EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Products

SCCP

OPINION ON
Hydroxyethyl-p-phenylenediamine sulfate

COLIPA n° A80

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCCP adopted this opinion at its 17th plenary of 30 September 2008

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

Scientific Committee members

Contact
European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

(ISSN)

ACKNOWLEDGMENTS

Dr. C. Chambers
Prof. V. Kapoulas
Prof. J.-P. Marty
Prof. T. Platzek (chairman)
Dr. S.C. Rastogi
Prof. V. Rogiers
Prof. T. Sanner
Dr. J. van Engelen
Dr. I.R. White

External experts

Dr. M.-L. Binderup	National Food Institute, Denmark (rapporteur)
Dr. H. Norppa	Institute of Occupational Health, Finland
Prof. K. Peltonen	Finnish Food Safety Authority, EVIRA, Finland
Dr. J. van Benthem	RIVM, the Netherlands

Keywords: SCCP, scientific opinion, hair dye, A80, hydroxyethyl-p-phenylenediamine sulfate, directive 76/768/ECC, CAS 93841-25-9, EINECS 298-995-1

Opinion to be cited as: SCCP (Scientific Committee on Consumer Products), Opinion on hydroxyethyl-p-phenylenediamine sulfate, 30 September 2008

TABLE OF CONTENTS

1. BACKGROUND

Submission I for Hydroxyethyl-*p*-phenylenediamine sulphate was submitted in February 1989 by COLIPA[1, 2].

The Scientific Committee on Cosmetology (SCC) adopted at its 46th plenary meeting of 19 February 1991 an opinion with the conclusion that:

"The SCC requires an adequate study for the induction of gene mutations in Salmonella assay."

Submission II for Hydroxyethyl-*p*-phenylenediamine sulphate was submitted in March 1992 by COLIPA[2].

The Scientific Committee on Cosmetology (SCC) adopted at its 54th plenary meeting of 10 December 1993 an opinion, just repeating its conclusion from the former opinion.

Submission III for Hydroxyethyl-*p*-phenylenediamine sulphate was submitted in August 2001 by COLIPA[2].

The Scientific Committee on Consumer Products (SCCP) adopted at its 2nd plenary meeting of 7 December 2004 an opinion (SCCP/0666/03) with the conclusion that:

"The SCCP 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."*

Submission IV for this substance was submitted in July 2005 by COLIPA. According to this submission Hydroxyethyl-*p*-phenylenediamine sulphate is used an oxidative hair colouring agent (precursor). The intended maximum on-head concentration is 1.5%. The oxidative colouring agent and the developer are mixed at ratios between 1:1 to 1:3. It is common practice to apply up to 100 g of the finished mixed product for a period of 30 minutes followed by rinse off with water and shampoo. The application may be repeated at monthly intervals.

Submission IV presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association
[2] According to records of COLIPA

2. TERMS OF REFERENCE

1. *Does the SCCP consider Hydroxyethyl-p-phenylenediamine sulphate safe for use as an oxidative hair dye with a concentration on-head of maximum 1.5% taken into account the scientific data provided?*

2. *Does the SCCP recommend any further restrictions with regard to the use Hydroxyethyl-p-phenylenediamine sulphate in oxidative hair dye formulations?*

3. OPINION

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

Benzeneethanol, 2,5-diamino-, sulfate (1:1) (salt) (CA index name, 9CI)
2-(2,5-Diaminophenyl)ethanol sulfate (1:1) (IUPAC)
3-(2-hydroxyethyl)-p-phenylenediammonium sulphate

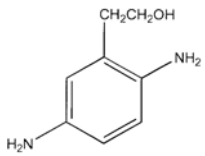
3.1.1.3. Trade names and abbreviations

Betoxol
COLIPA n° A80

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: 250.28 g/mol

3.1.4. Purity, composition and substance codes

Description \ Batch	WE 51	WE 68	100789 (R96003795	30/01 (R0012730)	36/37 (R0024480)
Identification	NMR, HPLC	NMR, HPLC	NMR, HPLC	NMR, HPLC	NMR, HPLC
NMR content / %, w/w	99.1	99.0	98.3	99.6	99.9
HPLC purity / area %**					
210 nm	99.7	99.7	99.4	99.8	99.8
254 nm	99.9	99.9	99.7	99.9	99.8
292 nm	100	100	100	100	99.8
HPLC content* / %, w/w	94.7	95.6	97.4	98.1	98.8
Impurities (content in ppm)					
p-Phenylenediamine	151	149	100	190	71
2-Methyl-1,4-benzenediamine	93	90	40	40	29
Metanilic acid	< 10°	< 10°	< 10°	< 10°	< 20°
Water content, % (w/w)	0.09	0.09	< 0.1	0.07	0.16
Loss on drying, % (w/w)	0.04	0.02	< 0.1	***	0.03
Sulfated ash, % (w/w)	0.02	0.02	0.05	0.01	0.16
Content of H_2SO_4, % (w/w)	39.1	39.1	39.2	***	39.1
Element screening / ppm	Na: 37 Si: 57	Na: 32 Si: 60	Na: 34 Si: 37	***	Na: 241 Si: 29 K: 29 Fe: 77

* The HPLC content refers to R0029 (100789 (R96003795)) with 99.9%, w/w; for each indicated value three measurements were made with a rather high standard deviation (always one measurement rather low)

** HPLC conditions: LiChrosphere 60 RP Select B; mobile phase consisting of ACN: buffer (KH_2PO_4 (0.02 M) + pentanesulfonic acid (0.01 M), pH 5.5) (5:95); Flow rate: 1 ml/min

*** not determined because lack of substance

° not detected, shown value indicates limit of detection

3.1.5. Impurities / accompanying contaminants

See 3.1.4

Solvent residues

Solvents, (i.e. solvents such as methanol, ethanol, isopropanol, n-propanol, acetone, ethyl acetate, cyclohexane, methyl ethyl ketone and monochlorobenzene < 100 ppm) were not detected.

3.1.6. Solubility

Water: 51.2 g/L (20 °C, pH 2.02) (measured according to EU method A6)
DMSO: 4.6% (w/w)
Acetone/water 1:1: 0.3% (w/w)

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 0.07 (measured according to EU method A8)

3.1.8. Additional physical and chemical specifications

Melting point:	250 °C (decomposition)	(EU – A.1)	*(Reference 7)*
Boiling point:	decomposition at melting point	(EU – A.2)	*(Reference 8)*
Flash point:	/		
Vapour pressure:	5.54 x 10^{-11} hPa (20°C)	(EU - A.4)	*(Reference 10)*
Density:	1.50 g/ml (20°C)	(EU - A.3)	*(Reference 9)*
Viscosity:	/		
pKa:	15.12 for R-CH_2OH (acidic)	*calculated*	*(Reference 6)*
	6.51 for R-C_6H4-NH_3^+ (basic)	*calculated*	
Refractive index:	/		
pH:	2.02 (saturated aqueous solution, 20°C)		*(Reference 5)*
UV_Vis spectrum:	/		

3.1.9. Stability

The test substance is considered to be stable for more than 3 years, if stored dry and protected from light at room temperature.

The stability of an approximately 5% (w/w) solution of hydroxyethyl-p-phenylenediamine sulfate in water and approximately 2% (w/w) in DMSO was tested over a period of 7 days. The test solutions were stored at room temperature and in the absence of light.
The test material was stable in these solutions over the period of seven days (Recovery: 95.5-100% of the original concentration).

General Comments to physico-chemical characterisation

- The stability of hydroxyethyl-p-phenylenediamine sulphate in the marketed products is not reported.

3.2. Function and uses

Hydroxyethyl-p-phenylenediamine sulfate is used as an oxidative hair colouring agent. The intended maximum on-head concentration is 1.5%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCP/0666/03

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:	/
Dose levels:	rat: 50, 100, 200, 300 and 400 mg/kg bw/day Mice: 50, 100 and 150 mg/kg bw
Route:	oral (gavage)
Observation period:	14 days
GLP:	not in compliance

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 LD50 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.: 17

Comment
The experiment was not performed in compliance with guidelines. It was performed with the dihydrochloride instead of the sulphate salt.

New Study, submission II

Guideline:	OECD 401 (1987)
Species/strain:	Mice/strain Him:OFl
Group size:	10/group (5 males and 5 females)
Test substance:	hydroxyethyl-p-phenylenediamine (1,4-diamino-2-ß-hydroxyethylbenzene-sulphate)
Batch:	Not indicated
Purity:	> 98% (according to the supplier)
Dose:	20, 36, 63, 112 and 200 mg/kg bw
Route:	oral (by gavage)
Exposure:	Once (in the morning), followed by a observation period of 2 weeks
GLP:	in compliance
Date:	July-August 1990

Five groups of 10 male and 10 female mice received the test item, once by gavage at 20, 36, 63, 112 or 200 mg/kg bw. This dosage-range was based on a preliminary study, in

which all animals (4/4) died after receiving 200 or 2000 mg/kg bw hydroxyethyl-p-phenylenediamine. Hydroxyethyl-p-phenylenediamine was dissolved in distilled water.
Behaviour, reactions and physical signs of the animals were observed and findings were recorded at 1, 10, 30 min, 1, 2, 4 and 6 hours post administration and then at least once a day for 2 weeks. Body weight was determined before administration, and 7 and 14 days post administration. Any found dead animal was dissected and submitted to a macroscopic *post-mortem* examination in order to identify the target organs. All surviving animals were sacrificed by CO_2 asphyxation at 14 days post administration and examined macroscopically.

Results
All animals in the 112 or 200 mg/kg bw group died early. Death occurred between 1 hr-4 days in the 112 mg/kg bw group and between 30 min-2 hr in the 200 mg/kg bw group. 1/10 of the animals in the 63 mg/kg bw died within 4 hr post administration.
No effects on body weight and body weight gain were observed. All animals showed signs of malaise (e.g. decreased motor activity, ruffled fur, closed eyes, hunched posture, decreased muscular tension or unconsciousness). In some animals ataxia, dyspnoea or low grade convulsions were observed. Effects diminished and returned to normal within 6hr – 1wk post administration. 7/20 of the animals that died early did not show any clinical signs at the *post-mortem* examination. Most of the other animals that died early showed signs of shock due to severe gastrointestinal irritations or hepatotoxicity.

Conclusions
Under the experimental conditions of this study, the LD50 was 80 mg/kg bw (71-89 mg/kg bw 95% CI), calculated for both sexes combined.

Ref.: 18

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:	Guinea pig, strain White Pirbright (SPF)
Group:	15 females
Substance:	1-β-hydroxyethyl-2,5-diaminobenzene dihydrochloride
Batch:	/
Purity:	/
Dose:	3% aqueous dilution thickened with 0.5% tylose
Vehicle:	water
GLP:	not in compliance
Date:	11 – 18 March 1985

A 3% dilution of 1-β-hydroxyethyl-2,5-diaminobenzene dihydrochloride in 0.5% aqueous tylose was applied onto the clipped flank region of 15 albino guinea pigs by means of a brush daily for 5 consecutive days. The skin was not covered, but animals were restrained from movement in order to avoid contact with the treated area (3 x 4 cm) during the first 5 hours after application. Possible skin reactions were evaluated for erythema and oedema 5 hours after each application.

Results
No skin reactions at all were observed at any observation time point.

Conclusion
No indication of a skin irritating potential of 1-β-hydroxyethyl-2,5-diaminobenzene dihydrochloride tested at a 3 % aqueous solution in a repeated application assay in guinea pigs was noted.

Ref.: 19

Comment
Although the experiment was not in compliance with guidelines and performed with the dihydrochloride instead of the sulfate, the data indicates that 1-β-hydroxyethyl-2,5-diaminobenzene dihydrochloride is not irritant to rabbit skin at concentrations up to 3%. This applies probably also for the sulfate.

3.3.2.2. Mucous membrane irritation

Guideline: OECD 405
Species: Rabbit, strain Albino New Zealand White (SPF)
Group: 3 males
Substance: Betoxol
Batch: "J. Robinson 10.7.89"
Purity: 99.7%
Dose: 60mg (0.1 ml), without rinsing
Vehicle: water to form paste
GLP: In compliance
Date: 17 November – 1 December 1999

A single sample of 0.1 ml of an aqueous paste of Betoxol containing 60 mg of the test item was applied into the conjunctival sac of the left eye of 3 male rabbits; the right eye served as control. The eyes were not rinsed, and evaluated and scored 1, 24, 48, and 72 hours as well as 7 and 14 days after application. Further readings by means of fluorescein-instillation took place 24 h and 48 h after substance application.

Results
Iridial irritation (grade 1 on a scale from 1-4) was observed in all animals after 1 and 24 hours. Irritation of the conjunctiva was seen as redness, chemosis and discharge, which had completely resolved within 7 days in two animals and within 14 days in one animal. No corneal opacity was observed, and treatment of the eyes with 2% fluorescein revealed no corneal epithelial damage in any of the animals. There was no evidence of ocular corrosion. No staining of peri-ocular tissues by the test item was observed.

Conclusion
Under the conditions of the test, undiluted Betoxol was irritant to rabbit eyes.

Ref.: 20

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline: OECD 406
Species: Mouse, strain CBA/Ca01aHsd
Group: 5 females per dose
Substance: hydroxyethyl-p-phenylenediamine sulfate
Batch: 100789
Purity: 99.7%

Dose: 0.5, 1.0 and 2.0 (w/v)
Vehicle: DMSO
Control: p-Phenylenediamine (PPD) at 1% in DMSO (in parallel)
GLP: in compliance
Date: 3 – 9 May 2000

The skin sensitising potential of hydroxyethyl-p-phenylenediamine sulfate was investigated in CBA/Ca01aHsd mice by measuring the cell proliferation in the draining lymph nodes after topical application on the ear.
3 test groups (3 different concentrations), 1 positive control group and 1 negative control (vehicle) group were tested. Twenty five µl of 0 (vehicle only), 0.5, 1.0 and 2.0% of hydroxyethyl-p-phenylenediamine sulfate in DMSO were applied to the dorsal surface of the ear to each of five mice per group for three consecutive days. p-Phenylenediamine (PPD) at 1% in DMSO was used as the positive control in parallel under identical test conditions.

On day 5, the mice received an intravenous injection into the tail vein of 250 µl phosphate buffered saline containing 20.0 µCi of [H^3] methyl thymidine. Approximately five hours later, the mice were sacrificed by CO_2-inhalation and the draining auricular lymph nodes were removed and weighed. After preparing a single cell suspension for each mouse, cells were precipitated by TCA and the radioactivity was determined (incorporation of [H^3] methyl thymidine in the pellets) by means of liquid scintillation counting as disintegrations per minute (dpm).
The mean dpm per treated group was determined and the stimulation index (test item compared to the concurrent vehicle control) was calculated. The proliferative response of lymph node cells was calculated as the ratio of ^{3}H-methyl thymidine-incorporation into lymph node cells of test group animals relative to that recorded for control group animals. A stimulation index, ratio of test substance / vehicle control, was calculated for each concentration.

Results
The mean stimulation indices were affected in a dose-dependent manner by the treatment with hydroxyethyl-p-phenylenediamine sulfate. With the test item in DMSO, mean stimulation indices of 2.8, 4.5 and 7.0 were obtained for the 3 test concentrations of 0.5, 1.0 and 2.0%, respectively. An EC3 value (equal to the concentration inducing a stimulation index of 3) of 0.57% for hydroxyethyl-p-phenylenediamine sulfate was calculated. The positive control (PPD, 1% in DMSO) caused a stimulation index of 10.1.

Conclusion
Hydroxyethyl-p-phenylenediamine sulfate induced a biologically relevant immune response in local lymph nodes after dermal application to the mouse ear when DMSO was used as vehicle. The EC3 value was 0.57%. The concurrent positive control demonstrated the sensitivity of the assay.
Based on these findings hydroxyethyl-p-phenylenediamine sulfate is evaluated to be a skin-sensitiser under the described test conditions.

Ref.: 22

Comment
The SCCP considers hydroxyethyl-p-phenylenediamine sulfate to be a strong sensitiser.

The previous submission contained a Magnusson-Kligman Maximisation test performed with hydroxyethyl-p-phenylenediamine sulfate, but the test design was not in line with current scientific and regulatory requirements. Specifically the concentrations used for both, the epidermal induction and the challenge were not based on the required thresholds for a minimum irritating/maximum non-irritating concentration. In addition, unspecified test material was used. The study results suggested that there was no indication of a sensitising potential of hydroxyethyl-p-phenylenediamine sulphate.

Ref.: 4 (subm. I)

Conclusion skin sensitisation
In the local lymph node assay, hydroxyethyl-p-phenylenediamine sulfate induced a biologically relevant immune response in the vehicle DMSO with an EC3 value of 0.57%, indicative of a strong sensitiser.

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428
Tissue:	Porcine back skin (thickness: 890 µm ± 40 µm); 1 male donor
Group size:	6 chambers
Diffusion cells:	Diffusion Teflon-chambers
Skin integrity:	tritiated water
Test substance:	*WR23361*
Batch:	36/37 sample no. R0024480
Purity:	99.8%
Radiolabel	2-(2,5-diamino[ring-U-^{14}C]phenyl)ethanol sulphate ; 544 MBq/mmol
Radiolabel batch	CFQ.6841
Radiolabel purity	97%
Test item:	Hair dye formulation VDE-0001, batch VDE-0001/1 containing WR23361 at 1.5%.
Doses:	100 mg/cm² formulation containing 1.5 mg/cm² WR23361
Receptor fluid:	physiological phosphate buffer containing NaCl and antibiotics
Solubility receptor fluid:	54.5 mg/L
Stability:	/
Method of Analysis:	liquid scintillation
GLP:	in compliance
Date:	28 February – 24 March 2005

The skin absorption of WR23361 at the maximum concentration intended for hair colorants (1.5%), was investigated with pig skin prepared from the back and the flanks. An area dose of 1.5 mg/cm² of the dye was applied once to the skin in a commercial oxidative hair dye formulation with a reaction partner (78 mg aqueous cream formulation containing 1.5% dye applied to 0.785 cm² skin, 14.7 mCi 2-(2,5-diamino[ring-U-14C]phenyl)ethanol sulphate in the presence of hydrogen peroxide.

The integrity of the skin was monitored at the beginning of the experiment using tritiated water. A diffusion Teflon-chamber was used. The receptor solution (physiological phosphate buffer containing NaCl and antibiotics) was pumped through the receptor chamber at a rate of 2.5 ml/h. Six chambers were investigated.
Sixty minutes after substance application, the test item was removed by washing the skin twice with 0.5 ml water, then once with 0.5 ml washing solution (shampoo-formulation diluted to approximately 16.7%) and again twice with water. The washing solutions were combined and the amount of dye was determined by radioactivity measurement.
Fractions of the receptor fluid were collected after 16, 24, 40, 48, 64 and 72 h, concentrated directly after the pump and analysed immediately. At termination of the experiment, the skin was heat-treated and the "upper skin" (stratum corneum and upper stratum germinativum) was mechanically separated from the "lower skin" (lower stratum germinativum and upper dermis). Both skin compartments were extracted separately and the dye content was quantified by means of measured radioactivity.

Results
The integrity of each skin preparation was demonstrated by examination of penetration characteristics with tritiated water resulting in 0.26 to 0.42% of the applied dose found in

the receptor fluids. All skin sample/diffusion chamber units were within the limit of acceptance (≤ 0.6%).
The total recovery of 93.6 ± 1.1% of the applied dose confirmed the validity of the test. The majority of the applied dose of WR23361 remained on the skin surface representing 98.14± 2.69%.

Under the condition of application 100 mg/cm² of an oxidative hair dye formulation containing 1.5% WR23361, an amount of 8.18 ± 1.22 µg/cm² (range 7.12 to 9.33 µg/cm²) was considered biologically available. (The percentage of the dose absorbed from the 100 mg/cm² application ranged from 0.57 to 0.72%).

Ref.: 23

Comment
The amount of formulation applied was excessive at 100 mg/cm²; 20 mg/cm² would be the adequate dose. Additionally, there was only 1 donor and too few chambers were used in the experiment. Therefore, the study is inadequate for the safety assessment.

3.3.5. Repeated dose toxicity

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

Guideline:	OECD 410 (1981)
Species/strain:	Rats/strain Wistar CRL:(WI) BR
Group size:	10 animals (5 males and 5 females) per dose group
Test substance:	Oxytol-B-sulphate (betoxolsulphate) 4% in koleston 2000
Batch:	/
Purity:	>98% (according to the supplier)
Dose:	0, 62.5, 250 and 1000 mg/kg bw
Route:	dermal
Exposure:	once a day on 5 days/week for 28 consecutive days
GLP:	in compliance
Study period:	26 September 1990 (first dosing)

Three groups (5 male and 5 female rats) received oxytol-B-sulphate in a concentration of 0 (vehicle only), 62.5, 250 or 1000 mg/kg bw once a day on 5 days per week for 28 consecutive days. This dose-range was based on a preliminary study, in which a non-significant reduction in body weight and food consumption was observed at a dose of 2000 mg/kg bw. The NOEL of this study was 500 mg/kg bw. Oxytol-B-sulphate was dissolved in distilled water.
Dermal administration was performed once a day on 5 days per week, on an area of the dorsal skin of about 5 cm x 6 cm, which is at least 10 % of the body surface. Hair of this region was clipped before first administration and then once a week. The test substance preparation was applied and spread using a plastic spatula. As far as necessary, some drops of distilled water were applied additionally to allow a distribution of the test substance on the whole administration area. The treated area was covered with a cellulose patch, which was held in place by an adhesive tape. Cellulose patch, tape and residual test substance were removed after 6 hours.
A negative control group (5 male and 5 female rats) did not receive the test item, but was treated with the same cellulose patch and adhesive tape as the dosed animals. In addition, two groups (5 male and 5 female rats) were treated with 0 or 1000 mg/kg BW in the same way as their corresponding groups, but were kept then for a further 14 days without test substance administration in an attempt to observe regression or progression of test substance induced lesions.
All animals were observed for clinical signs, behavioural changes and dermal alterations at least once per day. Body weight and feed consumption was determined regularly. Other investigations were ophthalmoscopy, haematology and clinical chemistry. Animals were killed by CO_2 and subjected to necroscopy including gross pathological examination, organ-

weight determination, histopathological examination and treated and untreated skin was dermally examined for erythema and oedema.

Results
Staining of skin, fur and bedding material in all dosed animals was observed: the incidence and the intensity of the staining increased with dose indicating that the staining was most probably due to oxytol-B-sulphate. These effects are regarded non-toxic.
No significant effect on body weight, organ weight and feed consumption was observed. No dose-related abnormalities with respect to ophthalmoscopy were observed. Chromodacryorrhoea (excessive secretion of coloured tears) was observed with increased dose.
In the 1000 mg/kg bw/day dose group 3/10 animals showed acanthosis indicating local irritation. In one of the animals showing acanthosis also an ulcer was observed. No other histopathological alterations were found.

Conclusions
Based on the local effects in this study the NOAEL was 250 mg/kg bw/day for both sexes. The NOAEL for systemic effects is 1000 mg/kg bw/day.

Ref.: 24

Comments
The test substance solution was not tested for stability.

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Guideline:	OECD 408 (1998)
Species/strain:	Rats / CRL:(WI) BR Wistar
Group size:	20 animals (10 males and 10 females) per dose group
Test substance:	hydroxyethyl-p-phenylenediamine sulfate (Betoxol II), in 1% aqueous methylcellulose (as a suspension)
Batch:	36/37
Purity:	~99.8%
Dose:	0 (vehicle only), 25, 35 and 55 mg/kg bw/day
Route:	oral, by gavage
Exposure:	daily for 90 consecutive days
GLP:	in compliance
Study period:	16 November 2004 – 15 February 2005

CRL:(WI) BR Wistar rats (n=10/sex/group) were treated daily on a 7/days/week basis by gavage with the test item at a dose of 0, 25, 35 and 55 mg/kg bw. This dose-range was based on data from a preliminary study which showed that a dose of 75 mg/kg bw/day caused significant body weight stagnation, body weight gain depression and decreased food consumption. In this range-finding study, the test item was found to cause an increase in AST activity at doses of 40 mg/kg bw/day (in females) and 60 mg/kg bw/day (in males and females).
In the 90-day oral toxicity study the test item was prepared in 1% aqueous methylcellulose, corresponding to a constant treatment volume of 10 ml/kg bw. The concentration of dosing suspensions was analysed on days 0, 7 and 90.
Mortality and morbidity of treated animals were checked twice daily, clinical observations were made once per day. Once before the first exposure, once a week thereafter and once on week 12, a modified Irwin test was performed, which included a functional observation battery (evaluation of reaction of auditory, visual and proprioceptive stimuli and assessment of grip strength and motor activity). Ophthalmoscopy was performed before treatment and in the control and 55 mg/kg bw dose groups on week 12.
Haematological examinations and clinical biochemical analyses were conducted before the first treatment and one day after the last treatment. Urinalysis was performed in week 11.

Animals were killed by pentobarbital and subjected to necroscopy including gross pathological examination, organ-weight determination and histopathology.

Results
No clinical signs have been recorded which were considered to be related to a toxic effect of the test substance. No difference was found between the experimental groups with respect to performance in the functional observational test battery before starting the study, one week thereafter and at termination of the study. Effects on body weight gain were observed, but these effects were not dose-related. Mean daily food consumption was comparable between the control and dosed groups. Ophthalmoscopy did not show any test item related alterations. Haematology showed some variations in RC, PT, WBC, SE, LY and RBC, but these effects were not dose-related. In the 55 mg/kg bw/day dose group, a test item-related and statistically and biologically significant increase in activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was found in male and especially in female animals. Histopathology did not reveal dose-related lesions of the examined organs.

Conclusion
Hydroxyethyl-p-phenylenediamine sulfate induced an increase in the AST and ALT activities in CRL:(WI) BR rats at a dose of 55 mg/kg bw/day for 90 days. The NOAEL in this study was 35 mg/kg bw.

Ref.: 25

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100 and TA 102
Replicates:	Triplicates in two independent experiments
Test substance:	Hydroxyethyl-p-Phenylenediamine Sulfate (WR 23361)
Solvent:	Deionised water
Batch:	36/37
Purity:	99.8 area% (by HPLC)
Concentrations:	Exp. I and II: 33, 100, 333, 1000, 2500 and 5000 µg/plate Exp IIa: 1000, 1500, 2000, 2500, 3000, 3500, 4000 and 5000 µg/plate (TA100 without S9-mix only)
Treatment:	Experiment I: Standard plate incorporation assay Experiment II: Pre-incubation assay Both assays with and without Phenobarbital/β-Naphthoflavone induced rat liver S9-mix
GLP:	in compliance
Study period:	6 December 2004 – 17 February 2005

To evaluate the toxicity of the test item a pre-study was performed with strains TA 98 and TA 100. Eight concentrations were tested up to 5000 µg/plate. The plates showed normal background growth up to 5000 µg/plate and there were no signs of toxicity, evident as a reduction in the number of revertants.

Results

A reduction in the number of revertants occurred in experiment II with metabolic activation in strain TA 1535 at 1000 µg/plate and in strain TA 1537 at 100 µg/plate and 333 µg/plate. Because this reduction was not dose dependent, it was not judged as a true toxic effect.

In both experiments, there were no indications of an increase in the mutant frequency at any concentration in the tester strains either with or without metabolic activation. The only exception was TA 100 in experiment two without metabolic activation at 2500 µg/plate. The number of revertants exceeded a doubling of the control value. No dose-dependency was observed, however, a confirmatory experiment was performed (IIa). This assay showed increases at 1500 and 4000 µg/plate compared to the control and the range of historical solvent and negative control were slightly exceeded. At both concentrations, a doubling of the number of revertants compared to the control was not achieved and the increases were not dose dependent. Therefore, the increases were not considered as biological relevant.

Conclusion
Under the test conditions used Hydroxyethyl-p-Phenylenediamine Sulfate (WR 23361) did not induce gene mutations in bacteria.

Ref.: 26

Comment
The historical range was slightly exceeded in the solvent control in both experiments of strain TA 102 with metabolic activation. In experiment one, the historical range was slightly exceeded in strain TA1535 (negative control) without metabolic activation. However, these findings did not influence the conclusion.

In vitro Mammalian Cell Gene Mutation Test (*hprt*- locus)

Guideline:	OECD 476 (1997)
Species/strain:	V79 Chinese Hamster cells
Replicates:	Two independent experiments with duplicate cultures
Test substance:	Hydroxyethyl-p-Phenylenediamine Sulfate (WR 23361)
Solvent:	Deionised water
Batch:	36/37
Purity:	99.8 area % (by HPLC)
Concentrations:	Exp. 1 (4-hours treatment) with S9-mix: 156.3, 312.5, 625.0, 1250.0, 1562.5 µg/ml without S9-mix (4-h treatment): 1.3, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 µg/ml Exp. 2 (24-hours treatment): without S9-mix: 0.3, 0.6, 1.3, 2.5, 3.8, 5.0, 7.5 and 10.0 µg/ml
Treatment	With and without Phenobarbital/β-Naphthoflavone induced S9-mix
GLP:	in compliance
Study period:	15 March – 20 May 2005

A pre-test on toxicity was performed with 8 concentrations between 19.5 and 2500 µg/ml. Without metabolic activation severe toxicity was observed at all the tested concentrations. Therefore, the pre-test was repeated in a lower concentration range (0.16 – 20 µg/ml) at both treatment intervals without metabolic activation.

Results
Although no precipitation was noted in the pre-experiments up to the maximum concentration, precipitation occurred in the first experiment at 1562.5 µg/ml in the presence of metabolic activation. Relevant toxic effects were observed in the first experiment at 30 µg/ml in the absence and at 1562.5 µg/ml in the presence of metabolic activation. In the second experiment, relevant toxic effects were observed at 5.0 µg/ml and above in both cultures.

No reproducible increases were observed in mutant colony numbers/10^6 cells in both experiments up to the maximum concentration with and without metabolic activation. The induction factor exceeded three times the mutant frequency of the corresponding solvent control at the following concentrations: experiment 1: second culture without metabolic activation at 15 µg/ml, experiment 1: second culture with metabolic activation at 1250 and 1562.5 µg/ml, Experiment 2: second culture at 5 µg/ml. However, there were no comparable effects in the parallel cultures and moreover the number of mutant colonies remained well within the historical range of negative and solvent controls.

Conclusion
Under the test conditions used Hydroxyethyl-p-Phenylenediamine Sulfate (WR 23361) did not induce gene mutations at the *hprt*-locus in V79 cells.

Ref.: 28

***In vitro* Micronucleus Test**

Guideline:	OECD 487 (draft 2004)
Species/strain:	Human peripheral blood lymphocytes
Replicates:	Duplicate cultures in one experiment
Test substance:	Hydroxyethyl-p-Phenylenediamine Sulfate (WR 23361)
Solvent:	Purified water
Batch:	36/37
Purity:	99.8 area % (by HPLC)
Concentrations:	with S9-mix: 600, 1350 and 1800 µg/ml; 3 h treatment 24 hours after mitogen stimulation without S9-mix: 75, 150 and 300 µg/ml, 20 h treatment 24 hours after mitogen stimulation
Treatment	With and without Aroclor induced S9-mix
GLP:	in compliance
Study period:	29 November 2004 – 4 January 2005

A preliminary cytotoxicity range-finding experiment was conducted both with and without metabolic activation at 3 and 20 hours treatment both 24 and 48 hours after mitogen stimulation. The experiment included 12 concentrations between 1.222 and 2503 µg/ml.

Results
Based on the range-finding experiment the highest concentration tested was 2503 µg/ml (10 mM) in the pre-test. Treatment of cells commenced approximately 24 hours following mitogen stimulation. In the absence of S9-mix this was for 20 hours followed by a 28-hour recovery period prior to harvest (20+28). Treatment in the presence of S9-mix for 3 hours was followed by a 45-hour recovery period prior to harvest (3+45). The dose levels for micronucleus analysis were selected by evaluating the effect of the test article on the replication index (RI). Micronuclei were analysed at three dose levels. The highest concentrations chosen for analysis, 300 µg/ml in the absence of S9-mix and 1800 µg/ml in the presence of S9-mix, induced approximately 67% and 60% reduction in RI respectively.

Treatment of cultures with the test article in the absence of metabolic activation resulted in frequencies of micronucleated binucleate (MNBN) cells that were higher in a concentration-related manner than those of the concurrent vehicle controls. There was a statistically significant increase in the frequency of MNBN cells after exposure to 150 and 300 µg/mL in the absence of S9, where the respective cytotoxicity values were 54% and 67%.
Treatment of cultures with 1350 and 1800 µg/ml in the presence of S9-mix, associated with 46% and 60% cytotoxicity respectively, resulted in frequencies of MNBN cells that were statistically significantly higher than those of the concurrent vehicle controls. There was a concentration-related increase and the positive response was more distinct compared to the

experiment in the absence of metabolic activation. Also much higher concentrations were tested in the presence than in the absence of S9, due to distinct difference in cytotoxicity with and without S9-mix.

Conclusion
Under the test conditions used, the increase in cells with micronuclei indicates genotoxic (clastogenic) activity of hydroxyethyl-p-phenylenediamine sulfate (WR 23361) in cultured human peripheral blood lymphocytes *in vitro*.

Ref.: 27

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1997)
Species/strain:	NMRI mice
Group size:	6 males per dose group
Test substance:	Hydroxyethyl-p-Phenylenediamine Sulfate (WR 23361)
Lot no:	36/37
Purity:	99.8 area% (by HPLC)
Dose level:	25, 50 and 100 mg/kg body weight
Route:	Orally, once
Vehicle:	Deionised water
Sacrifice times:	24 hours and 48 hours (only for the high dose level).
GLP:	In compliance
Study period:	28 February – 6 May 2005

A pre-experiment for acute toxicity was performed using two males and two females at three different doses (100, 150 and 200 mg/kg bw). The mice were examined for acute toxic symptoms at 1 h, 2-4 h, 6 h, 24 h, 30 h and 48 h after administration of 50, 100, 150 and 200 mg/kg bw. Animals died at both 150 and 200 mg/kg bw. 100, 50 and 25 mg/kg bw were estimated to be suitable. In the main study at least 2000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. As no sex-difference in toxicity was found in the pre-test, only male mice were used in the main experiment.

Results
The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that the test item had no cytotoxic properties in the bone marrow. The urine of the animals treated with the highest dose had taken the colour of the test item, indicating the bioavailability of the test item, which is confirmed by the toxicokinetics study (see point 3.3.9).
There were no indications of an increase in the frequency of cells with micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after treatment with A 80 were below or near to the value of the vehicle control group. The positive control (40 mg/kg bw cyclophosphamide) administered orally showed a statistically significant increase of induced micronucleus frequency.

Conclusion
Under the test conditions used, hydroxyethyl-p-phenylenediamine sulfate (WR 23361) did not induce an increase in the number of micronuclei in the bone marrow cells of mice.

Ref.: 29

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:	OECD guideline 414 (2001)
Species/strain:	Rats/CRL:(WI) BR Wistar
Group size:	22 females/group
Test substance:	hydroxyethyl-p-phenylenediamine sulfate (Betoxol II), in 1% methylcellulose
Batch:	30/01
Purity:	~99.9%
Dose:	0 (vehicle alone), 10, 30 and 70 mg/kg bw/day
Route:	oral, by gavage
Exposure:	daily from GD 6 up to GD 20
GLP:	in compliance
Study report:	13 October – 10 November 2004

CRL:(WI) BR Wistar rats (22 sperm-positive females/group) were treated daily from GD 6 up to GD20 by gavage with the test item at a dose of 10, 30 and 70 mg/kg bw prepared in 1 % aqueous methylcellulose. These dose levels were based on a preliminary dose-range finding study. The concentrations correspond to a constant treatment volume of 5 ml/kg bw. Control animals were treated with 1 % aqueous methylcellulose.
Cage observations were made twice daily. Individual examination of the animals was performed at least once a day and condition of the animals, including behaviour changes and signs of overt toxicity, were recorded. Body weight and food consumption was recorded on a regular basis. On GD21 sperm-positive females were asphyxated by CO_2 followed by cervical dislocation. Foetal weight, placental weight and corrected body weight was determined. The number of implantation sites, corpora lutea, number and position of live foetuses, early and late embryonic death and foetal death were counted. Also gross pathology, histology and foetal examination were performed.

Results
No mortality was observed. In the control and the 10 and 30 mg/kg bw/day dose groups no clinical symptoms were observed. In contrast, clinical symptoms were observed in the 70 mg/kg bw/day dose group. Animals in the 70 mg/kg bw/day dose group showed orange-coloured urine, reduced activity, hunched-position, salivation, piloerection, dyspnoea and reduced righting reflex. In three animals of the 70 mg/kg bw/day dose group, brownish discolouration around the mouth and nose was observed. Body weight and body weight gain in this dose group was significantly reduced in the first week of the treatment. These effects corresponded with clinical signs and reduced food consumption. Gravid uterine weight, corrected body weight and corrected body weight gain was not affected by the test item.
Necropsy did not reveal any treatment-related effects. Early embryonic death and post-implantation loss were higher in the 70 mg/kg bw/day dose group as a consequence of maternal toxicity. Foetal body weight and placental weight were similar to control values. Sporadic external, visceral and skeletal alterations were observed, but these effects were not considered treatment-related.

Conclusions
Based on this teratogenicity study a NOAEL for maternal and developmental toxicity of 30 mg/kg/day was determined. No specific compound-related teratogenic effects were observed in this teratogenicity study.

Ref.: 30

3.3.9. Toxicokinetics

Bioavailability across the intestinal barrier

Guideline:	Not indicated
Cells:	Human intestinal epithelial cell line TC-7, a sub-clone of the Caco-2 cell line
Test substance:	hydroxyethyl-p-phenylenediamine sulfate (Betoxol II)
Batch:	36/37
Purity:	~99.8%
Concentration:	50 µM in HBSS buffer containing 1 % DMSO
Incubation time	60 min
Number of experiments:	two independent experiments
GLP:	Not in compliance but QAU checked
Study period:	4 April – 2 May 2005

The bioavailability of hydroxyethyl-p-phenylenediamine sulfate across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells *in vitro*. The permeability from the apical (A, pH 6.5) to the basolateral (B, pH 7.4) side was investigated at 37° C in 96-well Multiscreen plates with shaking for a 60 min contact time. Analysis of the donor (apical) and receiver (basolateral) samples was done by means of HPLC-MS/MS, and the apparent permeability coefficient (P_{app}) was calculated for two independent experiments. ^{14}C-mannitol (4 µM) was used to demonstrate the integrity of the cell monolayer. Only monolayers with a mannitol permeability of < 2.5 x 10^{-6} cm/sec were used. Propranolol and ranitidine were used to validate the experimental conditions.
According to the laboratory's classification system, a low permeability is considered for test items revealing a P_{app} < 2 x 10^{-6} cm/sec. A P_{app} of 2 - 20 x 10^{-6} cm/sec and a $P_{app} \geq$ 20 x 10^{-6} cm/sec classify a substance to have a medium or a high permeability, respectively. Ranitidine, which has a 50 % absorption in humans, was used as low permeability reference compound, as recommended by FDA.

Results
The figures for the reference substances propranolol (P_{app} = 53.1 x 10^{-6} cm/sec), a high permeability reference compound with about 100 % absorption in humans, and ranitidine (P_{app} = 0.17 x 10^{-6} cm/sec) revealing an absorption of about 50 % in humans, were well within the typical range of 20 – 60 x 10^{-6} cm/sec and < 2 x 10^{-6} cm/sec, respectively.
Hydroxyethyl-p-phenylenediamine sulfate revealed a P_{app} of 77.5 x 10^{-6} cm/sec and thus was classified to be of high permeability, indicating a nearly 100 % absorption from the gastro-intestinal tract. As the absorption from the gastro-intestinal tract is likely to be permeability limited, the high permeability observed in this assay indicates a good absorption hydroxyethyl-p-phenylenediamine sulfate after oral administration.

Ref.: 31

Taken from SCCP/0666/03

Guideline:	/
Species/strain:	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 14C) in water 4.88%
Batch:	/

Purity:	/
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 crystal 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 aqueous solution, 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.

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

Ref.: 32

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

(hydroxyethyl-p-phenylenediamine sulfate)
(oxidative hair dye)

Calculation based on in vivo data (toxicokinetics study, reference 32)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**35 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**24.5 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60 mg/kg**	**=**	**0.408**
No observed effect level (mg/kg) (teratogenicity, oral, rat)	**NOAEL**	**=**	**30 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**74**

3.3.14. Discussion

Physico-chemical properties
Hydroxyethyl-p-phenylenediamine sulfate is used as an oxidative hair colouring agent. The intended maximum on-head concentration is 1.5%. The stability of hydroxyethyl-p-phenylenediamine sulphate in the marketed products is not reported.

General toxicity
The substance was considered to be moderately toxic. The LD_{50} ranging from 80 to 150 mg/kg bw in male and female rats; In mice, it was 90 mg/kg bw.
Based on the local effects in a 28-day study, the NOAEL was set at 250 mg/kg bw/day for both sexes. The NOAEL for systemic effects was set at 1000 mg/kg bw/day.
In a 90-day study, the NOAEL was set at 35 mg/kg bw based on an increase in the AST and ALT activities in rats.
The NOAEL for maternal and developmental toxicity was set at 30 mg/kg/day. No specific compound-related teratogenic effects were observed.

Toxicokinetics
Toxicokinetics were studied in rats *in vivo* after topical application and oral administration. After dermal application, the total amount absorbed corresponds to 15 µg/cm² for the formulation without hydrogen peroxide or to 35 µg/cm² with hydrogen peroxide, when considering the residual amount of material present in the skin at 72 hours. For the aqueous solution, the absorption was equivalent to 7.5 µg/cm². These data show clearly the influence of the formulation on the absorption of the dye *in vivo*.
In an *in vitro* test of bioavailability across the intestinal barrier, hydroxyethyl-p-phenylenediamine sulfate was considered to be of high permeability, indicating a nearly 100 % absorption from the gastro-intestinal tract. As the absorption from the gastro-intestinal tract is likely to be permeability limited, the high permeability observed in this assay indicates a good absorption hydroxyethyl-p-phenylenediamine sulfate after oral administration.

Irritation / sensitisation
In a test not in compliance with the guidelines, up to 3% 1-β-hydroxyethyl-2,5-diaminobenzene dihydrochloride were not irritant to rabbit skin. Under the conditions of the test, undiluted hydroxyethyl-p-phenylenediamine sulfate was irritant to rabbit eyes.
In the local lymph node assay, hydroxyethyl-p-phenylenediamine sulfate induced a biologically relevant immune response in the vehicle DMSO with an EC3 value of 0.57 %, indicative of a strong sensitiser.

Dermal absorption
Following application of 100mg/cm² of an oxidative hair dye formulation containing 1.5% hydroxyethyl-p-phenylenediamine sulfate, an amount of 8.18 ± 1.22 µg/cm² (range 7.12 to 9.33 µg/cm²) was considered biologically available. (The percentage ranged from 0.57 to 0.72%). The amount of formulation applied was excessive at 100 mg/cm²; 20 mg/cm² would be the adequate dose. Additionally, there was only 1 donor and too few chambers were used in the experiment. The study could not be used for the calculation of the Margin of Safety.

Therefore, for the safety assessment, the absorption as determined in the *in-vivo* rat-ADME study was used in the calculation of the MOS. It is known that dermal absorption through rat skin is higher than that through human skin. Therefore, an adequately performed *in-vitro* dermal absorption study will be required if a re-evaluation of the safe use of hydroxyethyl-p-phenylenediamine sulphate in oxidative hair dye formulations is requested.

Mutagenicity / genotoxicity
The genotoxicity is sufficiently investigated for the three types of genotoxic endpoints: gene mutation, structural and numerical chromosome aberration. Hydroxyethyl-p-phenylenediamine sulfate was negative in the bacterial gene mutation test as well as in the *in vitro* gene mutation test in mammalian cells. Positive results were reported in the *in vitro* micronucleus test.
As this clastogenic effects found *in vitro* was not confirmed in an *in vivo* bone marrow micronucleus test hydroxyethyl-p-phenylenediamine sulfate itself can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.
To reach a definitive conclusion, appropriate tests with hydroxyethyl-p-phenylenediamine sulfate in combination with hydrogen peroxide have to be provided.

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the information provided, a margin of safety of 74 has been calculated suggesting that the use of hydroxyethyl-p-phenylenediamine sulphate as an oxidative hair dye at a maximum concentration of 1.5% in the finished cosmetic product (after mixing with hydrogen peroxide) poses a risk to the health of the consumer.

The *in vitro* dermal absorption study was not carried out according to the basic criteria for dermal absorption of the SCCP (SCCP 0970/06). Therefore, for the safety assessment, the absorption as determined in the *in vivo* ADME study was used in the calculation of the MOS. It is known that dermal absorption through rat skin is higher than that through human skin.

The conclusion may be re-evaluated if an adequately performed *in vitro* dermal absorption study is available.

Hydroxyethyl-p-phenylenediamine sulfate is a strong sensitiser.

Hydroxyethyl-p-phenylenediamine sulfate itself has no mutagenic potential *in vivo*.
However, studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance.

5. MINORITY OPINION

Not applicable

6. REFERENCES

1. Tenberken-Pötzsch, B.; Identity, stability and purity test Hydroxyethyl-p-phenylenediamine sulfate (Betoxol II); WELLA AG; 2000
2. Dougoud, P.; Certificate of analysis for Hydroxyethyl-p-phenylenediamine sulfate (A080); COSMITAL SA; 2005
3. Geffke, Th.; Partition coefficient (n-octanol / water) using High Performance Liquid Chromatography (HPLC); NOACK; 2003
4. Geffke, Th.; Sieve analysis; NOACK; 2003
5. Geffke, Th.; Water solubility; NOACK; 2003
6. Lange, J.; Prediction of the pKa-value; NOACK; 2004
7. Geffke, Th.; Melting point / melting range; NOACK; 2003
8. Geffke, Th.; Boiling point; NOACK; 2003
9. Geffke, Th.; Determination of the density; NOACK; 2003
10. Smeykal, H.; Vapour pressure; SIEMENS AXIVA GMBH & CO. KG; 2002
11. Schneider, S.; Determination of the surface tension of an aqueous solution (1 g/l) of Betoxol II in accordance with OECD-Guideline 115 [and according to EEC-Guideline A.5]; ALLESSA CHEMIE GMBH; 2003
12. Geffke, Th.; Flammability of solids; NOACK; 2003
13. Smeykal, H.; Explosive properties; SIEMENS AXIVA GMBH & CO. KG; 2002
14. Smeykal, H.; Auto-flammability (solids - determination of relative self-ignition temperature); SIEMENS AXIVA GMBH & CO. KG; 2002
15. Geffke, Th.; Oxidizing properties of solids; NOACK; 2003
16. Scientific Committee on Consumer Products SCCP Opinion on Hydroxyethyl-p-phenylenediamine Sulfate Colipa n° A80; SCCP; 2004
17. Büsching, J.; Acute oral toxicity test of 1-ß-hydroxyethyl-2,5-diaminobenzene dihydrochloride; WELLA AG; 1979
18. Klein, W.; Acute oral toxicity of "Betoxol" in mice; SEIBERSDORF; 1990
19. Weide, J.; Skin compatibility test in the albino guinea pig with 1-ß-Hydroxyethyl-2,5-diaminobenzene dihydrochloride; WELLA AG; 1985
20. van Huygevoort, A. H. B. M.; Acute eye irritation/corrosion study with Betoxol in the rabbit; NOTOX; 2000
21. Contact sensitisation: Classification according to potency; ECETOC; 2003
22. Albrecht, A.; Test for sensitization (Local lymph node assay - LLNA) with Betoxol II; BIOSERVICE; 2001
23. Beck, H.; Cutaneous absorption of 1.5% Hydroxyethyl-p-phenylenediamine sulfate (=WR23361) in a typical hair dye formulation with peroxide and reaction partner through pig skin in vitro; COSMITAL SA; 2005
24. Hruby, R.; 28-Day dermal toxicity study with "Oxytol-B-Sulfat" in rats; SEIBERSDORF; 1991

25. Szakonyi, I. P.; Repeated dose 90-day oral toxicity study of Hydroxyethyl-p-phenylenediamine sulfate (Betoxol II, WR 23361) in CRL: (WI) BR rats; LAB INTERNATIONAL RESEARCH CENTRE HUNGARY LTD.; 2005
26. Sokolowski, A.; Salmonella typhimurium reverse mutation assay with Hydroxyethyl-p-phenylenediamine sulfate (WR 23361); RCC-CCR; 2005
27. Clare, G.; Hydroxyethyl-p-phenylenediamine sulfate (WR 23361): Induction of micronuclei in cultured human peripheral blood lymphocytes; COVANCE; 2005
28. Wollny, H.-E.; Gene mutation assay in Chinese hamster V79 cells in vitro (V79 / HPRT) with Hydroxyethyl-p-phenylenediamine sulfate (WR 23361); RCC-CCR; 2005
29. Honarvar, N.; Micronucleus assay in bone marrow cells of the mouse with Hydroxyethyl-p-phenylenediamine Sulfate (WR23361); RCC-CCR; 2005
30. Pàl-Kutas, K.; Teratology study: Prenatal developmental study of Hydroxyethyl-p-Phenylenediamine Sulfate (Betoxol II, WR 23361) in CRL(WI) BR rats; Lab International Research Centre Hungary LTD.; 2005
31. Kennedy, K. J.; ADME: A-B permeability - Study of Hydroxyethyl-p-phenylenediamine sulfate; CEREP; 2005
32. Reindl, E.; Toxicokinetics of "2,5-Diaminophenylethanol-Sulphate"; Seibersdorf; 1992
33. Kock, M.; Retrospective clinical study on skin compatibility of Hydroxyethyl-p-Phenylenediamine sulfate - containing hair colourant products in patients allergic to p-Phenylenediamine or Toluene-2,5-Diamine; WELLA AG; 2005
34. König, P.; Data base search for references for A080 - Hydroxyethyl-p-phenylenediamine sulfate: 93841-25-9; WELLA AG; 2005

Submission I (1989)

4. STERNER W., G. CHIBAMGUZA. Oxytol B. Prüfung auf sensibilisierende Eigenschaften am Meerschweinchen nach B. Magnusson und A.M. Kligman. IBR Forschungs GmbH, Südkampen, September 1985.