

OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD  
PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

P-PHENYLENEDIAMINE

Colipa n° A7

adopted by the SCCNFP during the 19<sup>th</sup> plenary meeting  
of 27 February 2002

## 1. Terms of Reference

### 1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

### 1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

- \* Is p-Phenylenediamine safe for use in cosmetic products?
- \* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

### 1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.

The extend to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

## 2. Toxicological Evaluation and Characterisation

### 2.1. General

#### 2.1.1. Primary name

p-Phenylenediamine (INCI name)

#### 2.1.2. Chemical names

1,4-Diaminobenzene	para-Benzenediamine
1,4-Benzenediamine	para-Aminoaniline
4-Aminoaniline	para-Diaminobenzene

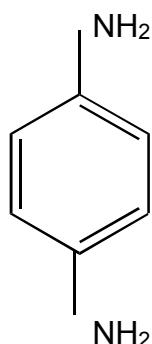
#### 2.1.3. Trade names and abbreviations

CI 76 060  
PPD

#### 2.1.4. CAS no.

106-50-3 (free base)

#### 2.1.5. Structural formula



#### 2.1.6. Empirical formula

Emp. Formula : C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>  
Mol weight : 108

**2.1.7. Purity, composition and substance codes**

≥ 99 %. (free base)

**2.1.8. Physical properties**

Appearance	:	white to light purple crystals
Melting point	:	140 °C
Boiling point	:	267 °C
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	<1 mm at 21°C (technical product)
Log P <sub>ow</sub>	:	/

**2.1.9. Solubility**

Slightly soluble in water, soluble in ethanol, ether, benzene, chloroform and acetone.

**2.2. Function and uses**

PPD is one of the key primary precursors of the oxidative hair dyes used as dark colours in permanent hair dye products. In permanent hair dye products, it is always used in combination with an oxidative agent.

It is currently in Annex III part 1 number 8 and is restricted to a maximum concentration of 6 % with certain warning on the label. The entry was last modified by 83/341/EEC.

**TOXICOLOGICAL CHARACTERISATION****2.3. Toxicity****2.3.1. Acute toxicity**

Acute toxicity has been investigated following oral, subcutaneous, intraperitoneal and topical application in a variety of species. The LD<sub>50</sub> following oral administration was 80-100 mg/kg in the rat, 290 mg/kg in mice, 250 mg/kg in rabbit and 100 mg/kg in cats.

The values following subcutaneous application were 170, 200 and 100 mg/kg for rat, rabbit and dog respectively.

The intraperitoneal and topical LD<sub>50</sub> values have each only been determined in the rabbits respectively. A variety of toxic effects have been reported with some variation between species.

There are several reports of deliberate or accidental para-phenylenediamine poisoning in humans but no details of the amount ingested were available. The symptoms reported include oedema of the glottis and acute renal failure.

Ref. : 67, 104, 114

### **2.3.2. Repeated dose oral toxicity**

A 14-day study was conducted according to OECD Guideline n° 408 (1981) in five groups of 20 (10 males and 10 females) rats from the Crl: CD (SD) BR strain (VAF plus) receiving PPD by gavage.

The animals received daily the test article dissolved in deionised, boiled water at dose levels of 5, 10, 20 and 40 mg/kg/day (free base) ; the animals of the control group were treated with the vehicle alone. All doses were given under the same volume 10 ml/kg bw.

- No treatment-related effects were noted on deaths, clinical observations, body weight growth, food intake, haematological parameters, macroscopic observations at necropsy.
- Treatment related blood chemistry changes resulted in both sexes at dose level of 5 mg/kg/day or greater (increased lactate dehydrogenase and creatine phosphokinase levels).
- Mean absolute and relative liver weights raised in males given 40 mg/kg bw/day while mean relative thyroid weights raised in females given 10 mg/kg bw/day and greater.
- Histopathological treatment findings were restricted to minimal myodegeneration noted in the skeletal muscle of 3 females given 40 mg/kg bw/day.

Under the experimental conditions adopted the NOAEL was < 5 mg/kg bw/day.

Ref. : 120

### **2.3.3. Sub-chronic oral toxicity**

A 13-week study was conducted in 150 Crl:CD(SD)BR rats (5 groups, 15 animals per sex) according to the OECD guideline N° 408 (1981). PPD was administered by gavage at corresponding dose levels of 2, 4, 8 and 16 mg/kg/day while the control group received the vehicle only (deionised boiled water). All doses were administered under a same 10 ml/kg bw volume. The animals were examined twice daily for mortality/viability and once daily for clinical signs. Food consumption and body weight were recorded weekly during pre-test and treatment period and before necropsy. Ophthalmoscopic examination was performed at pre-test and at week 13 (control and high-dose animals). Weeks 4 and 12/13, blood samples were collected for hematology and clinical biochemistry from all animals ; urine samples were collected for urinalysis. After 13 weeks, all animals were weighed and necropsied and descriptions of all macroscopic abnormalities were recorded. The major tissues and organ were collected from all animals ; absolute and relative weights were recorded at necropsy for adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thyroid, thymus and pituitary. Macroscopic and microscopic examination of a complete set of tissues from control and high dose animals were performed.

There were no mortalities or clinical signs considered related to the test article. No effect of PPD was recorded on the relative food consumption in any group. Body weights and body weight gain were unaffected by the treatment. No ophthalmologic findings related to the product tested were

noted. Concerning hematology, blood chemistry or urinalysis parameters, no changes were considered to be related to test article administration.

The mean absolute and body-weight-related liver weights were significantly increased for males given 8 mg/kg/day and 16 mg/kg/day. At the same dose levels, absolute and body weight-related kidney weights were increased for females. However, no associated histopathological changes were noted. No treatment macroscopic or microscopic findings were recorded. Histopathological examination restricted significant finding to minimal myodegeneration on skeletal muscle on 1 male and 1 female of the high dose group (16 mg/kg bw).

Based on these results, the NOAEL of PPD was established at 4 mg/kg/day.

Ref. : 121

A 12-week oral toxicity study was conducted in F344 rats (10 – 11 rats per group) with PPD administered in the diet at concentrations of 0.05 %, 0.1 %, 0.2 % and 0.4 % (or approximately 25, 50, 100 and 200 mg/kg/day respectively). Mortalities were observed at the theoretical dose of 200 mg/kg bw in 9 male rats and in 1 female rat. At the same dose level, a 50 % reduction in body weight in both sexes as compared to controls, as well as increased relative liver and kidney weights were noted. A trend toward these above observations was noted at the theoretical dose 100 mg/kg bw.

Based on these results, the NOAEL was obtained with dosing 50 mg/kg bw.

Ref. : 53

Another 13-week neurotoxicity study has been performed via gavage in young adult F344 strain rats to evaluate the potential neurotoxicity of PPD. Male and female ccl : CD BR Rats (10 rats/sex/group) were administered PPD at doses of 4, 8 and 16 mg/kg bw/day while the control group received the vehicle only (sterile water for injection). All doses were given under a same dosage volume of 10 ml/kg bw.

The animals were examined daily for mortality and clinical signs. Food consumption and body weights were recorded at least weekly. Ophthalmological examinations were performed before and at the end of dosing. Neurotoxicity evaluations were conducted before and after 4, 8 and 13 weeks of dosing according to a test battery consisting in motor activity and functional battery assessments.

There were no mortalities or clinical signs considered related to the test article. Food intake and body weight gain of treated group were similar to the controls. Neuropathology evaluations did not reveal abnormalities within the nervous system or skeletal muscle. There was no effect of the test substance on ocular tissue.

Based on these results, the NOEL was 8 mg/kg for both sexes and since the modifications observed can be assumed referring to pharmacological responses and not to neurotoxicity, the NOAEL was 16 mg/kg bw.

Ref. : 47

#### **2.3.4. Sub-chronic dermal toxicity**

A 90 day study has been carried out in the rabbit with the compound administered dermally twice weekly. Four hair-dye formulation containing 1, 2, 3 or 4 % of para-phenylenediamine and other hair-dye constituents were mixed with an equal volume of 6 % hydrogen peroxide. A dose of 1

ml/kg of this mixture was applied for 1 hour without occlusion to three application sites on six animals of each sex. The application sites were abraded prior to the first dose each week. No dose-related changes were observed on weight gain, clinical chemistry, haematology, urinalysis or on examination of the tissues at necropsy.

Ref. : 20

### **2.3.5. Chronic toxicity**

A 80-week study was performed in F344 rat in order to assess simultaneously the long term toxicity and potential carcinogenicity of PPD when administered daily in diet at concentrations of 0.05 % or 0.1 % , corresponding to approximately 25 or 50 mg/kg bw/day.

In absence of the full report of the study and basing on the information reported in Submission III, at concentrations of 0.05 % and 0.1 %, mean absolute spleen weight in females was lower than that of controls.

No other adverse effect related to a potential toxicity of PPD was observed at the dose level used.

Ref. : 53

### **2.4. Irritation & corrosivity**

#### **2.4.1. Irritation (skin)**

A 2.5% aqueous solution of PPD containing 0.05% sodium sulphite was mildly irritant when applied to abraded or intact rabbit skin covered by a gauze patch. The primary irritation index in a Draize rabbit test was estimated to be 0.3 out of a maximum score of 8.

Ref. : 67

#### **2.4.2. Irritation (mucous membranes)**

A 2.5% aqueous solution of PPD containing 0.05% sodium sulphite was not considered to be irritant when instilled into the rabbit eye (n=3) and then rinsed with water after 10 seconds. Minimal conjunctival irritation was seen in one animal only.

Ref. : 67

### **2.5. Sensitisation**

p-Phenylenediamine (PPD) is a very strong potential skin sensitisier and included in the European Standard Series for diagnostic patch testing of eczema patients.

The individual susceptibility to PPD seems to be under polymorphic control as acetylator phenotype may be important for metabolism and detoxification of PPD and represents a marker for determining individual susceptibility to PPD allergy. The submission contains a number of relevant references that support this statement. However, a number of important and more recent scientific publications is not included in the submission

p-Phenylenediamine sensitises 100% of laboratory animals (both guinea pigs and mice) used in predictive allergenicity testing if the concentration is high enough. The relative skin sensitising potency has been estimated in a mouse Local Lymph Node Assay (LLNA) by calculating the concentration of the chemical required to cause a stimulation index of 3 (EC3 value). Multiple

tests were performed in two laboratories to evaluate the intra- and inter-laboratory variation. The EC3 value for p-phenylenediamine varied between 0.06% and 0.20%.

Ref. : 126

There is plenty of evidence confirming that p-phenylenediamine is a strong clinical contact allergen in humans. The range of sensitisation responses is dependent on the vehicles, exposure condition and challenge concentration.

Standard patch tests in more than 36.000 eczema patients in Germany showed a sensitisation rate of PPD contact allergy of 4.8% after standardisation for age and sex. PPD was the 5<sup>th</sup> most frequently positive standard allergen after nickel, fragrance mix, balsam of peru and thimerosal. There was considerable regional difference in the sensitisation rates when different regions of Germany were compared.

Ref. : 112

The North American Contact Dermatitis Group reported in 1999 that in spite of its potential allergenicity, the PPD SPIN value (Significance Prevalence index Number), which corresponds to the quantitative measure of the relative clinical importance of contact allergens in the population, remained relatively stable through the periods 1984-1985 (191), 1992-1994 (197), and 1994-1996 (185). In the same time, the SPIN rank for PPD declined from 3 to 9 to 10, respectively, among the standard contact allergens. However, one should take into consideration that PPD sensitivity may be difficult to explain in some cases because PPD may cross react to the so-called para group of compounds, which contains chemicals with a similar structure used in rubber manufacturing, local anaesthetics, azo dyes used in textiles and certain drugs like sulfa antimicrobials.

Ref. : 71

In recent years several publications have reported series of cases of severe blistering dermatitis in patients who had used PPD containing skin paints (temporary tattoos). This “epidemic” of PPD contact allergies seen in young people who follow the trendy fashion of getting semi-permanent tattoos show how a new and different type of exposure to a potent contact allergen (skin paints with PPD containing henna) may lead to severe allergic contact dermatitis following the usual exposure through use of permanent hair dyes.

Ref. : 49, 66, 119, 125

There is marked inter-individual sensitivity to the PPD molecule on patch testing, with regard to both the exposure time and the concentration required. Experiments using PPD allergic patients showed that 6 of 16 reacted to 1% PPD after only 15 min exposure.

Ref. : 78

Further, recent reports have described serious contact anaphylaxis due to PPD in hair dyes. These immediate hypersensitivity reactions appear to be rare.

Ref. : 101

The prevalence of positive patch tests to PPD in consecutive eczema patients tested has remained rather stable over the last 30 years in spite of an increased usage of hair dyes. This finding may

be associated with higher dye purity, improved formulation technology, clear use instructions and warnings on package labels. However, hair dye allergic contact dermatitis is not infrequent and often leads to very severe bouts of oozing scalp dermatitis requiring specialist care and often treatment with systemic corticosteroids.

## **2.6. Reproduction Toxicity**

PPD had been tested in one group of 25 Mice receiving a subcutaneous dose of 28 mg/kg in aqueous solution on days 5 to 7, 8 to 10 or 11 to 14 of gestation. No teratogenic effect was observed.

Ref. : 96

Four hair dyes formulations containing 1, 2, 3 or 4 % PPD were rinsed with hydrogen peroxide before topical application at 2 ml/kg (corresponding to 20, 40, 60 and 80 mg/kg) to groups of 20 mated female rats on days 1, 4, 7, 10, 13, 16 and 19 of gestation.

No significant differences were found between control and treated group. No teratogenic activity was observed.

Ref. : 20

Hair dyes formulations containing 3 % PPD mixed with an equivalent solution of hydrogen peroxide were applied topically twice a week to female rats, 4 weeks prior mating and throughout mating and gestation.

No evidence of maternal toxicity or teratogenic effect was observed.

Ref. : 13

PPD was administered by gavage to pregnant female Sprague Dawley rats on day 6 through 15 of gestation at the dose levels of 5, 10, 15, 20 or 30 mg/kg/day. Pregnant animals were killed on day 20 of gestation ; visceral and skeletal malformations were recorded on the foetuses. Significant maternal toxicity was observed at 20 and 30 mg/kg/day (reduced body weight gain and decreased food consumption). No biologically or statistically significant increase in malformations or developmental variations was observed at any dose level.

Under the experimental conditions adopted, PPD revealed no teratogenic or embryo-toxic effects.

Ref. : 93

A one-generation reproduction toxicity study has been performed in the male Sprague Dawley Rat. 0.5 ml of hair dyes, one corresponding to a semi-permanent and the other to an oxidative dye, both containing 2.2 % PPD (approximately 11 mg/kg/day) was applied topically to the backs of 25 rats twice a week for 10 weeks. After the treatment period, each male ( $P_0$ ) was mated to 1 female each week for 3 weeks. One hundred F1 male offspring from these matings were mated to 1 female per week for 3 weeks. Female rats were killed between day 14 and 16 of gestation. There were no compound-related effects observed on  $P_0$  male body weight gains, percent fertility or total and average live pups per F1 litter. No evidence of reduced fertility was recorded in the F1 males.

No compound-related changes were noted in the number of implantations, dead foetuses and resorptions.

Ref. : 24

## **2.6.2. Two-generation reproduction toxicity**

A two-generation reproduction toxicity study has been performed in the male Sprague Dawley Rat (40 males and 40 females per group, 3 control groups). Hair dye formulations containing 2 %, 3 % or 4 % PPD were mixed with an equal volume of hydrogen peroxide and applied topically to the backs and necks of the animals twice a week (generation F<sub>0</sub>).

Treatment was continuous through growth, mating, gestation and lactation to the weaning of the F<sub>1A</sub> and F<sub>2B</sub> litters. No compound-related effects on survival, general appearance, food consumption, body weight gain, fertility of males or females or on gestation, lactation or weaning indices were observed.

Ref. : 26

## **2.7. Toxicokinetics (incl. Percutaneous Absorption)**

### **2.7.1. Toxicokinetics**

The distribution kinetics of 3H-labeled PPD were studied after intravenous and percutaneous administration in rabbits and in mice. Biphasic blood clearance was observed, with half-lives of 24 minutes and 43.5 hours being recorded. Rapid percutaneous absorption was observed in Rabbits. Subsequent tissue distribution studies were performed in Mice. Blood concentrations rose steadily for the first 24 h. 35 hours after application, 0.13 % of the applied radioactivity was detected per ml of blood.

Ref. : 94

The kinetics of 3H-labeled PPD hydrochloride were studied in the aqueous chamber of the rabbit eye after intraocular, subconjunctival, intravitreal (one eye only; the opposite eye served as control) or subcutaneous administration. There was rapid penetration of the dye from the application site ; detectable amounts of radioactivity were found in the aqueous fluid within the first 15-30 minutes after application. Peak concentrations in treated eyes were observed within 30 minutes (local drops or subconjunctival administration) to one hour (intravitreal or subcutaneous administration). Peak radioactivity was observed in the control eye within 30 minutes of application of test material. Clearance from the aqueous chamber was observed to be biphasic (minutes and hours for local drops, intravitreal injection and subconjunctival injection; hours and days for subcutaneous injection). About 5 % of the peak radioactivity remained in the aqueous chamber 4 days after application.

Ref. : 95

The absorption, distribution, metabolism and excretion of PPD in a 1:1 solution of ethanol and Emulphor EL-620 was studied in both sexes of F344 Rats and B6C3F1 Mice. Each species received single oral dose of either 60 or 600 µmol/kg 14C-radiolabeled PPD. In addition, animals from each species received an intravenous dose of 600 µmol/kg radiolabeled PPD. Data were collected from three animals per sex at each dose level at various time points over a 3-day period. Gastrointestinal absorption was nearly complete in both species. Excretion was not greatly affected by either the dose level or the route of administration. Initial tissue distribution was roughly proportional to estimated tissue volume in both species. Species and sex differences were observed with regard to clearance of tissue radioactivity, distribution and metabolism. PPD was readily absorbed, distributed to all major tissues examined and metabolised to several

metabolites rapidly cleared from the body, through the urine and through the feces to a lesser extent. No tissue specificity was seen.

Covalent binding of PPD or its metabolites to hepatic protein was observed in both sexes of each species. In contrast, no covalent binding with hepatic DNA was observed in either species.

Ref. : 55

## 2.7.2. Percutaneous absorption

### Percutaneous absorption *in vitro*

The experiments used Frantz static cells, and Dulbecco PBS containing antioxidant was used as receptor fluid. Female abdominal or breast skin was obtained at autopsy or from cosmetic surgery. Human hair was obtained from a female Caucasian volunteer. The integrity of skin membranes was tested by use of tritiated water prior to commencement of the study. Diffusion cells with high rates of water permeability or anomalously high values for PPD permeation were eliminated from the study. The percutaneous absorption of ring <sup>14</sup>C-labeled PPD to human skin over 48 hours was evaluated under 5 different dosing conditions (including a 30-minute post application aqueous rinse of the skin to mimic “in-use” conditions). The dosing conditions were :

- a) 100 mg/cm<sup>2</sup> of 1.3% PPD and other dyes in the presence of developer, but the absence of hair.
- b) 100 mg/cm<sup>2</sup> of 1.3% PPD and other dyes in the presence of developer and hair.
- c) 100 mg/cm<sup>2</sup> of 2.7% PPD, but no other dyes, developer or hair.
- d) 20 mg/cm<sup>2</sup> of 2.7% PPD, but no other dyes, developer or hair.
- E 100 mg/cm<sup>2</sup> of 1.3% PPD, but no other dyes, developer or hair.

30 cells were included in study A and 15 in the four remaining studies. 5 mg/cm<sup>2</sup> of hair was placed on the skin surface before addition of the formulation in the second dosing condition (B) described above.

### Results

The skin penetration was between 0.1% and 0.2% of the applied dose. This corresponded to a cumulative mass absorbed of about 1.9-2.4 µg/cm<sup>2</sup> for the complete dye formulations. The amount of radioactive material found in the skin itself ranged from 0.04-0.5% or 0.65-6.72 µg/cm<sup>2</sup>. For all formulations, the maximum cumulative absorption of PPD occurred 4 hours post application. This was followed by a slowing of the permeation caused by the removal PPD by the 30-minute aqueous rinse. Permeation was concentration and dose related. The presence of hair on the surface did not significantly affect the permeation process. A greater amount of PPD was found on or in the skin (but not in the receptor fluid) when it was applied in the presence of developer and other dyes and in the presence of hair. The study also included mass balance calculations showing a recovery rate between 83.6% and 104%. In conclusion this study has given results very close to the permeation levels found in vivo in humans (Wolfram and Maibach study, 1985). These percentage values corresponded to cumulative mass absorbed of about 1.9-2.4 µg/cm<sup>2</sup> for the in vitro studies and 4.5 µg/cm<sup>2</sup> for the in vivo studies.

Ref. : 2

### Percutaneous absorption *in vivo*

This study is published in a peer reviewed journal. A commercially available hair dye product (Nice'n Easy Blue Black) containing 2.7% of PPD was enriched with ring  $^{14}\text{C}$ -labelled PPD. Scalp penetration under condition of hair dye usage was evaluated for both rhesus monkey and man. The study included 5 human volunteers and 3 rhesus monkeys. The 2 species showed a remarkable similar pattern of dye penetration. Their amount absorbed was quantified on the basis of the percentage of radioactivity excreted in the urine following the application of known amount of labelled compound. Urine collection was continued as long radioactivity was recoverable in humans, and in the monkeys for 7 days. The total dose excreted in the urine in humans was  $0.190 \pm 0.06\%$ , and  $0.182 \pm 0.06\%$  in monkeys. Using these data, Kalopissis calculated the total exposure based on cumulative mass absorbed per scalp to be 3129  $\mu\text{g}$  equivalent to  $4.47 \mu\text{g}/\text{cm}^2$  based on an estimated scalp area of  $700 \text{ cm}^2$ .

Ref. : 58, 129

An analytical method was developed to determine PPD derivatives in urine samples collected from women after hair dyeing with commercial formulation. Five volunteers participated in the experiments. Metabolites of PPD were hydrolysed and measured using gas-chromatography mass-spectrometry. In the study the excretion of metabolites of PPD was followed for 2 days after the dye had been applied. The dose related excretion for PPD as measured by this method was comparable to that found by other authors who made use of tracer labelled material.

Ref. : 43

### 2.8. Mutagenicity/Genotoxicity

A paper indicates that the time between the preparation of the PPD solution and its use in the Ames test may influence the mutagenicity observed; DMSO used as a solvent may also influence the mutagenic response of the TA98 and TA1538 in the presence of a metabolic activation system.

*Comments :*

The absence of information about the chemical purity of the sample of PPD makes the results irrelevant.

Ref. : 25

A compound formed as a reaction product between Resorcinol and p-Phenylenediamine in the presence of an oxidising agent such as hydrogen peroxide, has been tested for mutagenicity in the Ames test, five strains (TA1538, TA100, TA1537, TA1538, TA98) in the absence and presence of an activation system provided by Aroclor treated rat liver microsomes.

The doses ranged from 5  $\mu\text{g}$  to 1000  $\mu\text{g}$  in two independent experiments.

The chemical tested was synthesised and purified by re-crystallisation, and analysed by nuclear magnetic resonance spectroscopy (NMR $^1\text{H}$ ). The results indicate in all strains and all conditions that the chemical (green dye) was not mutagenic. In the same 5 strains of *S. typhimurium*, and in all conditions Resorcinol (pure commercial product from Hoechst) was not mutagenic

*Comments :*

As p-Phenylenediamine, the other partner reactive chemical present in many hair dye formulations was not included in this test, no conclusions can be drawn about the possible origin of mutagenicity of PPD combined with other chemicals.

Shahin et al. mentioned that in their previous tests PPD was mutagenic in the Ames test when Aroclor induced S9 was used.

Ref. : 108

\* Commercial samples of analytical grade p-Phenylenediamine have been tested for mutagenicity on TA98 strain of *Salmonella typhimurium* in the presence and absence of S-9 mix obtained from rat-livers treated with Aroclor. Doses tested : 0-2 mg/plate. Replicate plates : 3  
*Results* : increase of ca. 100 fold the control value

\* Purified p-Phenylenediamine (from the commercial sample) was tested on the same strain in the same conditions

*Results* : no increase in mutagenicity.

\* A commercial sample of Resorcinol was tested on the same strain under the same conditions and was found non mutagenic.

\* An oxidation product obtained from a reaction between purified p-Phenylenediamine and Resorcinol , tested on the same strain of *Salmonella typhimurium*, in the same conditions was found to increase 5 fold the value of the control.

This reaction product has been identified as the “green compound”, an oxidised conjugation product of PPD and Resorcinol, distinct and different from the Brandowsky’s base.

\* The percutaneous absorption of the oxidation products administered topically to rats produced the presence in the urine of the treated animals of relevant mutagenicity (3 fold increase over the control urine from untreated animals).

*Conclusions*

- Commercial p-Phenylenediamine is mutagenic on strain TA98 of *Salmonella typhimurium* in the presence of a metabolic activation system.
- Purified p-Phenylenediamine is not mutagenic on strain TA98 of *Salmonella typhimurium*.
- Commercial Resorcinol is not mutagenic on TA98 strain of *Salmonella typhimurium*.
- An oxidised conjugation product between Resorcinol and p-Phenylenediamine (green compound) is mutagenic on TA98 strain of *Salmonella typhimurium*.
- The urine collected from rats treated topically with the “green compound” are mutagenic on TA98 strain of *Salmonella typhimurium*.
- Brandowsky’s base was not included in these experiments.

Ref. : 30

A technical grade sample of pure p-Phenylenediamine ( $\geq 99\%$ ) was mixed with Resorcinol and hydrogen peroxide at concentrations relevant to practical hair dyeing procedure : the mixture was assayed on the bacterial mutation system (TA98 + S9 mix), in the mammalian gene mutation

system (mouse lymphoma L5178Y cell), and in the chromosomal aberration system (human lymphocytes).

The mixture has been evaluated after preparation and after 30 min of incubation at 37° C.

#### *Bacterial gene mutation assay*

p-Phenylenediamine was mutagenic after 30 min of incubation when combined with H<sub>2</sub>O<sub>2</sub> on TA98 of *Salmonella typhimurium*.

In combination with Resorcinol this mixture was not mutagenic.

#### *Mammalian gene mutation assay*

p-Phenylenediamine was not mutagenic in the mouse lymphoma assay in the presence of H<sub>2</sub>O<sub>2</sub> alone, or in combination with Resorcinol .

#### *Chromosomal aberration assay*

p-Phenylenediamine was clastogenic when mixed with H<sub>2</sub>O<sub>2</sub>.

In combination with Resorcinol the same mixture was not clastogenic.

#### *Further experiments*

By this experiment M. Bracher et al. have demonstrated that p-Phenylenediamine alone, or in combination with Resorcinol, m-Aminophenol, 1-(2-hydroxyethyl)-amino-3,4 methylenedioxybenzene) or other couplers is mutagenic in the Ames test (TA98 + S9 mix) when the mixture is incubated for several hours.

#### *Comments*

From all these experiments it can be concluded that p-Phenylenediamine in mixture with H<sub>2</sub>O<sub>2</sub> or other complex is mutagenic on *Salmonella typhimurium* strain TA98 when the mixture is incubated for 30-60 min or more.

The mixture of p-Phenylenediamine with H<sub>2</sub>O<sub>2</sub> is clastogenic on human chromosomes tested *in vitro*.

Ref. : 16

C. Burnett et al. reported the results from testing of urine collected from female users of black hair dye of unknown formulation, before and 24 hours after hair dying.

The test for mutagenicity of the urine concentrates was performed on the strain TA1538 of *Salmonella typhimurium*.

The results from 30 persons were all negative.

*Comments*

The strain employed in the analysis (TA1538) is the less sensitive to the hair dye. The hair dye formulations employed in the analysis were different from those found mutagenic by Ames *et al.* (1975) to which paper Burnett *et al.*, refer in their study.

Ref. : 23

*In Vivo Micronucleus Assay of p-Phenylenediamine*

Animals	:	CD1 MICE, 5 animal/dose/group
Doses	:	25, 28, 100 mg/kg
Sampling times	:	24, 48, 72 hours
Positive control	:	TEM 1.5 mg/kg
Purity	:	commercial sample, not defined
Rationale for the doses :		200 mg/kg killed 3/5 animals. 100 mg/kg was the highest dose tested

*Results :*

p-Phenylenediamine did not induce micronuclei in mice. The ratio PCE/NCE in one treatment at 24h (100 mg/kg) in one at 48 h (25 mg/kg) and in two treatments at 72 h (50 and 100 mg/kg) was slightly reduced (1.2 in the control, 1.0 at 25 mg/kg, 0.9 at 50 mg/kg, 0.8/0.9 at 100 mg/kg). In some of these cases the no. of MN was higher than the control, although not statistically significant (1.6, 1.4, 1.6 vs. 1.3 in the control).

Ref. : 113

The studies, reference numbers 28, 59, 102, 105, 118, 128, were not relevant for the evaluation of the mutagenicity of PPD, as they did not report individual data concerning the chemical under evaluation.

*Summary of the results :*

1. p-Phenylenediamine when tested as a commercial chemical is mutagenic on *Salmonella typhimurium* in the presence of a metabolic activation system (TA98) and induces chromosomal aberrations on mammalian cells grown *in vitro*.  
The mechanism of the two mutagenic effects could be different.
2. Stored solutions (4 hours) of p-Phenylenediamine alone or mixed with H<sub>2</sub>O<sub>2</sub> or other couplers are mutagenic on *Salmonella typhimurium*.
3. The oxidised conjugation product (green chemical) obtained by the reaction between p-Phenylenediamine and Resorcinol, in the presence of an oxidising agent (hydrogen peroxide) is mutagenic on *Salmonella typhimurium* (TA98).  
When this reaction product is topically applied to shaved rats, the urine of these animals contain a fraction (green chemical) which is mutagenic on *Salmonella typhimurium*.
4. p-Phenylenediamine has been found positive for the induction of gene mutations in the mouse lymphoma assay (with and without metabolic activation, NTP: Colipa Subm.III)

5. p-Phenylenediamine has been found positive for the induction of chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells (Shelby and Stasiewicz, 1984; Colipa Subm.III and for the induction of chromosomal aberrations (dose related increase) in Chinese hamster ovary K-1 cells (Chung K.T. *et al.* Colipa Subm.III)
6. p-Phenylenediamine when tested as a purified chemical is not mutagenic on *Salmonella typhimurium*

### *Conclusions*

1. p-Phenylenediamine has been found positive for the induction of gene mutation in bacterial and mammalian cells grown *in vitro*, positive for the induction of chromosomal aberrations and sister chromatid exchanges in mammalian cells grown *in vitro*.  
The micronucleus assay on mice *in vivo* has produced negative results.
2. When combined with Resorcinol and oxidising agents (hydrogen peroxide) p-Phenylenediamine has given rise to a mutagenic reaction product on bacterial cells, and in the urine assay from rats topically treated with the reaction product;
3. p-Phenylenediamine, when combined with H<sub>2</sub>O<sub>2</sub> is mutagenic on bacterial cells and clastogenic in human lymphocytes;
4. p-Phenylenediamine when combined with other couplers, including Resorcinol, is mutagenic in bacterial cells, if the mixture is incubated for 1 hour or more.

The opinion expressed by SCC in the plenary meeting of 4-5<sup>th</sup> November 1991 has been taken into consideration when re-evaluating the dossier.

## **2.9. Carcinogenicity**

### **Short-term test for rat liver carcinogenesis**

The effect of PPD on liver carcinogenesis was investigated in male F344 rats initially treated with N-nitrosodiethylamine (DEN). Two weeks after a single dose of DEN, rats were given PPD at dietary levels of 110, 330 and 1000 ppm for 6 weeks. At week 3 following the N-nitrosodiethylamine treatment, all animals were subjected to partial hepatectomy. PPD did not significantly increase the level of  $\gamma$ -glutamyl transpeptidase-positive foci observed after nitrosoethylamine initiation. Increased levels were found after treatment with the positive control 3'-methyl-4-dimethylaminoazobenzene

Ref. : 47

### **Topical administration**

Three oxidation hair dye formulations containing 1.5% PPD, mixed with an equal volume of 6% hydrogen peroxide, were tested by topical application in groups of 100 mice weekly or once

every two weeks for 18 months. No evidence of carcinogenic activity was seen. One of the formulations contained in addition to PPD, 2,4-toluenediamine, the second contained 2,4-diaminoanisole, and the third contained m-phenylenediamine.

Ref. : 19

In a 2-year mouse-skin-painting (Swiss-Webster mice) study with a weekly dermal application of PPD at 1.5% in a hair dye formulation mixed with hydrogen peroxide, there were no significant differences between treated and control groups. The hair dye formulation did also contain 2,4-toluenediamine, 2,5-toluenediamine and resorcinol.

Ref. : 42

Swiss mice, 7 weeks old, groups of 50 females received 5 or 10% PPD applied in 0.02 ml (0.05 or 0.1 µg; 1 cm diameter; 0.13 µg/cm<sup>2</sup>) acetone twice weekly in a shaved area of interscapular skin. 100 untreated females served as controls and 40 DMBA females were kept as positive controls. The mice were allowed to die spontaneously or were killed when moribund. No treatment-related epidermal hyperplasia, ulceration or dermatitis were observed. The positive controls showed 67 tumours including papillomas. A number of treated mice had tumours in other organs, but the incidence was not statistical different from that of untreated controls.

Ref. : 115

Rabbits (strain not stated), 8 weeks old, groups of 5 females received 5 or 10% PPD applied in 0.02 ml acetone twice weekly to the inside of the left ear. The experiment was terminated at week 85. 5 untreated females served as controls and 5 DMBA females were kept as positive controls. No treatment-related local changes were observed in the ears. The positive controls showed 15 proliferating papillomas.

Ref. : 115

Four hair dye formulations containing 1,2,3 or 4% PPD mixed with an equal volume of 6% hydrogen peroxide just prior to use were evaluated for carcinogenic potential following skin application once weekly (1.5 cm<sup>2</sup>) to Swiss mice, 6 – 8 weeks old, groups of 50 males and 50 females for two years. No gross or histological abnormalities attributable to compound administration were observed.

Ref. : 3

The same hair dye formulation as above were applied to rats obtained from the first litter of a multigeneration reproduction study, twice weekly for two years. No compound-related or microscopic neoplastic lesions were observed.

Ref. : 54

A two-generation reproduction study and chronic toxicity-carcinogenicity study were conducted in Sprague-Dawley rats receiving topical applications of six oxidative hair-coloring formulations. PPD was one of the dyes tested the other dyes include 2,4-toluenediamine and 2,4-diaminoanisole. Each dye was mixed with an equal volume of 6% hydrogen peroxide prior to

application. In the reproduction study, PPD at a concentration of 2%, 3% or 4% was applied twice weekly throughout the growth, mating, gestation and lactation phases of the F<sub>0</sub> parents to the weaning of the F<sub>1a</sub> and F<sub>2b</sub> litters. Weanlings selected from the F<sub>1a</sub> litters were the subjects for the lifetime carcinogenesis study. For 24 months, they received topical administrations of PPD twice weekly. Five animals/sex/group were killed at 12 months; the remainder of the animals were killed at 24 months. All were necropsied; their tissues were subjected to histological evaluation. No compound-related increases in neoplasms were observed.

Ref. : 26

### **Topical administration and subcutaneous injection**

Wistar rats, a total of 40 males and 40 females were divided into four groups (10 males and 10 females each). Group 1 was painted on shaved skin on the back with 0.5 ml of a 1:1 mixture of 5% PPD (in 2% NH<sub>4</sub>OH) and 6% H<sub>2</sub>O<sub>2</sub> once a week for 18 months. Group 2 was given s.c. injection of 0.1 ml of a 1:1 mixture of 5% PPD (in 2% NH<sub>4</sub>OH and 1.8% NaCl) and 6% H<sub>2</sub>O<sub>2</sub> at the hips every other week for 18 months. Groups 3 and 4 received topical application and s.c. injection respectively, with corresponding vehicles only and served as controls.

#### *Application to skin*

The topical application resulted in a slight decrease in the bodyweights of the males after 30 weeks of exposure. No such effects were found among the females. The total PPD exposure of the rats during the eighteen months of treatment was 975 mg (12.5 mg/treatment). 40% (4/10) of the males developed tumours (1 cholangiocarcinoma and 1 adenoma of the liver, 1 nephroblastoma with lung and pancreas metastasis, 1 cortical adenoma of adrenal gland) and 60% (6/10) females developed tumours (1 fibromatosis and 5 mammary gland tumours which include fibrosarcoma, fibroadenoma and adenoma). The first mammary tumours in the female rats were observed after 47 weeks. The others were observed after 49, 60, 72 and 85 weeks. No tumour was found in the 10 male control rats, while 1 tumour (stromal cell sarcoma of the uterus) was found in the female control rats.

#### *Subcutaneous injection*

The total PPD dose during the eighteen months was 97.5 mg in the s.c. study. One male rat (14.3%; 7 effective animals) developed both a follicular carcinoma of thyroid and undifferentiated carcinoma of the lung. Among the 7 female rats, 6 (85.7%) developed tumours, 4 soft tissue tumours (includes unclassified sarcoma and lipoma) and 4 mammary gland tumours (ductectasia or adenosis), and 3 uterus tumours (includes adenocarcinoma, endometrial polyp and glandular cystic hyperplasia). No tumours were found among the control animals.

### *Conclusions*

The authors points out that it is of particular interest to notes that the mammary gland of female rats was the primary target organ of the oxidation product of PPD. This organ has also been shown to be highly susceptible to tumour induction by 2,4-diaminotoluene and a wide range of N-substituted aromatic amines. The mechanism by which these compounds induce the tumours

may be related to the presence of N,O-acyltransferases in the mammary gland which are responsible for the formation of N-acyloxyarylamin es. The latter compounds may in turn cause errors in the cellular genome by an introduction of arylamine-substituents at C-8 of guanine derivatives. Subsequently these errors are amplified by hormonal influences on the mammary gland. The authors also points out that after oxidation by H<sub>2</sub>O<sub>2</sub> in the presence of NH<sub>4</sub>OH, PPD becomes strongly mutagenic to *Salmonella typhimurium*.

Ref. : 97

A study was carried out using 4 hair dyes containing p-phenylenediamine and 1 hair dye containing 2,5-diaminotoluene. Type and composition is given in Table 1.

**Table 1.** Types and chemical composition of hair dyes

Sample	Type	Chemical composition	Content (%)
1	Water	PPD Resorcinol	4.65 3.25
2	Water	PPD Resorcinol	4.32 2.28
3	Water	PPD	5.93
4	Cream	2,5-Diaminotoluene Resorcinol 2-Nitro-1,2-diaminobenzene 4-Nitro-1,2-diaminobenzene	2.9 1.0 0.15 0.06
5	Powder	PPD	2.33

For application to skin, the hair dyes were oxidised with 6% hydrogenperoxide at the ratio 1:1 by volume or weight 20-30 min before test. For subcutaneous injection, hair dyes were oxidised with 6% hydrogenperoxide at the ratio 1:1 by volume and NaCl added to a concentration of 0.9% in the final oxidation mixture. Sample 5 was mixed with water to a final concentration of 125 mg/ml and NaCl added. Control group comprised 3% hydrogenperoxide, 0.9% NaCl and 2% NH<sub>4</sub>OH.

#### *Application to skin*

Groups of 10 males and 10 females Wistar rats were painted once a week for 18 months. The experiment was terminated after 24 months. The treatment did not influence the weight of the animals. The tumours induced are shown in Table 2.

**Table 2.** Incidence of tumours in rats treated with hair dyes by skin painting.

Sample	Sex	Rats with tumours %	Tumour type				
			Soft tissue	Mammary gland	Uterus	Thyroid	Others
Control	M	0 (0/10)			1		
	F	11 (1/9)					
1	M	20 (2/10)	1		1	1	
	F	89 (8/9)	3	6	1	1	
2	M	22 (2/9)					2 <sup>A</sup>
	F	60 (6/10)		4	2		
3	M	20 (2/10)	1	1			
	F	60 (6/10)		5	1	1	
4	M	30 (3/10)		1		1	1 <sup>B</sup>
	F	50 (4/8)		2	2		

<sup>A</sup>Kidney transitional cell carcinoma and bronchial adenoma<sup>B</sup>Angioma of liver*Subcutaneous injection*

Groups of 10 males and 10 females Wistar rats received subcutaneous injection in the back hip every 2 week for 18 months. The experiment was terminated after 24 months. The treatment did not influence the weight of the animals. The tumours induced are shown in Table 3.

**Table 3.** Incidence of tumours in rats treated with hair dyes by subcutaneous injections.

Sample	Sex	Rats with tumours %	Tumour type				
			Soft tissue	Mammary gland	Liver	Thyroid	Others
Control	M	0 (0/9)					
	F	0 (0/10)					
1	M	67 (6/9)	5	1			
	F	78 (7/9)	4	2			1 <sup>A</sup>
2	M	50 (4/8)	4				
	F	60 (6/10)	3	5	1	1	1 <sup>B</sup>
3	M	22 (2/9)			1		1 <sup>A</sup>
	F	100 (9/9)	4	8		1	2 <sup>C</sup>
5	M	20 (2/10)	1	1			
	F	56 (5/9)	2	3		1	

<sup>A</sup>Salivary gland<sup>B</sup>Mucin producing adenocarcinoma of small intestine<sup>C</sup>Includes glandular cystic hyperplasia of endometrium and hypertrophy

The authors conclude that all 5 samples of hair dyes caused benign and malignant tumours in various organs of exposed rats in both sexes. Particularly, soft tissue tumours were increased in both sexes and mammary gland tumours were increased in female rats. The mammary gland tumours found in some groups of rats were malignant type.

Ref. : 98

### **Oral administration**

Fischer 344 rats and B6C3F1 mice, groups of 50 males and 50 females (6 weeks old), were exposed to 625 or 1250 ppm PPD dihydrochloride for 103 weeks. After the 103 week period of compound administration, there were additional observation periods of 2 weeks for rats and 1 week for mice before the remaining animals were sacrificed. Twenty animals of each sex were used as controls. There were no significant positive association between the concentration of PPD administered and mortality in rats or mice of either sex. Slight dose-related mean body-weight depression was observed in female rats and the mean bodyweights among high dose male rats and dosed female mice were slightly depressed in relation to their respective controls, indicating that the concentrations of PPD dihydrochloride administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. It was concluded that under the condition of this bioassay, there were no convincing evidence that dietary administration of PPD was carcinogenic in Fischer 344 rats or B6C3F1 mice.

Ref. : 86

F344 rats, 6 weeks old of both sexes, were divided into 3 groups each. Group 1 and 2 of 63-66 rats of each sex were fed ad lib. diet containing 0.05% (500 ppm) and 0.1% (1000 ppm) PPD respectively. Group 3 of 24-25 rats of each sex were fed the basal diet for the control. The animals were killed after 80 weeks or when they became moribund. There was no relation between the average body weight and the concentration of PPD in male rats, but the body weight of the female rats given 0.1% PPD was slightly less than of the controls. The survival was not affected significantly by the treatment. It is concluded that PPD was not carcinogenic to F344 rats of either sex when given orally.

Ref. : 53

### **Intraperitoneal injection**

PPD was studied in strain A mice in two different laboratories (A and B). The experiment started when the animals were 6-8 weeks old. The animals received intraperitoneal injections 3 times a week for 8 weeks. Laboratory A. Groups of 10 or 20 males and 10 or 20 females received injections with 12.5 and 25 mg/kg with PPD. 10% (1/10) (0.10 tumours per mice) of the low dosed males and 11% (1/9) (0.10 tumours per mice) of low dosed females developed lung tumours. 20% (3/15) (0.20 tumours per mice) of the high dosed males and 29% (4/14) (0.36 tumours per mice;  $P<0.05$ ) of high dosed females developed lung tumours. 13% (7/54) (0.167 tumours per male) of control males and 11% (6/54) (0.11 tumour per mice) of control females developed lung tumours. Laboratory B. Groups of 30 males were used and the animals received injections with 6.4, 16 or 32 mg/kg PPD. The percent survivors with tumours were 13% (3/23) (0.13 tumours per mice), 27% (7/26) (0.31 tumours per mice), and 30% (7/23) (0.30 tumours per mice) among the low, medium and high dosed animals respectively. Among the control males 33% (8/24) (0.42 tumours per mice) developed tumours. Thus, with an exception of female mice in Laboratory A, all experiments were negative.

Ref. : 73

## Transplacental carcinogenicity

Pregnant NMRI mice (a group of 22 animals) were administered 30 mg/kg PPD in soy bean oil by gavage once a day from pregnancy day 10 through day 19 (a total of 10 administrations). A positive control group was administered urethane (300 mg/kg) and vehicle administered (10 ml/kg). The F1 generation numbered 95 males and 95 females in the PPD group, 110 males and 99 females in the urethane group and 77 males and 81 females in the vehicle control group. Total observation time was 137 weeks. PPD did not affect bodyweight or survival in dams or offspring, while the offspring of urethane treated dams had both lower survival rates and lower bodyweights compared to vehicle controls. Tumours occurred 31.2% of the PPD-treated animals as compared to 30.5% in vehicle control animals and 70.9% in the urethane-treated animals. The most commonly observed tumours were lymphomas and alveolar adenomas in all groups. When the incidence of alveolar adenomas was calculated in F<sub>1</sub> females, a slight statistically significant increase was observed in PPD-treated animals compared to the vehicle controls ( $p=0.04$ ). No increase in overall tumour incidence occurred in PPD treated dams or in their offspring.

Ref. : 50

## Neonatal carcinogenesis

In a neonatal carcinogenicity study, 5 day old male and female NMRI mice (51 males and 55 females) were injected intraperitoneally with 30 mg/kg/d PPD for 5 days. Positive control animals received 300 mg/kg/d urethane and vehicle control animals received 10 mg/kg/d soy bean oil. Total observation time was 130 weeks. Treatment with PPD did not affect survival or bodyweight. Tumours occurred in 30.1% of the PPD-treated animals as compared to 18.2% in vehicle control animals and 82.1% in urethane-treated animals. The most commonly observed tumours were lymphomas and alveolar adenomas in all groups. The incidence of these tumours (both sexes) is shown in Table 4.

**Table 4.** Tumour incidence.

Tumour type	Vehicle control (10 ml/kg/day)	pPD (30 mg/kg/day)	Urethane (300 mg/kg/day)
Lymphoma	10.4%	18.3%	12.5%
Alveolar adenoma	9.1%	10.8%	76.8%

PPD exposure did not change the frequency of lymphomas ( $P>0.10$ ) or alveolar adenomas ( $P>0.37$ ). A slight, statistically significant increase in overall tumour incidence was calculated for PPD-treated males ( $p=0.03$ ).

Ref. : 51

## Carcinogen classification

IARC has classified p-phenylenediamine as a category 3 carcinogen based on no data in human studies and inadequate data in animal studies. This classification was carried out in 1978. p-Phenylenediamine is classified by Germany as a 3 B carcinogen.

## Conclusion

Two skin painting and subcutaneous injection studies with PPD and hair dyes containing PPD showed and increase number of tumours in both male and females rats after administration of PPD + hydrogenperoxide. It was also found that this mixture was mutagenic in the Salmonella test. Other skin painting studies in mice with hair dye preparations containing PPD were negative. These studies did more or less mimic the use of hair dyes and the amounts of PPD used were very small. In similar experiments with known carcinogenic hair dyes no response was observed. No tumours were found in two oral studies, a neonatal study or after intraperitoneal injection of PPD in strain A mice.

There seems to be no evidence for carcinogenicity of PPD alone, however, the available data suggest that PPD together with hydrogenperoxide form a genotoxic carcinogen.

### **2.10. Special investigations**

#### **2.10.1. Rhabdomyolysis**

Skeletal muscle necrosis has been experimentally induced in 14 Dogs using single oral dose of 50, 80 or 100 mg/kg PPD. Marked increased serum creatine phosphokinase was observed in almost all animals, the effect appearing dose-related ; massive necrosis of the skeletal muscle was observed histopathologically dose-related. Animals receiving 80 mg/kg were the most severely affected.

Ref. : 125

These effects seem related to the disruptive effect of PPD on the respiration of skeletal muscle mitochondria

Ref. : 64

Another report quotes calcium leakage from the sarcoplasmic reticulum as a mechanism of skeletal muscle damage.

Ref. : 126

An additional study was performed in the Rat receiving subcutaneous administration of 40 µM/day of N-methyl derivatives of PPD for 3 days. Skeletal muscle necrosis was observed following mitochondrial oxidation. This parameter was considered as important in the initiation of the myotoxic activity observed.

Ref. : 79

#### **2.10.2. Biomedical markers in the skin**

Several studies have been performed in Guinea-pig to assess the effect of dermal application of 1 %, 3 % or 4 % PPD with or without hydrogen peroxide for up to 30 days on specific enzyme levels and other biochemical markers in skin. Statistical variations were observed in skin levels of acid phosphatase, alkaline phosphatase, tyrosinase, β-gluconidase, histamine and other enzymes. Histopathology of the skin was observed since the 1<sup>st</sup> day of dermal application with a preparation containing 1 % PPD, the deficiency being consistent after 7 days.

Ref. : 70, 71, 72

## 2.11. Safety evaluation

### CALCULATION OF THE MARGIN OF SAFETY

(p-PHENYLENEDIAMINE)

(Oxidative)

**The maximum concentration of 4.0 % of p-Phenylenediamine is mixed before use with H<sub>2</sub>O<sub>2</sub>. Thus the usage volume of 100 ml contains at maximum 2.0 %**

Highest penetration	PA ( $\mu\text{g}/\text{cm}^2$ )	=	4.47 $\mu\text{g}/\text{cm}^2$
Typical body weight of human		=	60 kg
Exposed area (scalp)		=	700 $\text{cm}^2$
Systemic exposure	PA x 700 $\text{cm}^2$	=	3.129 mg
Systemic exposure dose (SED)	PA x 700/ 60 x 1000	=	0.052 mg/kg bw
No observed adverse effect level (mg/kg) (species, route of application)	NOAEL	=	4 mg/kg bw

Margin of Safety	:	NOAEL / SED = 77
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## 2.12. Conclusions

PPD is used as an oxidising colouring agent for hair dyeing up to a maximum permitted concentration of 4% (free base) in the finished (marketed) cosmetic product. However, in combination with hydrogen peroxide, the maximum use concentration upon application is 2% (free base).

- \* PPD was not irritant or corrosive for the skin and the eye when applied in a 2.5% aqueous solution.
- \* PPD is a strong contact allergen, both experimentally and in clinical experience.
- \* Several studies on systemic toxicity have shown that the most sensitive target organ is skeletal muscle, with a rhabdomyolysis being experimentally observed following oral application in the rat at levels down to 10 mg/kg bw. From a 90 day study, a NOAEL of 4 mg/kg bw was obtained and is used as the basis for the safety evaluation. Reproductive toxicity studies clearly

established that PPD has no teratogenic or embryotoxic effects by oral route at doses up to 80 mg/ kg bw.

\* Several relevant studies have been performed on percutaneous absorption of PPD. The highest cumulative penetration obtained was 4.47 µg/cm<sup>2</sup>. This leads to a margin of safety of 77.

\* PPD is mutagenic in bacterial cells and mutagenic/clastogenic in mammalian cells *in vitro*. In combination with an oxidising agent or several couplers, PPD is also mutagenic in bacterial cells and clastogenic in mammalian cells *in vitro*. A micronucleus study in mice indicated the absence of clastogenicity *in vivo*, although some equivocal results.

*In vivo* studies with the combinations have not been submitted.

\* There are indications derived from experimental investigations in rats that PPD may be carcinogenic upon long-term topical application in combination with hydrogen peroxide.

## **2.13      Opinion**

The SCCNFP is of the opinion that the information submitted is insufficient to allow an adequate risk assessment to be carried out.

Before any further consideration, additional data would be required on carcinogenicity of PPD in combination with hydrogen peroxide and couplers (to simulate consumer exposure) upon application to rat's skin, and on *in vivo* mutagenicity/ genotoxicity of the said combinations.

## **2.14.     References**

1. Ames, B. et al (1975) Hair Dyes are Mutagenic : Identification of a Variety of Mutagenic Ingredients Proc. Nat Acad. Sci. 72,2423
2. An-Ex, Cardiff, UK (1997). Skin penetration of p-Phenylenediamine from hair dye formulations - *In vitro* assessment. Report n° CO5/19X/97
3. Antti Seppala. Evaluation of toxicity and carcinogenicity of 12 hair dye formulations. Eppley Institute for Research in Cancer, 1978.
4. Angelini G. et al (1985) Contact Dermatitis Due to Cosmetics. J. Appl. Cosmetol. 3, 223-236
5. Antti Seppala (1978) Evaluation of Toxicity and Carcinogenicity of 12 Hair Dye Formulations. Eppley Institute for Research in Cancer
6. Antunes M.A. et al (July 1998) Acute Dermatitis after Application of Hair Dyes. Fourth Congress of the European Society of Contact Dermatitis, Helsinki, P-9
7. Annstrong,, D.K.B. et al (1999) Occupational sensitisation to p-Phenylenediamine : a 17-year review. Contact Dermatitis 41, 348-349
8. Ashraf W. et al (1994) Systemic Paraphenylenediamine (PPD) Poisoning : A Case Report and Review. Human & Experimental Toxicology 13, 167-170
9. Averbukh, Z. et al (1989) Rhabdomyolysis and Acute Renal Failure Induced by Paraphenylenediamine. Human Toxicol. 8, 345-348
10. Baskettler D.A. and Goodwin, B.F.J. (1988) Investigation of the prohapten concept. Contact Dermatitis, 19, 248-53

11. Basketter, D. et al (1994). The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test
12. Baud, F. et al (1983) Rhabdomyolysis in Para-phenylenediamine Intoxication. Lancet 2 (8348), 514
13. Biodynamics Inc. (1977) A modified Segment II Teratology Study of Hair Dyes in Mice
14. Blijlevens (1977) Mutagenicity of Four Hair Dyes in *Drosophila melanogaster*. Mutation Research 48, 181-6
15. Blijlevens (1981) Mutation Research 90, 137-41
16. Bracher, M. et al (1990) Studies on the Potential Mutagenicity of p-phenylenediamine in oxidative hair dye mixtures. Mutation Research 241, 313-23
17. Brown, J. et al (1987) Chronic Renal Failure Associated with Topical Application of Paraphenylenediamine. British Medical Journal 294, 155
18. Buehler, E.V. (1985). A rationale for the selection of occlusion to induce and elicit delayed contact hypersensitivity in the Guinea Pig. Curr. Probl. Derm. 14, 39-58
19. Burnett, C. et al (1975) Long-term Toxicity Studies on Oxidation Hair Dyes. Fd. Cosmet. Toxicol. 13, 353
20. Burnett, C. et al (1976) Teratology and Percutaneous Toxicity Studies on Hair Dyes. Journal of Toxicology and Environmental Health 1, 1027-1040
21. Burnett, C. et al (1977) Dominant Lethal Mutagenicity Studies on Hair Dyes. Journal of Toxicology and Environmental Health 2, 657-662
22. Burnett C. Environmental Mutagen Society meeting Colarado Springs (1977).
23. Burnett, C. et al (1979) Mutagenicity studies on urine concentrates from female users of dark hair color product. Drug Chem. Toxicol. 2(3), 283-93
24. Burnett C et al (1981). Heritable translocation study on two hair dye formulations. Fundam Appl Toxicol 1, 325-328
25. Burnett, C. et al (1982) The Effect of Dimethylsulfoxide on the Mutagenicity of the Hair Dye p-Phenylenediamine. Mutation Research 103, 1-4
26. Burnett C M et al (1988). Multigeneration reproduction and carcinogenicity studies in Sprague-Dawley Rats exposed to oxidative hair-colouring formulations containing p-Phenylenediamine and other aromatic amines. Fd Chem Toxic 26(5), 467-474
27. Caspary W. J., Langenbach R., Penman B. W., Crespi C., Myhr B. C., Mitchell A. D. Mut. Res. 196: 61-81 (1988).
28. Chung, K.T. et al (1996) Effects of the nitro-group on the mutagenicity and toxicity of some benzamines. Environ. Mol. Mutagen. 27(1), 67-74
29. CIR Expert Panel (1985) Final Report of the safety assessment of p-Phenylenediamine Journal of the American College of Toxicology, 4(3), 203-266
30. Crebelli R., Conti L., Carere A., Mutagenicity of commercial p-Phenylenediamine and of an oxidation mixture of p-Phenylenediamine and Resorcinol in *Salmonella typhimurium* TA98. Zito R. Fd. Cosmet. Toxicol. 19: 79-84 (1981).
31. Dethloff, L A. et al (1996) Toxicological Comparison of a Muscarinic Agonist Given to Rats by Gavage or in the Diet. Food Chem. Toxicol. 34 (4), 407-22
32. Dooms-Goossens, A. et al (1989) Comparative Patch Testing with PPD-Base and PPD-Dihydrochloride : Human and Animal Data Compiled by the European Environmental Contact Dermatitis Research Group in : Current Topics in Contact Dermatitis, Springer-Vedag, , part 3, 281-285
33. Dunkel V. C., Schechtman L. M., Tu A. S., Swak A., Lubet R. A., Cameron T. P. Environ. Mol. Mut. 12: 21-31 (1988).
34. Dybing E., Thorgeirsson S. S. Biochem. Pharmacol. 26: 729-34 (1977).

35. Edman, B. et al (1982) Trends and forecasts for standard allergens in a 12-year patch test material. Contact Dermatitis 8, 95-104
36. El-Ansary, E. H. et al (1983) Systemic Toxicity of Para-phenylenediamine. Lancet 1(8337), 1341
37. Elsner, P. (Feb. 1995) Trends in Contact Sensitization in Zurich, Switzerland. American Contact Dermatitis Society Annual Meeting, Abstract 8, New Orleans, Louisiana
38. Fregert, S. et al (1968) Epidemiology of contact dermatitis Trans St. John's Hospital Dermatol. Soc. 55, 17-35
39. Gad, S. et al (1986). Development and validation of an alternative dermal sensitization test : the mouse ear swelling test (MEST). Toxicol Appl Pharmacol 84, 93-114
40. Garner R. C., Nutman C. A. Mut. Res. 44: 9-19 (1977).
41. Gentile J. M., Gentile G. J., Plewa M. J. Mut. Res. 188: 185-96 (1987).
42. Giles, A. L et al (1976) Dermal Carcinogenicity Study by Mouse Skin Painting with 2,4-Toluenediamine Alone or in Representative Hair Dye Formulations J. Toxicol. Environ. Health 1, 433-440
43. Goetz, N. et al (1988) Percutaneous absorption of p-Phenylenediamine during an actual hair dyeing procedure. Int J of Cosm Science 10, 63-73
44. Goh, C. L. (1987) Allergic Contact Dermatitis from Cosmetics. J. of Dermatol 14, 248 - 252
45. Gola, M. et al (1992) GIRDCA Data Bank for Occupational and Environmental Contact Dermatitis (1984 to 1988) American Journal of Contact Dermatitis 3, 179-188
46. Hammershoy, O. (1980) Standard patch test results In 3,225 consecutive Danish patients from 1973 to 1977. Contact Dermatitis 6, 263-268
47. Hagiwara, A., Tamono, S., Shibata, M.A., Arai, M., Tsuda, H. Lack of modifying effects of p-phenylenediamine on induction of gamma-glutamyl traspeptidase-positive foci in a medium-term bioassay system using F344 rats. Toxicol Lett 52: 261-268, 1990.
48. Haskell Laboratory for Toxicology and Industrial Medicine, Newark Delaware USA (1992). Subchronic oral neurotoxicity study of H.18508 in Rats. Report 854-91
49. Hausen B, Kaatz M, Jappe U, Stephan U, Heidbreder G. Henna/p-Phenylenediamin-Kontaktallergie Deutsches Arzteblatt. Jg 98. Heft 27. 6.7.2001
50. Holmberg, B., Kronevi, T., Ackevi, S., Ekner, A. Prövning av carcinogen aktivitet hos p-fenyllendiamin med peroral administrering på gravida möss (transplacentalförsök). Arbete och Hälsa 32: 1-44, 1983.
51. Holmberg, B., Kronevi, T., Ackevi, S., Ekner, A. Prövning av carcinogen aktivitet hos p-fenyllendiamin med intraperitoneal injektion på nyfödda möss (neonataförsök. Arbete och Hälsa 33: 1-35, 1983
52. Hossack, D. et al (1977) Examination of the Potential Mutagenicity of Hair Dye Constituents Using the Micronucleus Test. Experientia 33, 377
53. Imalda, K. et al (1983). Carcinogenicity and toxicity tests on p-Phenylenediamine in F344 Rats. Toxicol. Letter 16, 259-269
54. International Research and Development Corporation (1979) Lifetime Chronic Toxicity/Carcinogenesis Study in Rats. Final report
55. Ioannou, Y.M. et al (1985). p-Phenylenediamine dihydrochloride : comparative disposition in male and female rats and mice. J of Toxicol and Environ Health 16, 299-313
56. Ishihara, M. et al. (1983) Basic Studies on Contact Dermatitis Due to Hair Colorings and Cold Permanent Wave Solutions JNCI 70,443-446
57. Jouhar, A. J. (1979) Bristol Myers Company Limited
58. Kalopissis, G. (1986) Toxicology and Hair Dyes. In : The Science of Hair Care, Zviak, C (ed.), Marcel Dekker, New York, 287-308

59. Kerckaert, G.A., Leboeuf R.A., Isfort R.J. Assessing the predictiveness of the Syrian Hamster Embryo Cell Transformation Assay for determining the rodent carcinogenic potential of single ring aromatic/nitroaromatic amine compounds. *Toxicological Sciences* 41, 198-197, 1998.
60. Kiese, M. et al (1968) The Absorption of Some Phenylendiamines Through the Skin of Dogs *Toxicology and Applied Pharmacology* 12, 495
61. Korossy, S. et al (1969) Zür Revision des Allergenspektrums der Ungarn gebräuchlichen diagnostischen Standard-Reihe für Epikutantestung. *Berufsdermatosen* 17. 252-263
62. Krasteva, M. (1993) Study on the immuno-allergology of delayed contact dermatitis to paraphenylenediamine. PhD thesis, Medical Academy of Sofia, Bulgaria
63. Krause, W. et al (1991) Comparative Pharmacokinetics of Abecamil in Rat Following Single and Multiple Intragastric Treatment and Continuous Administration via the Diet. *Drug Metabolism and Disposition* 19 (1), 29-35
64. Kvelland I. *Hereditas* 100: 295-8 (1984).
65. Kwalek J. C., Hallmark R. K., Andrews A. W. *JNCI* 71: 293-8 (1983).
66. Le Coz C, Lefebre C, Keller F, Grosshans E. Allergic contact dermatitis caused by skin painting (pseudo tattooing) with black henna, a mixture of henna and p-phenylenediamine and its derivatives. *Arch Dermatol* 2000; 136: 1515-1517.
67. Lloyd, G. K. et al (1977) Assessment of the acute toxicity and potential Irritancy of hair dye constituents. *Fd. Cosmet. Toxicol.* 15, 607
68. L'Oréal, Aulnay-sous-bois., France (1996) Etude comparative de la réactivité in vitro de cinq colorants capillaires vis-à-vis de mitochondries isolées de muscles de Rat.. Report n° : TM 96/001
69. Lynde, C. W. et al (1982) Screening patch tests in 4190 eczema patents 1972-81 *Contact Dermatitis* 8, 417-421
70. Maibach, H. I. et al (1981) Percutaneous Penetration of Hair Dyes. *J. Soc. Cosmet. Chem.* 32, 223-229
71. Maouad, M. et al (1999) Significance-prevalence index number : A reinterpretation and enhancement of data from the North American Contact Dermatitis Group. *J. Am. Acad. Dermatol.* 41(4), 573-576
72. Marks, J. G. et al (1995) North American Contact Dermatitis Group Standard Tray Patch Test Results (1992 to 1994) *American Journal of Contact Dermatitis* 6, 160-165
73. Maronpot R.R., Simkin M.B., Witschi L.H., Smith L.H., Cline J.M. Strain A mouse pulmonary tumor test results for chemicals previously tested in the National Cancer Institute carcinogenicity tests. *JNCI* 76: 1101-1112, 1986.
74. Mascres, C. et al (1974) A study of the pathogenesis of muscle lesions induced by p-Phenylenediamine. *L'Union Médicale du Canada* 103, 672-677
75. Mathur, A.K. et al (1990) Biochemical and histopathological changes following dermal exposure to paraphenylenediamine in Guinea Pigs. *J Appl Toxicol* 10(5), 383-386
76. Mathur, A.K. et al (1992) Dermal toxicity of paraphenylenediamine. *Biomedical and Environmental Sciences* 5, 321-324
77. Mathur, A.K. et al (1995) Effects of dermal exposure to paraphenylenediamine in Guinea Pigs. *J. Toxicol –Cut. & Ocular Toxicol.* 14(3), 207-210
78. McFadden JP, Wakelin SH, Holloway DB, Baskett DA. *Contact Dermatitis* 39; 79-81,1998
79. Mitchell, A.D. et al (1988) *Env. Mol. Mut.* 1 (supl 13):37-101
80. Mitchell A. D., Rudd C. J., Caspary W. J. *Env. Mol. Mut.* 1 (suppl 13): 37-101 (1988).
81. Mohn. University of Leiden.

82. Moneghini, C. L et al (1980) La paraphénylénediamine dans les dermites allergiques de contact Med et Hyg. 38, 1577-1581
83. Müller, (1976) Untersuchung von 1,4-Diaminobenzol auf Mutagenität im Bakterientest. Batelle Institut, Frankfurt
84. Munday, R. et al (1989) Muscle necrosis by N-methylated p-Phebylenediamine in Rats: structure-activity relationships and correlation with free-radical production In Vitro Toxicology 57, 303-314
85. Modée, J. et al. (1962) A comparison of results of patch tests in 1951 and in 1961. Acta Dermato-venereol. 42, 280-289
86. National Cancer Institute (NCI) (1978) Bioassay of p-Phenylenediamine Dihydrochloride for Possible Carcinogenicity. DHEW Publication N° (NIH) 79-1730
87. Nethercott, J. K et al (1991) Patch Testing With a Routine Screening Tray in North America, 1985 Through 1989 : II. Gender and Response. Am. Journal of Contact Dermatitis 2, 130-134
88. Nielsen, N. H. et al (1993) Sensibilisation de contact aux constituants des cosmétiques dans une population danoise non sélectionnée. La Glostrup allergy study, Danemark. Ann. Dermatol. Venereol. 120, 33-38
89. Nishi K., Nishioka H. Mut. Res. 104: 347-50 (1982).
90. Nishioka H. Mut Res 38: 345 (1976).
91. Nohmi T., Miyata R., Yoshikawa K., Ishidate M. J. J. Tox. Sci. 7: 61-9 (1982)
92. Oliveira, H. et al (July 1998) Reactivity to 35 standard allergens in a normal population. Fourth Congress of the European Society of Contact Dermatitis, Helsinki P-105
93. Re, T.A. et al (1981) The absence of teratogenic hazard potential of p-Phenylene diamine in Sprague-Dawley Rats. Fund Appl Toxicol 1, 421-425
94. Rehani M.M. et al (1981). Distribution kinetics of 3H-labeled p-Phenylenediamine - A hair dye. Indian J Med Res 74, 129-134
95. Rehani M.M. et al (1979). Aqueous chamber kinetics of the 3H-labelled hair dye. Bull P.G.I. 13(4), 211-15
96. Reprotox (1978) Prüfung auf embryotoxische Wirkung an der Maus : p-Phenylendiamin
97. Rojanapo, W et al (1986) Carcinogenicity of an Oxidation Product of p-Phenylenediamine Carcinogenesis 7 (12), 1997-2002
98. Rojanapo, W et al (1986) Carcinogenicity of Hair Dyes used in Thailand. Thai Cancer Journal 12, 43-56
99. Romaguera, C. et al (1980) Statistical and comparative study of 4600 patents tested in Barcelona 1973-1977. Contact Dermatitis 6, 309-315
100. Rudner, E. J. et al (1975) The frequency of contact sensitivity in North America 1972-74. Contact Dermatitis 1; 277-280
101. Sahoo B, Handa S, Penchallaiah K, Kumar B. Contact Dermatitis 43; 244, 2000
102. Sasaki, Y.F., et al (1999) The alkaline single cell gel electrophoresis assay with mouse multiple organs: results with 30 aromatic amines evaluated by the IARC and U.S. NTP Mutation Research 440,1-18
103. Schutz, K. H. (1976) Comparable Studies of Sensitization of Different Hair Dye Ingredients. University of Hamburg
104. Segre, (1977) Rapport concernant les essais de toxicité aiguë chez la souris. Universita di Siena
105. Seiler, J.P. (1977) Inhibition of testicular DNA synthesis by chemical mutagens and carcinogens. Preliminary results in the validation of a novel short term test. Mutation Research 46(4), 305-10
106. Sertoli, A. et al. (1999) Epidemiological Survey of Contact Dermatitis in Italy (1984-1993)

- by GIRDCA (Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali). American Journal of Contact Dermatitis 10, 18-30
107. Shahin, M. et al (1979) Personal communication, L'Oréal Laboratories
108. Shahin, M.M. et al (1980) Studies on the mutagenicity of resorcinol and hydroxy-3-(p-amino)anilino-6,N-[(p-amino)phenol]benzoquinone-monoimine-1,4 in *Salmonella typhimurium*. Mutation Research 78(3), 2113-18
109. Sharma, P. P. et al (1990) Dose-dependent Pharmacokinetics and Teratogenic Activity of Topical Retinoids. Toxicologist 10 (1), 237
110. Shelby, Stasiewicz (1984)
111. Sir Hashim, M. et al (1992) Poisoning from henna dye and para-phenylenediamine mixtures in children in Khartoum. Annals of Tropical Paediatrics 12, 3-6
112. Schnuch A, Geier J, Uter PJ et al. National rates and regional differences in sensitisation to allergens of the standard series. Contact Dermatitis 1997; 37: 200-209.
113. Soler-Niedziela, L. et al (1991) Studies on three structurally related phenylenediamines with the mouse micronucleus assay system. Mutation Research 259, 43-48
114. Spector, W. S, ed. (1956) Handbook of Toxicology, Volume 1. Acute toxicities of solids, liquids and gases to laboratory animals. W.B, Saunders Co., Philadelphia, 232
115. Stenbeck, F. G. et al (1977) Noncarcinogenicity of Hair Dyes : Lifetime Percutaneous Applications in Mice and Rabbits. Fd. Cosmet. Toxicol. 15, 601-606
116. Storrs, F. J. et al (1989) Prevalence and relevance of allergic reactions in patients patch tested in North America - 1984 to 1985. J. Am. Acad. Dermatol. 20,1038-1045
117. Thompson C. Z., Hill L. E., Epp J. K., Probst G. S. Environ. Mut. 15: 803-11 (1983).
118. Topham, J.C. (1980) The detection of carcinogen-induced sperm-head abnormalities in Mice. Mutation Research 69(1), 149-55
119. Tosti A, Pazzaglia M, Betazzoni M. Contact allergy from temporary tattoos. Arch Dermatol 2000; 136: 1061-1062.
120. Toxicol Laboratories Limited, Herefordshire, England (1993). Paraphenylenediamine. 14-day oral (gavage) range-finding toxicity study in the rat. Report n° LRL/43/93
121. Toxicol Laboratories Limited, Herefordshire, England (1995). Paraphenylenediamine - 13-week oral (gavage) toxicity study in the rat. Report n° LRL/44/94 (1995)
122. Uter, W. et al (1998) Epidemiology of contact dermatitis. The information network of Departments of Dermatology (IVDK) in Germany. Eur. J. Dermatol 1, 36-40
123. Veien, N. K. et al (1992) Patch Test Results From a Private Dermatologic Practice for Two Periods of 5 Years With a 10-Year Interval. American Journal of Contact Dermatitis 4,189-192
124. Venitt S., Searle C. E. INSERM 52: 263-72 (1975).
125. Wakelin SH, Creamer D, Rycroft RJG, White IR, McFadden JP. Contact dermatitis from paraphenylenediamine used as a skin paint. Contact Dermatitis 1998; 39: 92-93.
126. Warbrick EV, Dearman RJ, Basketter DA, Kimber I. J Appl Toxicol 19; 255-260, 1999
127. Watanabe T., Hirayama T., Fukui S. Mut. Res. 245: 15-22 (1990).
128. Williams, G.M. et al (1982) Reliability of the hepatocyte primary culture/DNA repair test in testing of coded carcinogens and noncarcinogens. Mutation Research 97(5):359-70
129. Wolfram, L. J. et al (1985) Percutaneous Penetration of Hair Dyes. Arch Dermatol Res 277, 235-241
130. Yabe, K et al (1991) An Experimental Rhabdomyolysis due to Paraphenylenediamine Contained in Hair Dyes. Res Pract Forens Med 34, 109-115
131. Yabe, K. et al (1992) The effect of a p-Phenylenediamine containing hair dye on the  $\text{Ca}^{2+}$  mobilization in the chemically skinned skeletal muscle of the Rat. Nippon Hoigaki Zasshi 46(2), 132-140