EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Hydroxyethyl-p-phenylenediamine Sulfate

COLIPA N° A80

Adopted by the SCCP
during the 2nd plenary meting of 7 December 2004

TABLE OF CONTENTS

1. BACKGROUND

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.

2. TERMS OF REFERENCE

The SCCNFP is requested to answer the following questions:

* Is Hydroxyethyl-p-phenylenediamine sulfate safe for use in cosmetic products?

* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

3. ASSESSMENT

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Hydroxyethyl-p-phenylenediamine sulfate (INCI)

3.1.1.2. Chemical names

2-(2-Hydroxyethyl)-1,4-phenylenediammonium sulfate
2-(2-Hydroxyethyl)-p-phenylenediammonium sulfate
3-(2-Hydroxyethyl)-p-phenylenediammonium sulfate
2,5-Diammonio-phenylethanol sulfate 1-β-Hydroxyethyl-
2,5-diammoniobenzene sulfate
1,4-Diammonio-2-β-hydroxyethyl-benzene sulfate

The respective older synonyms below, which do not comply with the IUPAC nomenclature rules, are used in the submitted toxicological studies:

2,5-Diamino-phenylethylalcohol sulfate
1-β-Hydroxyethyl-2,5-diaminobenzene sulfate
1,4-Diamino-2-β-hydroxyethyl-benzene sulfate

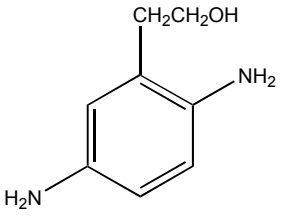
3.1.1.3. Trade names and abbreviations

Oxytol B (sulfate salt)
Oxytol A (dihydrochloride salt)

3.1.1.4. CAS / EINECS number

CAS : 93841-25-9
EINECS : 298-995-1

3.1.1.5. Structural formula



. H_2SO_4

3.1.1.6. Empirical formula

Formula : $C_8H_{12}N_2O$. H_2SO_4

3.1.2. Physical form

Grey powder

3.1.3. Molecular weight

Molecular weight : 248 (sulfate)

3.1.4. Purity, composition and substance codes

Purity : circa 99%
It exists as a free base, a hydrochloride and a sulfate. It is used as a sulfate.

3.1.5. Impurities / accompanying contaminants

No data submitted

3.1.6. Solubility

Soluble in water and methanol; slightly soluble in ethanol, insoluble in isopropanol, acetone, chloroform.

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow} : /

3.1.8. Additional physical and chemical specifications

Organoleptic properties	:	/
Melting point	:	198-202 °C (with decomposition)
Boiling point	:	/
Flash point	:	/
Vapour pressure	:	/
Density	:	/
Viscosity	:	/
pKa	:	/
Refractive index	:	/

General comments on analytical and physico-chemical characterisation

The following properties do not or poorly comply with the basic requirements for proper characterisation:

* No data are given on impurities
* No data are given on the stability of the substance (as such and in a formulation)
* No data on Log P_{ow} and on the density of the test substance
* No quantitative data were reported on its solubility

3.2. Function and uses

Hydroxyethyl-p-phenylenediamine sulfate is used as an oxidative hair dye at a maximum use concentration of 3 % (1.5 % in combination with H_2O_2).

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline	:	/
Species/strain	:	Wistar rat (SPF); CF1 mice (SPF)
Group size	:	5 male + 5 female rats; 10 female mice
Test substance	:	Hydroxyethyl-p-phenylenediamine dihydrochloride
Purity	:	/
Batch no	:	/
Dose levels	:	rat : 50, 100, 200, 300 and 400 mg/kg bw/day, by gavage mice: 50, 100 and 150 mg/kg bw
Observation period	:	14 days
GLP	:	/

A 1.0 % aqueous test solution was administered by gavage to 25 female (circa 187 g) and 25 male (circa 194 g) Wistar rats and 50 female CF1 mice (circa 26 g). Single doses of 50, 100, 200, 300 and 400 mg/kg bw were administered to groups of 5 male and 5 female rats; single doses of 50, 100 and 150 mg/kg bw to groups of 10 female mice.

During the observation period of 14 days, mortalities and signs of toxicity were recorded. All animals were dissected.

Results
20 minutes after administration, the test compound caused moderate sedation and ataxia. No changes were observed in organs.
The LD_{50} was calculated as 150 mg/kg bw in male and female rats and as 90 mg/kg bw in mice.
The substance was considered to be moderately toxic.

Ref.: 1

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline	:	/
Species/strain	:	Pirbright white guinea pig (SPF)
Group size	:	15 female
Test substance	:	Hydroxyethyl-p-phenylenediamine dihydrochloride
Purity	:	/
Batch no	:	/
Dose levels	:	3 % aqueous test solution
Observation period	:	daily, 5h p.a. for 5 days
GLP	:	

Draize test. The compound, as dihydrochloride (3 % in aqueous solution) applied daily for 5 days to the clipped skin area (3 x 4 cm), without washing off, of 15 female Pirbright White guinea pigs resulted not irritating (skin reactions evaluated daily 5 h post treatment).

Comment: the hair dye is used as sulfate.

Ref.: 3

3.3.2.2. Mucous membrane irritation

Guideline	:	/
Species/strain	:	Pirbright white guinea pig (SPF)
Group size	:	5 female
Test substance	:	Hydroxyethyl-p-phenylenediamine dihydrochloride
Purity	:	/
Batch no	:	/
Dose levels	:	0.1 ml of 1.5 % aqueous test solution

Observation period	:	0.5, 1, 2, 3, 4, 6, 7 and 24 hours after application
GLP	:	/

Draize test. The compound as dihydrochloride instilled (1.5 % in water, 0.1 ml) into the conjunctival sac of one eye (without washing) of 5 female Pirbright guinea pigs resulted not irritating after a 24-hour (examinations with 0.1 % fluoroscein sodium solution) observation period (eye reactions evaluated at 0.5, 1, 2, 3, 4, 5, 6, 7 and 24 hours).

Comment: the hair dye is used as sulfate.

Ref.: 2

3.3.3. Skin sensitisation

Guideline	:	/
Species/strain	:	Pirbright white guinea pig (SPF)
Group size	:	20 test and 20 control (10 positive and 10 negative)
Test substance	:	1-β-Hydroxyethyl-2,5-diamino benzene
Purity	:	/
Batch no	:	/
Dose levels	:	3 % dermal injection Challenge: 1, 2 and 3 % in distilled water
Observation period	:	24 and 48 h
GLP	:	/

Guinea pig maximisation test of Magnussen and Kligman. Sensitisation was tested in 10 male and 10 female Pirbright guinea pigs treated with 3 % intradermal injections and closed dermal topical application (including Freund's complete adjuvant FCA) of test compound on the clipped shoulder area. Challenge reaction by closed patch test on day 14 after the last exposure with 1 %, 2 % and 3 % in distilled water. The compound caused no skin reactions (reading at 24 and 48 hours).

Ref.: 4

Comment: the hair dye is used as sulfate.

3.3.4. Dermal / percutaneous absorption

Guideline	:	/
Species/strai	:	Sprague Dawley rats
Group size	:	3 males and 3 females per group
Method:		urine and faeces excretion, carcass and organs analysis after topical application and oral administration by gavage
Test substance	:	Hydroxyethyl-p-phenylenediamine sulfate (radiolabelled ^{14}C) in commercial formulations with and without hydrogen peroxide 1.47 %
Reference	:	Hydroxyethyl-p-phenylenediamine sulfate (radiolabelled ^{14}C) in water 4.88 %
Batch no	:	unknown
Purity	:	unknown
Dose levels	:	1.63 mg/cm² for the formulations with or without hydrogen peroxide (total area treated 9 cm²)

		1.67 mg/cm² for the aqueous solution (total area treated 9 cm²) 3 mg for the aqueous solution (0.3 %) administered orally by gavage
Contact duration	:	30 minutes, then washing of the skin and monitoring of the diffusion during 72 hours
Analysis	:	liquid scintillation
GLP	:	in compliance

Results

The experimental variability is very high. The mean percutaneous absorption *in vivo* calculated from the excretion and residual amounts in the carcass is low : 0.063 ± 0.063 % of the dose when the substance is applied without hydrogen peroxide, and 0.077 ± 0.074 % of the dose when the substance is applied with hydrogen peroxide. This is corresponding to 1.03 to 1.26 µg/cm². For the control formulation, the amount absorbed is 0.124 ± 0.097 % of the applied dose (2.07 µg/cm²). The radioactivity was excreted predominantly via urine (75 to 86 %) than via the faeces (14 to 25 %).

After topical application, the concentrations in the organs were near the detection limit (thyroids, adrenals, brain, testes, bones).

After oral administration the test substance is excreted via urine (86 %) and to a less extent via faeces (14 %). Highest concentrations were obtained in thyroids, liver and adrenal. Lowest were detected in the testes, fat and femur.

When considering the residual amount of material present in the skin at 72 hours, the total amount absorbed is corresponding to 15 µg/cm² for the formulation without hydrogen peroxide or to 35 µg/cm²with hydrogen peroxide. For the control formulation, the absorption is equivalent to 7.5 µg/cm². These data show clearly the influence of the formulation on the absorption of the dye.

Conclusion

This study is inadequate for the measurement of the percutaneous absorption.

Ref.: 19

Guideline	:	Draft OECD, May 1995
Tissue	:	Porcine skin dermatomed 1000 µm
Method	:	Flow through diffusion cells
Test substance	:	1,4-Diamino-2-(2-hydroxyethyl) benzene sulfate (radiolabelled ^{14}C) in commercial formulation
Batch no	:	L 728444
Purity	:	unknown
Receptor fluid	:	"physiological" unknown composition, solubility of the test substance in this liquid not documented, flow 5 ml/hour.
Skin temperature	:	32°C
Dose levels	:	1.618 % (with or without hydrogen peroxide)
Contact duration	:	15 minutes, then washing of the skin and monitoring of the diffusion during 72 hours
Replicate cells	:	6 cells for each condition (with and without peroxide)
Skin integrity	:	checked with tritiated water
GLP	:	in compliance

The skin penetration of ^{14}C 1,4-Diamino-2-(2-hydroxyethyl) benzene sulfate was evaluated in a flow through diffusion cell system. Porcine skin was dermatomed 1000 µm thick and kept

frozen. The test substance was prepared at a concentration of 1.618 % in a commercial formulation with or without hydrogen peroxide. Circa 100 mg of the formulation were applied per cm^2 of skin. Integrity of the epidermal membrane was checked by measurement of tritiated water diffusion before the study.

Results

The quantity of test substance penetrating through the skin to the receptor fluid corresponded to:
- 0.02 ± 0.006 % of applied dose (0.324 ± 0.097 µg/cm²) in the absence of peroxide
- 0.017 ± 0.005% of applied dose (0.275 ± 0.081 µg/cm²) in the presence of peroxide.

In the skin at the end of the 72 hours the amount of substance recovered are:
- 0.147 ± 0.023 % of applied dose (2.378 ± 0.372 µg/cm²) in the absence of peroxide
- 0.317 ± 0.091% of applied dose (5.129 ± 1.472 µg/cm²) in the presence of peroxide.

Because the stratum corneum was not separated from the living epidermis, the total amount recovered in the skin concerns the full tissue. In this case the total amount absorbed is:
- 0.167 % of applied dose (2.702 µg/cm²) in the absence of peroxide
- 0.334 % of applied dose (5.404 µg/cm²) in the presence of peroxide.

For a surface of contact of 500 cm² and a body weight of 60 kg, the body load is corresponding to:
- 22.5 µg/kg body weight in the absence of peroxide
- 45.0 µg/kg body weight in the presence of peroxide.

This study is characterized by an insufficient time (15 minutes) allowed for contact between the skin and the formulation. So the data obtained cannot be used for a correct evaluation of the body load after a 30 minutes exposure.

Ref.: 21

Remark: the studies performed *in vivo* in human volunteers (ref. 14) and *in vitro* on pig skin (ref. 20) are considered inadequate.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data submitted

3.3.5.2. Sub-chronic (90 days) oral toxicity

Guideline	:	/
Species/strain	:	Sprague Dawley albino rat
Group size	:	50 male and 50 female

Test substance	:	Oxytol B (Hydroxyethyl-p-phenylenediamine sulfate)
Purity	:	/
Batch no	:	C281 183
Dose levels	:	0, 5, 25, 40 and 40 (recovery) mg/kg
Observation period	:	90 day
GLP	:	/

Hydroxyethyl-p-phenylenediamine sulfate (10 ml/kg) was administered by gavage daily to groups of 10 male and 10 female Sprague Dawley rats at doses 0, 5, 25, 40 and 40 (recovery) mg/kg/day for 90 days.

Food consumption and body weight gain were normal. From the 11^{th} to 13^{th} week, orange coloured urine was observed and the frequency of this observation increased with the higher doses. No ophthalmoscopic and haematological changes, weight deviations and macroscopic changes of the organs were found. All histomorphological findings were not test substance dependent. The mean GOT and GTP values of the highest dose group increased during week 13 in comparison with the control group. The no-effect level (NOEL) was set at 25 mg/kg bw.

Ref.: 5

Guideline	:	OECD 408 (1981)
Species/strain	:	Borchen Dawley rat
Group size	:	12 male and 12 female per test and control group
Test substance	:	Hydroxyethyl-p-phenylenediamine dihydrochloride
Purity	:	99 %
Batch no	:	/
Dose levels	:	5 ml/kg (control), 25mg/kg (test)
Observation period	:	12 week application
GLP	:	/

The substance (hydrochloride) was administered daily by gavage to 12 male and 12 female SPW Wistar rats for 12 weeks at a dose of 25 mg/kg/bw. A control group of the same size received 5 ml/kg water only.

Food and water consumption and body weight gain were normal. There were no haematological, clinico-chemical and ophthalmoscopical changes. No urine coloration was observed. There were no macroscopical findings in the organs and no weight deviations. A complementary histological examination of the organs of 5 males and 5 females did not show any significant difference with the control group. The no effect level was set at 25 mg/kg/bw.

Ref.: 6, 7

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

Bacterial Reverse Mutation Test

Guideline, not described
No dose range finding assay has been performed;
No description of toxicity;
No description of the results;

The substances tested were different (free base and sulfate *salt)*

Conclusions
Safety assessment cannot be performed due to the inadequacy of this dossier. It should be noted that AT tester strains have not been used as requested by OECD guidelines 471.

Ref.: 15, 17, 18

Guideline	:	/
Species/strain	:	*Salmonella typhimurium*, TA 97, TA98, TA100,
Replicates	:	Only one test
Test substance	:	OxytolB (1,4 diamino-2-beta-hydroxyethylbenzene sulfate)
	:	OxytolA (1,4 diamino-2-hydroxymethylbenzene dihydrochloride)
Batch no	:	Not described (or unreadable; purity not described)
Concentrations	:	OxytolB 0.5 –500 µg/plate with and without metabolic activation
GLP	:	in compliance

Liver S9 fraction from rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system.

Results
COLIPA A80 has been tested for gene mutation in *Salmonella typhimurium* using a plate incorporation protocol. No dose range finding assay has been performed; no description of toxicity; no description of the results; no replicates; only 3 tester strains have been used instead of the battery of 5.

Conclusions
Assessment cannot be performed due to the inadequacy of the dossier.

Ref.: 16

***In Vitro* Mammalian Cell Gene Mutation Test**

Guideline	:	/
Species/strain	:	L5178Y cell line / $TK^{+/-}$ Locus
Replicates	:	yes but no independent exp.
Substance	:	BW 26 12 (Hydroxyethyl-p-phenylenediamine sulfate)
Batch no	:	not indicated
Purity	:	> 99 %
Treatment time	:	not described
Replicate	:	only 1 experiment with or without metabolic activation
GLP	:	in compliance

Liver S9 fraction from rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system.

Results
Some sporadic increase was observed at 500 µg/ml in the absence of activation system. However, due to the extremely low survival rate (6 %) associated at this positive result, its biological relevance is questionable. For the remaining concentrations/replicates, no other increases were observed in the presence or in the absence of activation.

Conclusions
From the results generated in only one experiment, it might be concluded that A 80 give negative results in this test. However, no independent experiment was performed.
The study is considered inadequate.

Ref.: 9

***In Vitro* Mammalian Chromosome Aberration Test**

Guideline	:	/
Species/strain	:	Chinese Hamster Ovary (CHO) cells
Replicates	:	Duplicate cultures, only one test
Test substance	:	BW 2612
Batch no	:	/
Purity	:	> 99 %
Concentration	:	62.5 – 250 µg/ml with metabolic activation 0.625 – 2.5 µg/ml without metabolic activation
Exposure	:	2 h : +S9 mix 24 h : – S9 mix.
GLP	:	in compliance

Liver S9 fraction from Wistar rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system.

Results
100 cells with 19 chromosomes were scored for aberrations. (2n=19). With or Without S9 mix, no statistically significant increased in the number of cells with structural chromosomal aberration were observed.

Conclusions
The study provided gives negative results for all doses tested. However, no independent experiment was performed. The study is inadequate.

Ref.: 10

***In Vivo* Mammalian Erythrocyte Mouse Micronucleus Test**

Guideline	:	/
Species/strain	:	Mice, CD-1
Group size	:	5 male + 5 female per dose
Test substance	:	Hydroxyethyl-p-phenylenediamine sulfate in 1% carboxymethylcellullose

Batch no	:	V 8580
Dose levels	:	0, 60, 120, 240 mg/kg bw
Administration	:	Two repeated oral gavage, 24 h interval
Sacrifice times	:	6 hours post dosing
GLP	:	not in compliance

The test substance has been investigated for induction of micronuclei in the bone marrow cells of CD-1 mice. The substance was administered twice by single gavage at 60, 120 & 240 mg/kg bw and the bone marrow harvested after 6 hours post last dosing. Negative and positive controls were in accordance with the OECD guideline.

Results
Mean values of micronucleated PCE
No statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic cells over the concurrent vehicle control values was observed.

PCE/NCE ratio
Groups of mice treated with the test substance did not exhibited variation of the PCE/NCE ratio and it cannot be estimated if the test substance has reached the bone marrow.

Conclusions
Under the conditions of the test, it can be concluded that there was no evidence of induced chromosomal or other damage leading to the micronucleus formation in polychromatic erythrocytes treated mice. No evidence that the compound had reached the bone marrow cells was indicated.

Comment: The study on toxicokinetics (ref. 19) indicates the distribution of the test substance into the bone marrow.

Ref.: 11

In Vivo Mammalian Sister Chromatid Exchanges Test

Guideline	:	/		
Species/strain	:	Chinese Hamsters		
Replicates	:	no		
Substance	:	A80		
Batch no	:	/		
Administration	:	Single intraperitoneal injection	:	1, 20, 50, 66, 80 mg/kg
		Oral gavage	:	1, 5, 20, 50, 80 mg/kg
		Epicutaneous	:	128, 640, 5 x 128 mg/kg
GLP	:	not in compliance		

Results
No statistically dose related increase in SCEs frequencies was observed. However, the number of animals is low (intraperitoneal: 2 males and 2 females for the 3 lower doses; 1 male and 1 female for the 2 upper doses) (similar situation for oral gavage and worth for epicutaneous).

Conclusions
The study provided gives negative results.

However, the individual values are given with the standard error of mean while the group mean values are noted with the standard deviation. In addition, only 25 cells per animal have been scored. Too few animals per dose.
Moreover, the use of 5-BrdU allows to differentiate between cells having passed through 1, 2 or more DNA synthesis phases. This allows to check the proliferation rate index and gives indications on cytotoxicity or mitotic delay. Such information is not given in this study.
This study is not acceptable for the above mentioned reasons.

Ref.: 12

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline	:	/
Species/strain	:	Sprague Dawley rat (SPF)
Group size	:	25 mated female rats
Test substance	:	Oxytol B (Hydroxyethyl-p-phenylenediamine sulfate)
Purity	:	/
Batch no	:	C 281 183
Dose levels	:	10 mg/kg
GLP	:	/

1-(β-Hydroxyethyl)-2,5-diaminobenzene-sulphate administered daily by gavage to 25 mated female Sprague-Dawley rats from day 6 to 15 of gestation at oral doses of 10 mg/kg/day (10 ml/kg in distilled water) did not show embryo-toxicity and teratogenicity on day 20 of gestation.

Ref.: 13

3.3.9. Toxicokinetics

See. 3.3.4. dermal / percutaneous absorption

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

3.3.14. Discussion

On the basis of the results of acute toxicity studies, A80 should be classified as 'toxic' (EC criteria). A80 does not show eye- or skin irritation at the 'in-use concentration' and was negative in a maximisation test. A sub-chronic study showed a NOEL of 25 mg/kg bw/day and the substance was not teratogenic in rats at 10 mg/kg bw/day.

The *in vitro* and *in vivo* studies on percutaneous absorption are all inadequate; an assessment could not be done.

The *in vitro* studies on mutagenicity are all inadequate; an assessment could not be done.

4. CONCLUSION

The SCCNFP is of the opinion that the information submitted is inadequate to assess the safe use of the substance.

Before any further consideration, the following information is required:

* complete physico-chemical characterisation of the test substances used, including data on stability.

* data on percutaneous absorption following the SCCNFP Notes of Guidance

* data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance.

5. MINORITY OPINION

Not applicable

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