Evaluation on Ocular Irritation Potential of Marketed Permanent Hair Colour

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Abstract: The evaluation of eye irritation potential is essential to ensuring the safety of individuals in contact with wide range of substances designed for industrial, pharmaceutical or cosmetic use. The present paper is an attempt to investigate the eye irritation potential of marketed permanent hair colour contains *para*-phenylenediamine (PPD) and ammonia as per the OECD guideline 405 (Organization for Economic Cooperation and Development). The clinical signs of body weight and ocular reaction (Cornea, Chemosis, Conjunctivae and Iris) were recorded at various intervals of time such as 1, 24, 48, and 72 hours post instillation. Test material produced no clinical signs of toxicity and mortalities noticed during the study. In the both initial and confirmatory test, eye irritation was observed. The permanent hair colour classified as an "**Irritant**" (Category 2B).

1. Introduction

Eyes are one of the most delicate parts of our body. They can be exposed to cosmetic products and their ingredients through either use of products directly (Hair Dyes, Mascaras, Eye Creams,) or accidentally (which may enter the eye). The evaluation of eye irritation potential for a cosmetic product and its ingredients is essential to provide reassurance that a product is safe for consumers to use through intended and foreseeable uses and accidental exposures to the eye. The conventional method for determination of the irritant or corrosive potential of chemicals is an acute eye irritation test and has become the international standard assay for acute eye irritation and corrosion. This test involves an examination of cornea, conjunctiva, chemosis and iris post application of test item to the eyes of white rabbits.[1]

The colouring of hair is an amazing and transforming experience. It's a process of bringing hair to life in a new way through beauty of chemistry - the way product formulas react with Hair.[2] Therefore, the application of hair colour is a combination of Art and Science that requires imagination, creative expression, intuition and technical skill. The combination is inspiring and the possibilities are endless. Hair dye use is very common among both the genders, today millions use it. Colouring of hair is performed not only by professionals but also a popular cosmetic procedure at home. Hair dyes are widely used, either to cover up grey hairs, or simply by those wanting to change their natural hair colour.[3]

Hair Dyes can be classified based on chemical composition, mechanism of action namely (1) Permanent Hair Colour, (2) Temporary Hair Colour, (3) Semi-permanent Hair Colour, (4) Demi-permanent Hair Colour. In permanent Hair Colour, formulation consists of primary intermediates (Eg: *para*-phylenediamine, para-aminophenol) are mixed with couplers (Eg: Resorcinol, m-aminophenol) to generate coloured oxidation product through chemical reaction that binds irreversibly within the hair shaft. Permanent hair colour consists of two components namely "Colorant" and "Developer".[4] To achieve Permanent Hair Colour, the cuticle should be opened usually an alkaline solution is used (Eg: Ammonia). This alkaline solution not only opens the hair shaft, but also causes swelling of the shaft, making absorption of the dye easier. Hydrogen peroxide is commonly used as an oxidant in hair dyeing process which allows the diffusion of precursors into hair cortex and catalyses the oxidation of precursors into large coloured molecules that are infuse within the hair shaft. The combination of various dye precursors with different couplers are required to produce a variety of colours.[5] Due to their basic reactive chemistry, the safety evaluation of hair

dyes has always been a major consideration. Hair dyes are therefore one of the most studied and regulated consumer products on the market with an overwhelming amount of safety data.[6]

The Objective of this study was to assess the Eye Irritation/Corrosion Potential of "Marketed Permanent Hair colourant" contains *para*-phenylenediamine (PPD) and Ammonia to the Eye of New Zealand White rabbits. The study has been approved by the Institutional Animals Ethics Committee (IAEC) and performed as per OECD Test Guideline 405".[7]

2. Material and Methods[8, 9,10]

Evaluation performed on marketed Permanent Hair Colour contains the following ingredient. The product contains two components namely "Hair Colourant" and "Developer".

Hair Colourant: Water, Cetearyl Alcohol, Propylene Glycol, Laureth-12, Ammonium Hydroxide, Lauric Acid, Glycol Distearate, Ethanolamine, Polyquternium-22, Silica dimethyl silylate, Ascorbic acid, Ammonium Thiolactate, Dimethicone, Pentasodium Pentetate, Carbomer, **p-Phylenediamine, N, N-Bis (2 -Hydroxyethyl)-p-phenylenediamine sulfate, Resorcinol, 2, 4-Diaminophenoxyethanol HCl, m-Aminophenol**, Parfum.

Developer: Water, Hydrogen Peroxide, Cetearyl alcohol, Sodium Stannate, Trideceth-2 Carboxamide MEA, Pentsasodium Pentetate, Phosphoric acid, Ceteareth-25, Tetrasodium Pyrophosphate, Glycerin.

Physical appearance of the product was "White to Off-white Coloured" for both Colourant and Developer. It was manufactured on Feb-2018 (Batch No: LHC3423) and the expiry declared on packaging was Dec-2020. The product was stored at room temperature of 25 ± 2^0C without opening the seal. An amount of 100 mg of test item mixture (1:1) was instilled in to the eye as per OECD test guideline 405.

The acute eye irritation study was performed in accordance with the OECD Guidelines 405. Healthy 12 months old 2 female New Zealand White Rabbits were used for the study. Two Females selected were nulliparous and non-pregnant. Animals were kept under acclimatization for eight days before application. Animals were housed in stainless steel cages having facility for holding pelleted feed and drinking water in water bottle fitted with stainless steel sipper tube. The cage was provided with a card showing the details of cage number, test formulation, animal number, sex of animal and the study number. The animals were maintained at ambient temperature, relative humidity of 25 deg and 40 - 63 % respectively. The animals were exposed to 12 hours light/dark cycle. Standard laboratory rabbit feed was provided *ad libitum* throughout the experimental period. Reverse Osmosis (RO) purified water was provided *ad libitum* throughout the experimental period with help of water bottles.

Both eyes of each experimental animal provisionally selected for testing and were examined one hour before test item instillation using ophthalmoscope. Animals with normal eyes were used for experiment. Sixty minutes prior to test item instillation, buprenorphine (0.01 mg/kg) was administered by subcutaneously (SC) to provide a therapeutic level of systemic analgesia. Five minutes prior to instillation, two drops of a topical ocular anesthetic (0.5% proparacaine hydrochloride) was instilled to each eye. The test item was placed in the conjunctival sac of left eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The right eye, which remained untreated, served as control. The eye was washed with water after 24 hours of test item instillation.

As no irritant effect was observed, post instillation procedure was not carried out. Initial test was performed using single animal. 100 mg of test item was instilled in to the conjunctival sac of left eye and observed for eye lesions. Since, no corrosive or irritant effect was found in initial test, the response was confirmed using two additional animals. Animals were evaluated for the entire duration of the study for clinical signs of pain and/or distress (e.g. repeated pawing or rubbing of the eye, excessive blinking, excessive tearing) twice daily, with a 6 hours gap between observations. All animals were observed twice daily for mortality and morbidity during experimental period.

Individual animal body weight was recorded on day 1 of the experiment and on the day of termination. The grades of ocular reaction (conjunctivae, chemosis, cornea and iris) were recorded at 1, 24, 48, and 72 hours following test item instillation as shown in **Table 1**. As no ocular lesions were observed, all animals were terminated after 72 hours observation.

Table 1: Grading of Ocular Lesions

Cornea (Opacity: degree of density - readings will be taken from most dense area)	**Grade**
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible	1
Easily discernible translucent area; details of iris slightly obscured	2
Nacrous area; no details of iris visible; size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Maximum possible: 4 *The area of corneal opacity will be noted	

Iris	**Grade**
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect	1
Hemorrhage, gross destruction, or(no)reaction to light	2
Maximum possible: 2	

Conjunctivae (Redness - refers to palpebral and bulbar conjunctivae; excluding cornea and iris)	**Grade**
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse,crimson colour; individual vessels not easily discernible	2
Diffuse beefy red	3
Maximum possible: 3	

Chemosis (Swelling - refers to lids and/or nictating membranes)	**Grade**
Normal	0
Some swelling above normal	1
Obvious swelling, with partial eversion of lids	2
Swelling, with lids about half closed	3
Swelling, with lids more than half closed	4
Maximum possible: 4	

Based on Globally Harmonized System of Classification and Labelling of Chemicals (GHS), a substance that produces in at least one animal effects on the cornea, iris, chemosis or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or in at least 2 of 3 tested animals, a positive response of corneal opacity ≥ 3; and/or iritis > 1.5; calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material. The substances are classified as "**Category 1**".

Substances that have the potential to induce reversible eye irritation classified as "**Category 2A**". Substances that produce in at least 2 of 3 tested animals a positive response of:

(a) Corneal opacity ≥ 1; and/or

(b) Iritis ≥ 1; and/or

(c) Conjunctival redness ≥ 2; and/or

(d) Conjunctival Oedema (Chemosis) ≥ 2 calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material, and which fully reverses within an observation period of normally 21 days. Whereas in the case of "Category 2B", when the effects listed above are fully reversible within 7 days of observation.

3. Results and Discussions

3.1 Clinical Observations

In the both initial test and confirmatory tests, erythema of left eye was observed. The reversibility was noticed on day 6 (initial test) and 7 (confirmatory test) as shown in **Table 2**.

Table 2: Individual Animal Clinical Observations

Study Type	Animal No.	Sex	Days													
			1	1	2	2	3	3	4	4	5	5	6	6	7	7
Initial Test	01	F	23++	23++	23++	23++	23++	23++	23++	23++	23+	23+	0	0	0	0
Confirmatory Test	02	F	23++	23++	23++	23++	23++	23++	23++	23++	23+	23+	23+	23+	0	0

3.2 Body Weight

There were no signs of toxicity and mortalities noticed in the study. Loss of body weight is an important marker of gross toxicity which drastic or interference with absorption of nutrient will be reflected in body weight reduction. There were no statistically significant mean weight differences in body weights between the control and the treated groups from the first day of patch application through the end of the experiment as shown in **Table 3**. It can be inferred that the test item has no tendency to produce drastic tissue destruction nor does it seem to interfere with absorption of the nutrients.

Table 3: Individual animal Body Weight (kg) and Body Weight Gain (%)

Study Type	Animal No.	Sex	Body weight on days		% Body weight gain
			1	7	1-7
Initial Test	01	F	2.41	2.45	1.7
Confirmatory Test	02	F	2.50	2.54	1.6

3.3 Eye Reactions

In both initial test and confirmatory tests, eye irritation was observed. Mean score of "2" for conjunctival redness and chemosis of left eye was observed and reversibility was noticed within 7 days as shown in **Table 4**.

Table 4: Individual animal Eye Grading

Study Type	Animal No.	Sex	Observation	1 h		24h		48h		72h	
				RE	LE	RE	LE	RE	LE	RE	LE
Initial Test	01	F	Cornea	0	0	0	**0**	0	0	0	0
			Iris	0	0	0	**0**	0	0	0	0
			Redness	0	2	0	**2**	0	2	0	2
			Chemosis	0	2	0	**2**	0	2	0	2
Confirmatory Test	02	F	Cornea	0	0	0	0	0	0	0	0
			Iris	0	0	0	0	0	0	0	0
			Redness	0	2	0	2	0	2	0	2
			Chemosis	0	2	0	2	0	2	0	2

Note: LE=Left Eye; RE=Right Eye

The mean eye irritation score was presented in **Table 5**. It was calculated as shown below

Mean Eye Irritation Score = Grades of 24 h+ 48 h + 72 h / Number of observation (3)

Mean Eye Irritation Score = 2+2+2 / 3 = 2

Table 5: Mean Eye Irritation Score

Mean Score for Cornea	RE - 0	LE - 0
Mean Score for Iris	RE - 0	LE - 0
Mean Score for Redness	RE - 0	LE - 2
Mean Score for Chemosis	RE - 0	LE - 2

4. Conclusion

Based on the results of the study, the marketed permanent hair colour contains *para*-phylenediamine has been classified as an "**Irritant**" (Category 2B) as per OECD guidelines and Globally Harmonized System of Classification and Labelling of Chemicals (GHS). This article spotlights the need of fundamental research for the development of safer and gentle hair colour which is sustainable and eco-friendly.

5. Acknowledgment

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6. References

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