



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

2-METHYL-5-HYDROXYETHYLAMINOPHENOL

COLIPA N° A31

Adopted by the SCCP
during the 7th plenary meeting of 28 March 2006

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1. BACKGROUND

Submission I for 2-Methyl-5-hydroxyethylaminophenol (1-Methyl-2 hydroxy-4-(β -hydroxyethyl)-amino benzene) was submitted in May 1983 by COLIPA^{1,2} according to COLIPA.

Submission II for this substance was submitted in January 1988 by COLIPA.

The Scientific Committee on Cosmetology (SCC) has, at its 48th meeting on 4 October 1991, expressed its opinion with the conclusion:

“The short-term oral study on rats (one-dose: 150 mg/kg bw and only 10 male + 10 female animals) is not adequate for defining the No Effect Level, due also to the presence of some toxic effects. The SCC requires a subchronic toxicity study (90 days) on rats to define the No Effect Level.”

Submission III for this substance was submitted in February 1994 by COLIPA.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at its 8th plenary meeting on 23 June 1999 the opinion (SCCNFP/0132/99) with the conclusion that:

“The SCCNFP is of the opinion that 1-Methyl-2-hydroxy-4-(β -hydroxyethyl)-amino-benzene can be used safely in permanent hair dye formulations at a maximum concentration of 2.0%. Since permanent hair dyes are mixed with hydrogen peroxide before application, the in-use concentration is 1.0%. Since A31 is a sensitiser, cosmetic products containing this substance must carry a label warning of a risk of sensitisation.”

The substance is currently regulated by of the Cosmetics Directive (76/768/EC), Annex III, Part 2 under entry 21 on the List of substances provisionally allowed, which cosmetic products must not contain except subject to restrictions and conditions laid down.

Submission IV for this substance was submitted by COLIPA in July 2005. According to this submission 2-Methyl-5-hydroxyethylaminophenol is used as an ingredient of oxidative hair colouring products at a maximum on-head concentration of 1.5%, after mixing the hair dye formulation with a hydrogen peroxide preparation typically in 1:1 proportions.

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 the records of COLIPA

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider 2-Methyl-5-hydroxyethylaminophenol safe for use as an oxidative hair dye with an on-head concentration of maximum 1.5 % taken into account the scientific data provided?*
2. *Does the SCCP recommend any further restrictions with regard to the use of 2-Methyl-5-hydroxyethylaminophenol 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

2-Methyl-5-hydroxyethylaminophenol (INCI name)

3.1.1.2. Chemical names

Phenol, 5-[(2-Hydroxyethyl)amino]-2-methyl- (CAS name)

Synonyms

1-Methyl-2-hydroxy-4-(β -hydroxyethyl)-amino-benzene

2-Hydroxy-4-(β -hydroxyethyl)-aminotoluene

2-methyl-N- β -hydroxy-ethylaminophenol

6-methyl-3- β -hydroxyethylaminophenol

3.1.1.3. Trade names and abbreviations

Trade name: Imexine[®] OAG (Chimex), N^o C 3267, 3267 PAN, Orex 119

COLIPA n^o: A31

Other code : 2948

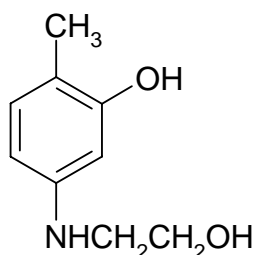
3.1.1.4. CAS / EINECS number

CAS: 55302-96-0

EINECS: 259-583-7

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3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₉H₁₃NO₂

3.1.2. Physical form

Beige powder, odourless

3.1.3. Molecular weight

Molecular weight: 167.21

3.1.4. Purity, composition and substance codes

Description	Batch No.				
	0121442	Op. 169/2	Op. 202	Op. M686	CFQ9704
Characterisation/ Identification *	NMR, IR, MS, UV- spectrometry	HPTLC	HPTLC	-	MS, HPTLC
HPLC purity, area %	99.3%	-	-	-	98.7%
Content determined by potentiometric titration (g/100 g)	99.8	99.0	99.3	98.9	-

* characterisation / identification of batches Op. 169/2, Op. 202 and Op. M686 is inadequate

3.1.5. Impurities / accompanying contaminants

5-amino-2-methylphenol (<0.5 g/100 g)
 2-chloroethyl-3-hydroxy-4-methylphenylcarbamate (<500 µg/g)
 3-(3-hydroxy-4-methylphenyl)-1,3-oxazolidin-2-one (<10 µg/g)

Water content: < 0.2 g/100 g
 Loss on drying: < 0.5 g/100 g
 Ash: < 0.5 g/100 g
 Methanol: < 100 ppm
 As, Sb, Hg: < 5 ppm each
 Cd: < 10 ppm

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Pb: < 20 ppm

3.1.6. Solubility

Water: 37.0 ± 1.8 g/L at $20 \text{ }^\circ\text{C} \pm 0.5 \text{ }^\circ\text{C}$

Ethanol: ≥ 200 g/L

DMSO: ≥ 200 g/L

3.1.7. Partition coefficient (Log P _{ow})

Log P_{ow}: 0.77 at 23 °C, pH 7.5

3.1.8. Additional physical and chemical specifications
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organoleptic properties

- melting point: 88.6°C - 93.6°C, for 3 different batches
- flash point: /
- vapour pressure: /
- boiling point: /
- density at 20 °C: /
- viscosity: /
- pKa: /
- UV absorption spectrum: λ_{max} 206.4 nm, 243.4 nm, 295.8 nm
- Refractive index at 20 °C: /

3.1.9. Stability

The dosages of 2-Methyl-5-hydroxyethylaminophenol used in various tests were stable during the study period.

The suspensions of 2-Methyl-5-hydroxyethylaminophenol in CMC, used in various tests, were homogeneous.

General Comments on Physico-chemical characterisation

- 3 of the 5 batches of 2-methyl-5-hydroxyethylaminophenol test materials are not adequately characterised;
- 2-methyl-5-hydroxyethylaminophenol is a secondary amine, and thus is prone to nitrosation. Nitrosamine content in 2-methyl-5-hydroxyethylaminophenol is not reported.
- Stability of the test material in marketed products is not reported.

3.2. Function and uses

2-Methyl-5-hydroxyethylaminophenol is used as an ingredient of oxidative hair colouring products at a maximum on-head concentration of 1.5%, after mixing the hair dye formulation with a hydrogen peroxide preparation typically in 1:1 proportions.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: OECD 420
Species/strain: Sprague-Dawley rats
Group size: 5 females
Test substance: 2-Methyl-5-hydroxyethylaminophenol in 0.5 % aqueous carboxymethyl-cellulose
Batch: 01211442
Purity: 99.8%
Dose: 2000 mg/kg bw by gavage
GLP: in compliance

The acute oral toxicity of 2-Methyl-5-hydroxyethylaminophenol was investigated using the fixed dose method. The test substance in 0.5 % carboxymethylcellulose was administered to 5 female rats (one for the sighting test) at the dose 2000 mg/kg bw. The animals were observed for mortality, clinical signs and body weight for 2 weeks and were then subjected to necropsy.

Results

No death occurred. One animal showed noisy breathing associated with pilorection and swollen abdomen. The maximum non-lethal dose is > 2000 mg/kg bw.

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: OECD 405
 Species: New Zealand White Rabbits
 Group: 3 females
 Substance: Imexine OAG
 Batch: Op.169/2
 Purity: 99%
 Dose: 0.1 g in conjunctival sac
 GLP: in compliance

A 0.1 g sample of undiluted Imexine OAG was instilled in the conjunctival sac of the left eye of each animal. The eye was not rinsed-off after instillation of the test item, and the untreated right eye served as control. The ocular reactions were assessed 1 hour, 24, 48, and 72 hours after instillation.

Results

A slight swelling and a slight redness of conjunctivae were noted 1 hour after instillation of Imexine OAG. At 24 and 48 hours, animals showed significant swelling with eyelids half or completely closed. At 72 hours, a marked decrease in chemosis was observed. At 24 through 72 hours, significant redness was noted. Grading of the iris was not possible resulting from corneal opacity. From 1 hour after instillation, corneal translucency was easily identifiable. The cumulative mean indices of chemosis, redness, and cornea were 3.33, 2.00, and 2.33, respectively.

Conclusion

Imexine OAG is an irritant to rabbit eyes when tested undiluted.

Ref.: 3

3.3.2.2. Mucous membrane irritation

Guideline: OECD 405
 Species: New Zealand White Rabbits
 Group: 3 males
 Substance: Imexine OAG
 Batch: 0121442
 Purity: 99.8%
 Dose: 0.1 ml of 2% Imexine OAG suspended in 0.5% aqueous carboxymethylcellulose
 GLP: in compliance

A 0.1 ml aliquot of diluted (2%) Imexine OAG in an aqueous solution of carboxymethylcellulose (0.5%) was instilled in the conjunctival sac of the left eye of each animal. The eyes were not rinsed-off after instillation of the test item preparation, and the untreated right eye served as control. The ocular reactions were assessed 1 hour, 24, 48, and 72 hours after instillation.

Results

There were no ocular reactions for any of the three animals treated.

Conclusion

The single instillation of 0.1 ml of 2% Imexine OAG in a 0.5% carboxymethylcellulose suspension was non-irritating to rabbit eyes.

Ref.: 4

3.3.3. Skin sensitisation

Local Lymph Node Assay

Guideline: OECD 429
 Species: CBA/J mice
 Group: 28 females
 Substance: Imexine OAG
 Batch: 0121442
 Purity: 99.8%
 Dose: 2.5, 5, 10, 25 and 50% Imexine OAG w/v in dimethylformamide
 GLP: in compliance

Twenty-eight (28) female CBA/J mice were used in the main study, separated in seven groups of 4 animals each. The seven groups used in the study consisted of:

5 treated groups receiving Imexine OAG at the concentrations of 2.5, 5, 10, 25 and 50% (w/v) in dimethylformamide. This vehicle was selected in a previous solubility study showing that Imexine OAG was non-soluble in other recommended vehicles, and that 50% (w/v) Imexine OAG in dimethylformamide was the maximal practicable concentration. This concentration was non-irritant in a preliminary test.

A negative control group receiving dimethylformamide alone.

A positive control group receiving alpha-hexylcinnamaldehyde at 25 % (v/v) in dimethylformamide.

The test substance Imexine OAG, dimethylformamide or alpha-hexylcinnamaldehyde was applied over the ears (25 µL per ear) for three consecutive days designated as days 1, 2 and 3. After 2 days of resting, the proliferation of lymphocytes in the lymph nodes draining the application sites was measured by incorporation of tritiated methyl thymidine on day 6. The values obtained were used to calculate stimulation indices (SI). The irritant potential of the test item was assessed in parallel by measurement of ear thickness on days 1, 2, 3 and 6.

Results

No cutaneous reactions and increases in ear thickness were observed in the animals of the treated groups. No lymphoproliferative responses were observed in Imexine OAG groups, while lymphoproliferation was observed with alpha-hexylcinnamaldehyde at 25% (SI value of 8.39).

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substance	Dilution %	SI
Imexine OAG	2.5	0.81
Imexine OAG	5	1.31
Imexine OAG	10	0.61
Imexine OAG	25	0.53
Imexine OAG	50	0.44
alpha-hexylcinnamaldehyde	25	8.39

Conclusion

Under the conditions of this murine local lymph node assay, Imexine OAG did not induce delayed contact hypersensitivity.

Ref.: 5

3.3.4. Dermal / percutaneous absorption

***In Vitro* Percutaneous Absorption Study in Human Dermatomed Skin**

Guideline: /
 Species: Human
 Group: 4 female donors (between 6 and 8 samples per experiment)
 Substance: Imexine OAG
 Batch: Op.M686
 Purity: 98.9% and test batch CFQ9704 of 5-(2-hydroxyethylamino)-2-methyl [U-¹⁴C] phenol (98.7% radiochemical purity)
 Dose: 1.5% Imexine OAG in 3 experiments with primary intermediate and peroxide, with peroxide alone, and by itself
 GLP: in compliance

Human skin samples were obtained from four (4) female donors subjected to plastic surgery. They were kept frozen at about -20°C until use.

Test Procedure

Skin samples (486 ± 121 µm in thickness) were dermatomed and mounted in 2cm² flow-through diffusion cells, using phosphate-buffered saline as the receptor fluid (flow rate 6 ml/h). Their integrity was checked before application of the formulation by measuring the trans-epidermal water loss. The skin was maintained at 32°C.

The test item (coupler) was tested under three different conditions:

Imexine OAG + primary intermediate (p-phenylenediamine, PPD) in the presence of developer (hydrogen peroxide, (H₂O₂): use conditions; 8 diffusion cells used

Imexine OAG alone in the presence of H₂O₂: oxidative conditions; 6 diffusion cells used

Imexine OAG in the presence of water: non-oxidative conditions; 6 diffusion cells used

For the in use conditions, Imexine OAG was incorporated into a typical hair colouring formulation at 3% (w/w) associated with the primary intermediate PPD at 1.94%, before mixing with developer (1:1, w/w) to yield a final concentration of 1.5% Imexine OAG (w/w). In other conditions, it was incorporated at 3% into the same formulation devoid of primary intermediate

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before mixing with developer or water (1:1, w/w) to give a similar final concentration of 1.5% Imexine OAG (w/w).

About twenty (20) mg/cm² of each hair dye mixture was applied to the skin surface for about 30 minutes. After this time period, the remaining formulation on the skin surface was removed using a standardized washing procedure, simulating use conditions. Twenty-four (24) hours after application, the percutaneous absorption of 5-(2-hydroxyethylamino)-2-methyl [U-¹⁴C] phenol was estimated by measuring its concentration by Liquid Scintillation Counting in the following compartments: skin excess, stratum corneum (isolated by tape strippings), living epidermis + dermis and receptor fluid.

Results

All diffusion cells yielded data that could be analysed. Most of the radio-labelled Imexine OAG applied on the skin surface was removed with the washing procedure (skin excess, 95.7%, 93.4%, and 94.4% of the applied dose for a total recovery rate of 96.8%, 94.4%, and 95.7% in use, oxidative and non-oxidative conditions, respectively).

The penetrated amounts (amounts measured in the receptor fluid) were 1.11 ± 0.62 (0.42% of the applied dose), 1.18 ± 0.34 (0.39%), and 2.38 ± 1.68 $\mu\text{g}_{\text{eq}}/\text{cm}^2$ (0.83%) in use, oxidative, and non-oxidative conditions, respectively.

The absorbed amounts (dermal delivery, sum of the amounts measured in the living epidermis/dermis and receptor fluid) represented 2.58 ± 1.03 (0.95% of the applied dose), 2.35 ± 0.79 (0.77%), and 3.44 ± 1.89 $\mu\text{g}_{\text{eq}}/\text{cm}^2$ (1.21%) in use, oxidative, and non-oxidative conditions, respectively.

Conclusion

The dermal absorption (sum of the amounts measured in the living epidermis, dermis and receptor fluid) of Imexine OAG incorporated at 1.5% (final concentration) in a typical oxidative hair dye formulation was estimated to be 2.58 ± 1.03 $\mu\text{g}/\text{cm}^2$ (1.49-4.56 $\mu\text{g}/\text{cm}^2$) in use conditions. The maximal penetration was in non-oxidative conditions was 3.44 ± 1.89 $\mu\text{g}/\text{cm}^2$ (1.10-5.55 $\mu\text{g}/\text{cm}^2$).

The maximum absorption in a typical oxidative hair dye formulation was 4.56 $\mu\text{g}/\text{cm}^2$. This figure is used for the calculation of the Margin of Safety.

Ref.: 14

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity
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No data submitted

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity
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Guideline:	OECD 408
Species/strain:	Sprague-Dawley rats
Group size:	10 males and 10 females per dose group
Test substance:	2-Methyl-5-hydroxyethylaminophenol in 0.5 % aqueous carboxymethyl-cellulose
Batch:	Op.202
Purity:	99.3%
Dose:	0, 50, 220 and 1000 mg/kg bw by gavage
GLP:	in compliance

2-Methyl-5-hydroxyethylaminophenol in 0.5 % carboxymethylcellulose in water was administered to 10 male and 10 female rats by gavage at the doses 50, 220 and 1000 mg/kg bw 7 days a week for 13 weeks, the control group received the vehicle only, no recovery group was included. Mortality, clinical signs, feed consumption and body weight were recorded. Ophthalmoscopy was performed on all animals of the control and the high dose group before the first treatment and in week 13. Haematological and blood biochemical parameters were evaluated in week 13 as well as urinalysis in all animals. At term all animals were inspected macroscopically and a range of organs was examined microscopically in high dose and control animals and in addition on the animals with macroscopic findings.

Results

Treatment related findings in the 1000 mg/kg group were loud breathing, hypokinesia, ptyalism and regurgitation. Yellow-orange coloured urine was found in all substance-treated animals, brown fur and/or tail in all high and middle dose group animals and in 6/10 males of the low dose group. No death occurred. Feed consumption was not changed. A treatment-related decrease in mean body weight gain was observed in females of the high dose group. Differences in some haematological parameters were within the range of historical controls. Proteinuria was observed in animals of both sexes in the 1000 mg/kg group. With the exception of colouring effects no further substance-related findings were noted at the lower doses.

The NOAEL of subchronic toxicity is 220 mg/kg bw.

Ref.: 6

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity <i>in vitro</i>
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Bacterial gene mutation assay

Guideline:	OECD 471
Species/strain:	<i>Salmonella typhimurium</i> , TA98, TA100, TA1535, TA1537, TA102
Replicates:	Two independent tests

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Test substance:	2-Methyl-5-hydroxyethylamino-phenol
Batch:	0121442
Purity:	99.8%
Concentrations:	312.5 - 5000 µg/plate without metabolic activation 312.5 - 5000 µg/plate with metabolic activation
GLP:	in compliance

2-Methyl-5-hydroxyethylamino-phenol has been investigated for the induction of gene mutations in *Salmonella typhimurium*. Liver S9 fraction from rats induced with Aroclor was used as the exogenous metabolic activation system. A preliminary study revealed no toxicity in the absence and presence of S9 mix and therefore the concentration range was based on the recommended maximum of 5000 µg/plate. Negative and positive controls were in accordance with the OECD guideline.

Results

2-Methyl-5-hydroxyethylamino-phenol did not induce gene mutations in *S. typhimurium* in any of the five strains. No toxicity was noted in the two experiments.

2-Methyl-5-hydroxyethylamino-phenol is not mutagenic in the bacterial gene mutation assay.

Ref.: 7

***In vitro* micronucleus test**

Guideline:	/
Cells:	Human lymphocytes
Replicates:	2 independent tests with and without S9 mix, 24 and 48 hours after PHA stimulation
Test substance:	2-Methyl-5-hydroxyethylamino-phenol
Batch:	0121442
Purity:	99.8%
Concentrations:	547.2-1420 µg/ml without metabolic activation; treatment for 20 h 684 - 1670 µg/ml with metabolic activation for 3 h
GLP:	In compliance

2-Methyl-5-hydroxyethylamino-phenol has been investigated for induction of micronuclei in cultured human lymphocytes with a harvest time of 48 hours (20 h exposure without metabolic activation, 3h exposure with metabolic activation). Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. Toxicity was determined by measuring the reduction in the replication index (RI). Negative and positive controls were in accordance with the draft of the OECD guideline.

Results

The test substance induced a significant increase in the frequency of micronuclei in tests without S9 mix and a treatment for 20 hours. Under the experimental conditions used, 2-Methyl-5-hydroxyethylamino-phenol was mutagenic / clastogenic in mammalian cells (human lymphocytes) *in vitro*.

Ref.: 10

***In vitro* mammalian cell gene mutation test (TK+/-)**

Guideline:	OECD 476
Cells:	L5178Y mouse lymphoma cells (TK+/-)
Replicates:	2 independent tests with and without S9 mix
Test substance:	Methyl-5-hydroxyethylamino-phenol
Batch:	0121442
Purity:	99.8%
Concentrations:	0.313 – 10 mM without metabolic activation 0.313 – 10 mM with metabolic activation
GLP:	In compliance

Methyl-5-hydroxyethylamino-phenol has been investigated for induction of gene mutations at the TK-locus in L5178Y mouse lymphoma cells after exposure for 3 hours without and with metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. Test concentrations were based on the level of toxicity. Since the test substance was only moderately toxic in the preliminary test, the highest concentration was 10 mM (equivalent to 1670 µg/ml). Negative and positive controls were in accordance with the OECD guideline.

Results

The test substance induced significant increases in the mutant frequencies after the 3-hour treatment in the absence of S9 mix. Under the experimental conditions used, Methyl-5-hydroxyethylamino-phenol was mutagenic in mammalian cells (L5178Y mouse lymphoma cells) *in vitro*. Colony sizing was not performed for the induced mutant colonies and, therefore, no information is available on the mechanism of mutation induction (i. e. induction of point mutations or chromosomal effects).

Ref.: 8

***In vitro* mammalian cell gene mutation test (HPRT)**

Guideline:	OECD 476
Cells:	L5178Y mouse lymphoma cells (HPRT)
Replicates:	2 independent tests with and without S9 mix
Test substance:	Methyl-5-hydroxyethylamino-phenol
Batch:	0121442
Purity:	99.8%
Concentrations:	150 –1670 µg/ml (10 mM) without metabolic activation 150 –1670 µg/ml (10 mM) with metabolic activation
GLP:	in compliance

Methyl-5-hydroxyethylamino-phenol has been investigated for induction of gene mutations at the HPRT-locus in L5178Y mouse lymphoma cells after exposure for 3 hours without and with metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. Test concentrations were based on the level of toxicity. Since the test substance was only moderately toxic in the preliminary test, the highest concentration was 10 mM (equivalent to 1670 µg/ml). Negative and positive controls were in accordance with the OECD guideline.

Results

The highest concentration tested led to about 30% relative survival in the absence and in the presence of S9 mix. The test substance did not induce significant and / or reproducible increases in the mutant frequencies after the 3-hour treatment in the presence or absence of S9 mix. Under the experimental conditions used, Methyl-5-hydroxyethylamino-phenol (A31) was not mutagenic in the *in vitro* HPRT gene mutation test with L5178Y mouse lymphoma cells.

Ref.: 9

3.3.6.2	Mutagenicity/Genotoxicity <i>in vivo</i>
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Rat bone marrow micronucleus test

Guideline:	OECD 474
Species/strain:	Rat, CrI:CD (SD)BR
Group size:	5 males + 5 females
Test substance:	Methyl-5-hydroxyethylamino-phenol
Batch:	0121442
Purity:	99.8%
Dose:	500, 1000 and 2000 mg/kg bw (once by gavage)
Sacrifice time:	24 and 48 (highest dose group only) hours after the treatment
GLP:	in compliance

Methyl-5-hydroxyethylamino-phenol has been investigated for induction of micronuclei in the bone marrow cells of rats. Since preliminary toxicity tests did not indicate toxic effects, 2000 mg/kg bw was selected as the top dose-level. Negative and positive controls were in accordance with the OECD guideline.

Results

In all treated groups, the PCE/NCE ratio was not lower than in the negative control group but the applicant suggested target cells exposure by the results of plasma analysis after administration of 2000 mg/kg. The mean MNPCE frequencies were not significantly increased in any of the groups treated with the test substance. The positive control substance gave the expected result. Methyl-5-hydroxyethylamino-phenol did not induce chromosome aberrations or damage to the mitotic apparatus in bone marrow cells of rats after oral treatment under the appropriate test conditions used.

Ref.: 11

3.3.7.	Carcinogenicity
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Mice

CGJ:BDF₁ mice (7 weeks old), groups of 50 males and 50 females received 0%, 1% or 2% 2-methyl-5-hydroxyethylaminophenol (94.6% purity) in the drinking water for 92 and 96 weeks, respectively. The males were sacrificed after 96 weeks and the females after 104 weeks. The drinking water was renewed three times a week. Moribund animals and animals that survived to the end of the study were exsanguinated and necropsied. The average body weights of the treated and control females were similar throughout the study, but those of the treated males were slightly lower than the controls. The mortalities of the treated males were higher than that of the controls, but those of the females were similar in the three groups throughout the study. It is stated that the mean intake of 2-methyl-5-hydroxyethylaminophenol among the male mice were

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0, 1330 and 2620 mg/kg/d and among the female mice were 0, 1530 and 3100 mg/kg/d. Among the females, the frequency pituitary chromophobe adenoma was 19% (8/43) in the control, 5% (2/42; $p < 0.05\%$) in the low dose group and 5% (2/44; $p < 0.05\%$) in the high dose group and the frequency of leukaemia/lymphoma was 33% (14/43) in the control, 40% (17/42) in the low dose group and 16% (7/44; $p < 0.05\%$) in the high dose group. Otherwise, no significant different in tumour induction were found between the control groups and the exposed groups.

Rats

F344/DuCrj rats (7 weeks old), groups of 50 males and 50 females received 0%, 0.5% or 1% 2-methyl-5-hydroxyethylaminophenol (94.6% purity) in the drinking water for 104 weeks and subsequently sacrificed. The drinking water was renewed three times a week. Moribund animals and animals that survived to the end of the study were exsanguinated and necropsied. The average body weights of the treated and control groups were similar throughout the study. The mortalities of the treated males and controls were similar, but that of control females was higher than those of the treated females. It is stated that the mean intake of 2-methyl-5-hydroxyethylaminophenol among the male rats were 0, 246 and 485 mg/kg/d and among the female rats were 0, 349 and 752 mg/kg/d. The frequencies of fibroma of the skin in male rats were 6% (3/49) in the control, 0 (0/47) in the low dose group and 17% (8/47; $p < 0.01$) in the high dose group. The frequency of leukaemia/lymphoma was 22% (9/41) in the control, 7% (3/44) in the low dose group and 2% (1/43; $p < 0.01\%$) in the high dose group. Otherwise, no significant different in tumour induction were found between the control groups and the exposed groups.

Ref.: 12

Comment

2-Methyl-5-hydroxyethylaminophenol was not carcinogenic in a two-year study with the substance added to the drinking water of rats and mice. The two-year oral carcinogenicity bioassays were not conducted according to GLP, and only summary study reports are available. These studies, however, followed the US National Cancer Institute (NCI) guidelines and were conducted according to standardized methodologies by the Japanese Cancer Institute. The effects on weight and mortality suggest that the concentration of the substance in the high dose groups should have been higher.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Embryo-foetal developmental toxicity study by the oral route in rats, study from 1981

Guideline:	/
Species/strain:	Sprague-Dawley rats
Group size:	20 mated females per dose group
Test substance:	2-Methyl-5-hydroxyethylaminophenol in 0.5 % aqueous carboxymethyl-cellulose
Batch:	/
Purity:	no data
Dose:	0, 500, 1000 and 2000 mg/kg bw by gavage
Exposure period:	day 6 to 15 of gestation
GLP:	in compliance

Evaluation was taken from SCCNFP/0132/99.

A preliminary toxicity study was carried out at doses of 500-1000-2000 mg/kg/day bw with the following results: decrease in body weight gain at 2000 mg/kg/day.

The compound was therefore administered orally to Charles River rats (10 males and 10 females per group) at doses of 50-300-1800 mg/kg/day (0.5 % w/v in CMC, 1 ml/100 g bw) from day 6 to 15 of pregnancy. No death and sign of toxicity was noted to be due the treatment. At the doses of 300 and 1800 mg/kg there was a production of brownish coloured saliva after treatment and a brown staining of the fur. At the dose of 1800 mg/kg: brown staining of body extremities; brown/orange discoloration of the urines. In the dams no apparent changes of internal organs due to the treatment have been observed at post mortem exams, although staining of the fur was still evident. Litter parameters: 300 mg/kg/day: post-implantation loss slightly higher than control; mean litter weight (NS) and mean foetal weight (P <0.05) lower than control; 1800 mg/kg/day: mean pre-implantation loss slightly higher than control (associated with the higher mean number of corpora lutea). Only one major malformation reported at 300 mg/kg/day, was considered to be spontaneous and not compound related. Embryonic and foetal development (evaluated by incidence of minor internal organ changes and skeletal malformations) were not affected by the treatment. There is no evidence for teratogenicity, embryotoxicity or foetotoxicity.

The No Effect Level in this study was 50 mg/kg bw/day, based on embryotoxicity. There was no evidence of compound related teratogenicity at any dose level.

Comment

The evaluation was adequate, but the derivation of the NOAEL was erroneous. The NOAEL of both maternal and embryo-/foetotoxicity was 1800 mg/kg bw/d. But no data on purity and on the batch were provided.

Ref.: 26

Embryo-foetal developmental toxicity study by the oral route in rats, 2004

Guideline:	OECD 414
Species/strain:	Sprague-Dawley rats

Opinion on 2-methyl-5-hydroxyethylaminophenol

Group size:	24 mated females per dose group
Test substance:	2-Methyl-5-hydroxyethylaminophenol in 0.5 % aqueous carboxymethyl-cellulose
Batch:	0121442
Purity:	99.8%
Dose:	0, 100, 300 and 1000 mg/kg bw/d by gavage
Exposure period:	day 6 to 19 of gestation
GLP:	in compliance

2-Methyl-5-hydroxyethylaminophenol in 0.5 % carboxymethylcellulose in water was administered to 24 mated female rats per dose group by gavage at the doses 100, 300 and 1000 mg/kg bw from day 6 to 19 of gestation, the control group received the vehicle only.

The animals were observed daily for clinical signs. Individual body weights were recorded at days 0, 3, 6, 9, 12, 15, 18 and 20 post coitum. Food consumption was measured for the day-intervals 0-3, 3-6, 6-9, 9-12, 12-15, 15-18 and 18-20 p.c.. All mated females were sacrificed at day 20 of gestation. Immediately following sacrifice, the uterus was removed, weighed and the number of (non)viable foetuses, early and late resorptions and the number of total implantations and corpora lutea was recorded. A macroscopic examination of the organs was carried out. All foetuses were individually weighed and the sex of the foetuses was determined. One half of the foetuses was examined for skeletal defects and variations of the ossification process by Alizarin Red staining and one half was evaluated for visceral imperfections (organic defects).

No animal died during the study. During treatment all treated females of the 1000 mg/kg group had brown discoloured urine (due to the test substance), 1/24 at 100 mg/kg and 19/24 at 300 mg/kg. No clinical signs of toxicity were observed. Feed consumption and body weight gain were not affected in a dose-related way. No treatment related effects were found with regard to litter data, external and visceral observations. No treatment-related changes in the frequency of variations and malformations were registered.

The NOAEL of maternal toxicity and embryo-/foetotoxicity were both 1000 mg/kg bw/d.

Ref.: 13

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation
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No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

Opinion on 2-methyl-5-hydroxyethylaminophenol

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(2-Methyl-5-hydroxyethylaminophenol)
(Oxidative/permanent)

Maximum absorption through the skin	A ($\mu\text{g}/\text{cm}^2$)	=	4.56 $\mu\text{g}/\text{cm}^2$
Skin Area surface	SAS (cm^2)	=	700 cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	3.192 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	0.053 mg/kg
No observed adverse effect level (mg/kg) (rat, 90 day, oral)	NOAEL	=	220 mg/kg

Margin of Safety	NOAEL / SED	=	4150
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3.3.14. Discussion

Physico-chemical specifications

Three of the five batches of 2-methyl-5-hydroxyethylaminophenol test materials are not characterised adequately. 2-methyl-5-hydroxyethylaminophenol is a secondary amine, and thus, it is prone to nitrosation. Nitrosamine content in the test material is not reported. Stability of 2-methyl-5-hydroxyethylaminophenol in the marketed products is not reported.

General toxicity

The maximum non-lethal dose in rats is > 2000 mg/kg bw. The NOAEL of subchronic toxicity in rats is 220 mg/kg bw.

The NOAEL of maternal toxicity and embryo-/foetotoxicity in rats were both 1000 mg/kg bw/d.

Irritation/sensitisation

When tested undiluted, 2-methyl-5-hydroxyethylaminophenol was non-irritant to rabbit skin.

It is an irritant to rabbit eyes when tested undiluted. The single instillation of 0.1 ml of 2 % 2-methyl-5-hydroxyethylaminophenol in a 0.5 % carboxymethylcellulose suspension was non-irritating to rabbit eyes.

2-Methyl-5-hydroxyethylaminophenol did not induce delayed contact hypersensitivity in a Local Lymph Node Assay.

Dermal absorption

The maximum dermal absorption (sum of the amounts measured in the living epidermis, dermis and receptor fluid) of 2-methyl-5-hydroxyethylaminophenol incorporated at 1.5% (final concentration) observed in a typical oxidative hair dye formulation was 4.56 µg/cm².

Mutagenicity

2-Methyl-5-hydroxyethylaminophenol is mutagenic *in vitro*. It induced gene mutations in cultured mammalian cells (TK locus). It also induced micronuclei in mammalian cells *in vitro*. The test substance did not induce damage to chromosomes or the mitotic apparatus in the *in vivo* micronucleus test. Thus, the mutagenic potential of 2-Methyl-5-hydroxyethylaminophenol seen *in vitro* does not lead to genotoxic or mutagenic effects *in vivo* under appropriate test conditions. The results of plasma analysis suggested systemic availability, but no clear evidence of bone marrow exposure was demonstrated.

There is, however, no evidence that the bone marrow was exposed.

Carcinogenicity

2-Methyl-5-hydroxyethylaminophenol was not carcinogenic in a two-year study with the substance added to the drinking water of rats and mice.

Taking the data submitted on mutagenicity/genotoxicity and on carcinogenicity into account, there is no concern regarding the carcinogenic potential of 2-Methyl-5-hydroxyethylaminophenol, when used in hair dyes.

4. CONCLUSION

The SCCP is of the opinion that the use of 2-methyl-5-hydroxyethylaminophenol itself as an oxidative hair dye at a maximum concentration of 1.5 % in the finished cosmetic product (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer.

2-Methyl-5-hydroxyethylaminophenol is a secondary amine, and thus is prone to nitrosation. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.

Studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken following the relevant SCCNFP opinions and in accordance with its Notes of Guidance.

5. MINORITY OPINION

Not applicable

6. REFERENCES

References in italics [15-31, 33] are not submitted as full reports in the present dossier. They consist of reports for studies considered to be inadequate, reports for range finding toxicity studies or publications. They can be provided upon request. Appropriate data bases were searched for relevant safety data on 2-M-5-HAP. No reports were identified in the literature that provided new information which is reasonably expected to substantially alter the human risk assessment performed in the present submission. In addition, the majority of published studies were performed with test articles of unknown purity and/or impurity profile, which does not permit to put the results into proper perspective. Therefore, the studies were not included in the present submission. However, results of the literature search can be provided upon request.

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7. ACKNOWLEDGEMENTS

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