# Assessment on Dermal Irritation Potential of Permanent Hair Colour with *para*-Phenylenediamine (PPD)

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**Abstract:**

Study performed to access the irritation potential of marketed permanent hair colourant contains *para*-phenylenediamine (PPD) and ammonia as per the OECD Guidelines. The test item was applied to New Zealand white rabbit skin and covered with a gauze patch, which was held in place with non-irritating tape. The clinical signs of toxicity, mortality, body weight change and skin reactions of erythema and oedema were evaluated at various intervals of time (1 hr, 24 hr, 48 hr and 72 hr). The test material produced a "Mild Irritation" and no corrosive effects were observed.

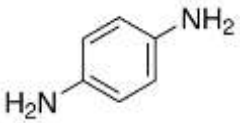
## 1. Introduction

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.[1] 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.[2]

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".[3] 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.[4] 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.

According to many dermatologists[5], there are many cases of men and women who suffered from allergic reaction to permanent chemical dyes, PPD (*para*-phenylenediamine). PPD is an aromatic amine with a chemical formula $C_6H_8N_2$. Present in the form of white crystals, PPD oxidizes in the air turning from red to brown and finally black. Most of the marketed product contains PPD which is known to be contact allergic dermatitis. People can become allergic to PPD at any time, even if they have been exposed to it before without problems. A mild reaction to PPD might involve dermatitis to the upper eye lids or the rims of

the ear. In severe cases, there may be reddening or swelling of the scalp and face these eyelids may completely close and the reaction may spread. In some allergic reactions, anaphylaxis can occur which in some cases can lead to death.[6] Physicochemical properties of p-phenylenediamine (PPD) as shown below.[7]

| | | |
|---|---|---|
| INCI | : | p-phenylenediamine |
| Synonyms | : | Benzene-1, 4-diamine<br>1, 4 -Benzene diamine<br>1, 4-Diaminobenzene<br>1, 4-Phenylenediamine<br>p-amino aniline |
| Empirical Formula | : | $C_6H_8N_2$ / $C_6H_4(NH_2)_2$ |
| Structure | : |  |
| Molar Mass | : | 108.2 g/mol |
| CAS No | : | 106-50-3 |
| EINCS No | : | 612-028-00-6 |
| Boiling point | : | $267^0$C |
| Melting point | : | $139\text{-}147^0$C |
| Relative density | : | 1.10 |
| Solubility in water, g/100 ml at 25°C | : | 4.00 |
| Vapour pressure, Pa at 100°C | : | 144 |
| Relative vapour density (air = 1) | : | 3.70 |
| Flash point | : | 156°C c.c. |
| Auto-ignition temperature | : | 400° C |

The Objective of this study was to assess the Irritation/Corrosion Potential of "Marketed Permanent Hair colourant" contains *para*-phenylenediamine (PPD) and Ammonia to the Skin of New Zealand White rabbits. The study has been approved by the Institutional Animals Ethics Committee (IAEC) and performed as per the OECD Guideline for the Testing of Chemicals (No. 404, Section 4: Health Effects) "Acute Dermal Irritation/Corrosion" adopted on 28th July 2015.[8]

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

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 ± $2^0$C without opening the seal. An amount of 0.5 g of test item (0.25 g of Colourant and 0.25 g of Developer) was applied per site as per OECD test guideline 404.

The acute dermal irritation study was performed in accordance with the OECD Guidelines 404 "Acute Dermal Irritation/Corrosion". Healthy 12 months old 3 female New Zealand White Rabbits were used for the study. Three 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.

Twenty four hours before the test, fur was removed by closely clipping the dorsal area of the trunk of the animals using electric hair clipper. Care was taken to avoid abrading the skin, and only animals with healthy, intact skin were used. The test item was applied to a small area (approximately 6 $cm^2$) of skin and covered with a gauze patch, which was held in place with non-irritating tape. The patch was loosely held in contact with the skin by means of a suitable semi-occlusive dressing for the duration of the exposure period. The patch was attached to the skin in such a manner that there was good contact and uniform distribution of the substance on the skin. Access by the animal to the patch and ingestion or inhalation of the test item was prevented by wrapping with crepe bandage. After completion of exposure period patch was removed and test site was cleaned with cotton dipped in water.

All animals were examined for signs of erythema and oedema, and the responses were scored. Erythema is redness of the skin or mucous membranes caused by hyperemia of superficial capillaries whereas Oedema is swelling caused by fluid in body's tissue. The initial test was performed by applying three test patches sequentially to the one animal. The first patch was applied at site 2 and removed after three minutes. As no skin reaction was observed, second patch was applied at site 3 and removed after one hour. As no skin reaction was observed, third patch was applied and removed after four hours, and the response was graded. At control site (site 1) distilled water was applied and removed during third patch removal. Since, no erythema and oedema found in initial test, the response was confirmed using two additional animals. For both the animals test item was applied at site 2 for an exposure period of four hours. Control site (Site 1) was applied with distilled water. The test was performed using three female rabbits as shown below in **Table 1**.

**Table 1: Study Design**

| Test | | Initial Test | | | | Confirmatory Test | |
|---|---|---|---|---|---|---|---|
| Animal No. | | 1 | | | | 2 and 3 | |
| Site No. | | 1 | 2 | 3 | 4 | 1 | 2 |
| Treatment | Distilled water | Yes | No | No | No | Yes | No |
| | Test item | No | Yes | Yes | Yes | No | Yes |

All animals were observed once daily for clinical sings and twice daily for mortality and morbidity during treatment period. Individual animal body weight was recorded on day 1 of the experiment and on the day of termination. For the initial test, test sites were examined immediately after the patch removal. Site 1 and 4 was scored at 60 minutes, 24, 48 and 72 hours after patch removal. In confirmation test site was scored at 60 minutes, and then at 24, 48 and 72 hours after patch removal. Dermal reactions (Erythema and Oedema) were evaluated and recorded according to the evaluation criteria of Draize Scale as shown in **Table 2[9].**

As per OECD guidelines 404[9], "**Mild Irritation**" (Category 3) are classified if the mean score of Erythema ≥ 1.5 and <2.3 for erythema/eschar or for oedema from grading in at least 2 of 3 tested animals from grades at 24, 48 and 72 hours or if the reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions. However "**Irritation**" (Category 2) are classified if the mean score of Erythema ≥ 2.3 and ≤ 4.0 for erythema/eschar or for oedema from grading in at least 2 of 3 tested animals from grades at 24, 48 and 72 hours or if the reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions.

**Table 2: Rating of Erythema and Oedema[9]**

| Erythema and Eschar Formation | Score |
|---|---|
| No erythema | 0 |
| Very slight erythema (barely perceptible) | 1 |
| Well defined erythema | 2 |
| Moderate to severe erythema | 3 |
| Severe erythema (beef redness) to eschar formation preventing grading of erythema | 4 |
| Maximum possible: 4 | |
| | |
| Oedema Formation | Score |
| No oedema | 0 |
| Very slight oedema (barely perceptible) | 1 |
| Slight oedema (edges of area well defined by definite raising) | 2 |
| Moderate oedema (raised approximately 1 mm) | 3 |
| Severe oedema (raised more than 1 mm and extending beyond area of exposure) | 4 |
| Maximum possible: 4 | |

At the end of experiment all animals were sacrificed by intravenous injection of sodium thiopentone. Animal were not subjected to necropsy and gross pathology as animals did not shown any signs of equivocal response.

## 3. Result and Discussions:

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. In the initial test, "well defined erythema" was noted at two treated skin sites at 24 and 48-hrs observations. In confirmatory test, again "slight erythema" was observed at one hour after patch removal and "well defined erythema" observed at two treated site at 24 and 48-hrs observations as shown in the **Table 4** and **Fig 1-3**. Skin sites appeared normal at 72-hr observation in all the cases. Mean value of Erythema were calculated based on 24, 48, and 72 hours evaluations as shown in **Table 4**.

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

| Study Type | Animal No. | Sex | Body weight on days | | % Body weight gain |
|---|---|---|---|---|---|
| | | | 1 | 4 | 1-4 |
| Initial Test | 01 | F | 1.99 | 2.10 | 5.5 |
| Confirmatory Test | 02 | F | 1.92 | 2.11 | 9.9 |
| | 03 | F | 2.15 | 2.26 | 5.1 |

**Table 4: Individual Animal Skin Grading**

| Study Type | Animal No. | Sex | Time points (hrs.) | | | | | | | | Average Score for 24, 48 and 72 (hrs.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | | 24 | | 48 | | 72 | | | |
| | | | ER | ED | ER | ED | ER | ED | ER | ED | ER | ED |
| Initial Test | 1 | F | 0 | 0 | 2.5 | 0 | 2.5 | 0 | 0 | 0 | 1.7 | 0 |
| Confirmatory Test | 2 | F | 1 | 0 | 2.5 | 0 | 1.5 | 0 | 0 | 0 | 1.3 | 0 |
| | 3 | F | 1 | 0 | 2.5 | 0 | 2.5 | 0 | 0 | 0 | 1.7 | 0 |
| Mean Score for ER | | | 1.6 | | | | | | | | | |
| Mean Score for ED | | | 0 | | | | | | | | | |

ER=Erythema; ED=Oedema

**Fig 1**

**Fig 2**

**Fig 3**

**Fig 1:** Control (Placebo)
**Fig 2:** Erythema after 24 Hour of test item exposure
**Fig 3:** Erythema after 48 hour of test item exposure

## 4. Conclusion

Based on the above results, the marketed permanent hair colorant having *para*-phenylenediamine and ammonia has been classified as "**Mild Irritation**". Most of the hair colorant having *para*-phenylenediamine (PPD) which is well recognized as skin allergen and sensitizer by CDC (Centers for Disease Control and Prevention). Generally exposure routes of PPD might through inhalation, skin absorption, ingestion, and skin and/or eye contact, symptoms of exposure include throat irritation (pharynx and larynx), bronchial asthma, and sensitization dermatitis. This evaluation of the marketed product not only spotlights the irritation potential of *para*-phenylenediamine (PPD) but also pinpoints the need of fundamental research for the development of safer and gentle hair colorant which is free from harsh chemicals like PPD, 2-NPPD (2-Nitro *para* Phenylenediamine), Oleic acid, ammonia etc. This basic research would create awareness for the need of eco-friendly hair colour.

## 5. Acknowledgment

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