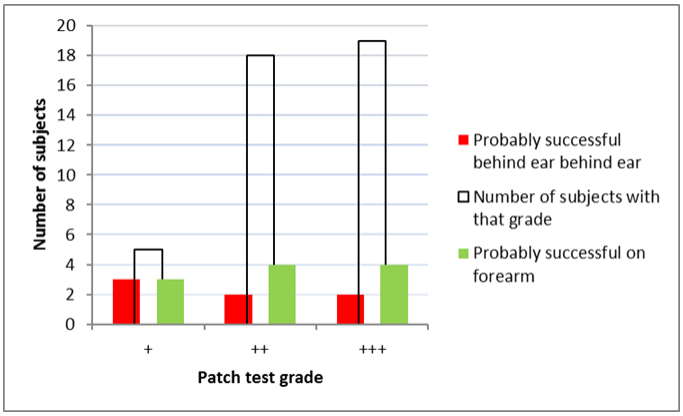
AAT probably successful/true negative
Probably successful: Negative AAT to an experimental product with concentrations equivalent or lower than the mean concentration of declared real-life eliciting group of shades but the subject was not available for the use test at the end of the study.

Subjects eligible but not available for use tests:
AU04, AU5, AU6, NL04, NL06, NL14, NL15, UK01, UK02, UK05, UK06, DE02

In all subjects



In subjects with different patch test grades



Comparison of dermatological evaluation and self-evaluation by the subject
Comparison of judgement behind the ear

AAT reactivity (positive AAT to any product at Day 2)

		Dermatologist reading (positive or doubtful)	
		+	-
Self-perceived	+	39	0
	-	0	3

Meets success criteria

		Dermatologist reading (positive or doubtful)	
		+	-
Self-perceived	+	35	0
	-	1	6

3.4 SAFETY EVALUATION

No adverse events related to the application of experimental Products A, B, C and D or to the control Product Y were observed. No serious adverse events were observed. No safety issues related to the AAT procedure, experimental and control products were observed in PPD-positive subjects with history of allergic reactions to hair colouring products and in control PPD-negative subjects.

Adverse events reported were infrequent, not serious, mild or moderate in severity and most importantly, unrelated to the product applications. The allergy alert test reactions to the experimental products themselves were not severe and were easily managed by the PPD-positive subjects. No subject interrupted the study because of the severity of the reaction.

Long-term effects such as the induction of contact sensitisation by an AAT application were not investigated in this study. None of the PPD-negative subjects who were also hair dye consumers, reported manifestations compatible with active sensitisation after participating in the study.

Table 3.4 – 1: Experimental group (PPD-positive subjects) (N=46)

	Mild		Moderate		Severe		Total	
	Related	Not related	Related	Not related	Related	Not related	Related	Not related
Body system Eye disorders	0	0	0	1 (2.2%) UK04	0	0	0	1 (2.2%)
Body system Skin and subcutaneous tissue disorders	0	1 (2.2%) UK09	0	0	0	0	0	1 (2.2%)

Table 3.4 – 1: Control group (PPD-negative subjects) (N=48)

	Mild		Moderate		Severe		Total	
	Related	Not related	Related	Not related	Related	Not related	Related	Not related
Body system Renal and urinary disorders	0	0	0	1 (2.1%) AU29	0	0	0	1 (2.1%)

6. REFERENCES

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7. GLOSSARY OF TERMS

See SCCS/1602/18, 10th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 141

8. LIST OF ABBREVIATIONS

See SCCS/1602/18, 10th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 141