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

HYDROXYBENZOMORPHOLINE

COLIPA N° A25

Adopted by the SCCP
during the 8th plenary meeting of 20 June 2006

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1. BACKGROUND

Submission I for Hydroxybenzomorpholine was submitted in May 1983 by COLIPA[1,2].

The Scientific Committee on Cosmetology (SCC) has at its 54th meeting on 10 December 1993 expressed its opinion and its revised opinion on 6-hydroxybenzomorpholine (CAS 977067-94-9) with the conclusion:

"*The possibility of nitrosamine formation with this compound should be considered. The SCC requires a chromosomal aberration test in mammalian cells grown in vitro*. "

Submission II for this substance was submitted in December 1993 by COLIPA[2].

The SCC has at its 66th meeting on 18 July 1996 expressed its opinion (SCC/1302/95) on hydroxybenzomorpholine with the conclusion:

"COLIPA A25 (6-hydroxybenzomorpholine; INCI: hydroxybenzomorpholine) is used in direct hair dye formulations at concentrations up to 2 %. Since the oxidative hair dyes are mixed with hydrogen peroxide before use, the concentration at application is 1 %.
Information supplied in Submission II indicates that the nitrosamine content of COLIPA A25 exceeds the maximum allowed for cosmetic ingredients. COLIPA should be asked to explain this anomaly. Overall evaluation of the data presented in Submission I and II does not show evidence of genotoxicity. Other data provided in this submission were included in the previous SCC evaluation or do not modify the previous conclusions or calculation of the safety margin. Classification: 2. Further classification of the contamination with nitrosamines is required. Information should be provided on the representativeness of the batch used in relation to the commercial product".

Submission III for this substance was submitted in May 1997 by COLIPA[2].

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at its plenary meeting of 23 June 1999 the opinion (SCCNFP/0131/99) with the conclusion:

"The SCCNFP is of the opinion that hydroxybenzomorpholine 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%. The sensitisation data in the dossier was generated with a method not conforming to OECD[3] n° 406. However, no further sensitisation data are requested provided that cosmetic product containing this substance carry a label warning of a risk of sensitisation."

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association
[2] According to records of COLIPA
[3] OECD Organisation for Economic Co-operation and Development

The substance is currently regulated by the Cosmetics Directive (76/768/EC), Annex III, Part 2 under entry 22 on the List of substances provisionally allowed, which cosmetic products must not contain except subject to restrictions and conditions laid down.

COLIPA submitted Submission IV for this substance in July 2005. According to this submission Hydroxybenzomorpholine is used as an ingredient of oxidative hair colouring formulations at a maximum on-head concentration of 1.0%, 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://pharmacos.eudra.org/F3/cosmetic/doc/HairDyeStrategyInternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider Hydroxybenzomorpholine safe for use as an oxidative hair dye with an on-head concentration of maximum 1.0 % taken into account the scientific data provided?*

2. *Does the SCCP recommend any further restrictions with regard to the use of Hydroxybenzomorpholine 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

Hydroxybenzomorpholine (INCI)

3.1.1.2. Chemical names

6-Hydroxybenzomorpholine
3,4-dihydro-2H-1,4-benzoxazin-6-ol

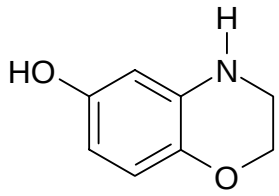
3.1.1.3. Trade names and abbreviations

Trade name: Imexine OV (Chimex)
COLIPA n°: A25

3.1.1.4. CAS / EINECS number

CAS: 26021-57-8
EINECS: 247-415-5

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_8H_9NO_2$

3.1.2. Physical form

Pink-mauve to brown powder

3.1.3. Molecular weight

Molecular weight: 151.16

3.1.4. Purity, composition and substance codes

Purity and impurities in various batches of hydroxybenzomorpholine:

Description	**Batch No.**						
	Op.45	**Op.90**	**E70 CXB**	**Op. T142**	**Op. T145**	**Op.59**	**0508918**
Chemical identification and chemical characterisation						IR, NMR, MS, elemental analysis of batch no. 0508918 conforms with the molecular formula	
UV spectrum		Comparable UV spectra					
HPLC profile*		Comparable HPLC profile				Comparable HPLC profile, purity > 99% based on HPLC peak area	
HPTLC profile**	Comparable HPTLC profile			Comparable HPTLC profile			
Melting point (ºC)	112	113.5	113.5	111.7	110.5	115	115
Alkalinity titre by potentiometry ($HClO_4$) % w/w	99.0	99.2	99.5	99.4	100	>98	98.3
2,5-dimethoxyaniline % w/w						Not detected <0.1	Detected < 0.1

Description	Batch No.						
	Op.45	**Op.90**	**E70 CXB**	**Op. T142**	**Op. T145**	**Op.59**	**0508918**
2-(2,5-dimethoxy-phenylamino)-ethanol % w/w						Not detected <0.1	Detected < 0.1
Isopropanol, ppm						10	Detected <100
Ethanol, ppm							Detected <100
Ethyl acetate, ppm							150
Ash content, % w/w							011
Water content, % w/w							0.61
Loss on drying, % w/w	0.6, unidentified batch						
Metal content	For batch no. 0508918: 23 ppm Fe; ≤1 ppm Ni; 2 ppm Zn; <1 ppm each of As, Ba, Bi, Cd, Co, Cu, Mn, Mo, Pb, Pd, Pt, Sb, Se, Sn, Ti, V; and <0.1 ppm Hg						

* HPLC profile of only batch no. 0508918 is provided
** HPTLC profile is not provided for any batch

3.1.5. Impurities / accompanying contaminants

See 3.1.4. 'Purity, composition and substance codes'
Nitrosamine content in ten different batches of hydroxybenzomorpholine is reported to be between 492 and 2616 ppb.

Ref.: submission IV: 12, 13, 14

3.1.6. Solubility

Water: 19.0 ± 1.4 g/l (20°C) (unidentified batch)
Ethanol: ≤ 1 g/100 ml
DMSO: ≥ 20 g/100 ml

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 0.22 at 23°C and pH 7.45 (batch no. 0508918)

3.1.8. Additional physical and chemical specifications

organoleptic properties

- melting point: /
- flash point: 110.5 – 115 °C for various batches (see 3.1.4.)
- vapour pressure: /
- boiling point: /
- density at 20 °C: /
- viscosity: /
- pKa: /
- UV absorption spectrum: /
- Refractive index at 20 °C: /

3.1.9.	Stability

Hydroxybenzomorpholine 1 mg/ml and 200 mg/ml in 0.5% CMC was shown to be stable up to 9 days at +4°C, protected from light and under inert gas atmosphere.
Hydroxybenzomorpholine 0.1 mg/ml, 50 mg/ml and 500 mg/ml in DMSO was shown to be stable up to 4 hours at room temperature, protected from light and under inert gas atmosphere.

General Comments on Physico-chemical characterisation

- Nitrosamine content was reported to be 10 to 50 times higher than permitted in the Cosmetics Directive in a previous submission. No nitrosamine data was provided in this submission.
- Stability of hydroxybenzomorpholine in marketed products is not provided.
- Adequate chemical identification /characterisation has been performed only for two batches (Op.59 and 0508918) of hydroxybenzomorpholine. Chemical identification and characterisation of batch Op.45 as hydroxybenzomorpholine is based only on HPTLC.

3.2. Function and uses

Hydroxybenzomorpholine is used in oxidative hair dye formulations at a maximum on-head concentration of 1%, after mixing with the developer containing hydrogen peroxide.

3.3. Toxicological Evaluation

3.3.1.	Acute toxicity

3.3.1.1.	Acute oral toxicity

Guideline:	OECD 401 (1987)
Species/strain:	Rat, Sprague-Dawley
Group size:	5 per sex
Test substance:	Imexine OV
Batch:	op. 90
Purity:	99.2%
Dose levels:	5000, 2000, 1000 or 500 mg/kg bw (20 ml/kg) as 25, 10, 5 and 2.5 (w/v) suspension
Vehicle:	0.5% aqueous carboxymethylcellulose (w/v)
Observation:	15 days
GLP:	in compliance

A single oral (gavage) 5000 mg/kg bw dose of the test substance to 5 week old rats (5 per sex) after overnight fasting.
Due to the mortalities, 5 days later, further groups were dosed at 2000 and 1000 mg/kg bw as 10 and 5% (w/v) suspension, followed by a final group 14 days later, at 500 mg/kg bw dose as 2.5% (w/v) suspension

Animals were observed at 15 min, 1, 2 and 4h and then daily for clinical signs and mortality. Body weights were recorded on the Day -1, 1, 8 and 15. Post-mortems were carried out on all animals. No organ/tissue samples were taken.

Results
All animals given 5000 mg/kg were found dead within 4 h. The initial effect of clonic convulsions and lethargy were noted in 15 minutes of dosing leading to coma and death.
All females and 1 male given 2000 mg/kg were found dead within 4 h. The remaining males were dead by Day 2. Lethargy and prostration were noted in the first 4 hours of dosing leading to coma and death after showing unconsciousness and clonic convulsions.
At 1000 mg/kg, 2 females were found dead on day 2, and all animals showed unconsciousness and prostration mainly on the day of dosing. Surviving animals resumed normal appearance and behaviour within day 8.
There were no deaths at 500 mg/kg, no body weight changes, and clinical signs were limited to prostration on the day of dosing.

Conclusion
Under the conditions of this study, the maximal non-lethal dose of hydroxybenzomorpholine was 500 mg/kg, the minimal lethal dose was 1000 mg/kg, and the median lethal dose (LD_{50}) was between 1000 and 2000 mg/kg.

Ref.: 1

3.3.1.2.	Acute dermal toxicity

/

3.3.1.3.	Acute inhalation toxicity

/

3.3.2.	Irritation and corrosivity

3.3.2.1.	Skin irritation

Guideline: OECD 404
Species: New Zealand rabbits
Group: 3 male
Substance: IMEXINE OV
Batch: T145
Purity: 100%
Dose: 500 mg
GLP: in compliance

Approximately 24 hours prior to the application of hydroxybenzomorpholine, the flanks of each animal were clipped free of fur. A 500 mg sample of neat hydroxybenzomorpholine moistened with 0.5ml water was applied to a 6 cm^2 area of the right flank of each animal (the untreated left flank served as control). It was held in contact with the skin for 4 hours by means of a semi-occlusive dressing. Subsequently, the dressings were removed, any residual test substance was removed and the treated area was observed 1, 24, 48 and 72 hours afterwards.

Results
There were no skin reactions at any observation time-point.

Conclusion
Under the conditions of this study, hydroxybenzomorpholine was non-irritating to rabbit skin when tested undiluted.

Ref.: 2

3.3.2.2. Mucous membrane irritation

Eye Irritation in Rabbits (neat test item)

Guideline:	OECD 405
Species:	New Zealand rabbits
Group:	3 male
Substance:	IMEXINE OV
Batch:	T145
Purity:	100%
Dose:	100 mg
GLP:	in compliance

A 100 mg sample of neat hydroxybenzomorpholine was instilled into the conjunctival sac of the left eye of the animals after gently pulling the lower lid away from the eye ball. The lids were then held together for about one second to avoid any loss of test substance, and the eyes were not rinsed after instillation. The untreated right eye served as control, and ocular reactions were assessed 1, 24, 48, 72 and 96 hours after instillation.

Results
Marked conjunctival reactions (marked chemosis, slight to moderate redness) were observed in all animals one hour after instillation. These conjunctival reactions fully disappeared within 72 hours of instillation. Moderate to marked corneal opacity was observed in all animals on the day after instillation and fully disappeared within 96 hours. Isolated ocular discharge and minimal iris lesions were also observed.

Conclusion
Under the conditions of this study, hydroxybenzomorpholine was considered to be moderately irritating to rabbit eyes when tested undiluted.

Ref.: 3

Eye Irritation in Rabbits (diluted test item)

Guideline:	OECD 405
Species:	New Zealand rabbits
Group:	3 male
Substance:	IMEXINE OV
Batch:	0508918
Purity:	98.3%
Dose:	0.1 ml of 2% IMEXINE OV (diluted in a 0.5% aqueous solution of carboxymethylcellulose)
GLP:	in compliance

A 0.1 ml aliquot of a 2% solution of hydroxybenzomorpholine in 0.5% aqueous carboxymethylcellulose was instilled into the left conjunctival sac of test animals. The eyes were not rinsed following instillation of the test item. The non-treated right eye served as control, and ocular reactions were assessed 1, 24, 48 and 72 hours after instillation.

Results
Ocular reactions were seen in 1 animal with slight chemosis (grade 1) and slight redness of conjunctivae (grade 2) at 1 hour after instillation only.

Conclusion
Under the conditions of this study, hydroxybenzomorpholine at 2% in 0.5% aqueous carboxymethylcellulose was transiently irritating to rabbit eyes.

Ref.: 4

3.3.3. Skin sensitisation

Guinea-Pig Maximisation Test (Magnusson/Kligman Test Method)

Guideline:	OECD 406
Species:	Dunkin-Hartley guinea pigs
Group:	10 female (treated) and 5 female (control)
Substance:	IMEXINE OV
Batch:	T145
Purity:	100%
Dose:	1% IMEXINE OV intradermally with FCA; 25% IMEXINE OV topically (pre-treatment with sodium lauryl sulphate)
GLP:	in compliance

The dilutions of IMEXINE OV used were selected on the basis of the results from preliminary intra-dermal and topical experiments in which:

- 1% (w/w) hydroxybenzomorpholine in paraffin oil was the maximum practicable concentration for intra-dermal injection; only slight irritation was observed at this concentration;
- 25% (w/w) hydroxybenzomorpholine in paraffin oil was the maximum practicable concentration for topical induction and challenge and it did not provoke any signs of irritation.

The application sites were clipped free of fur before each induction or challenge treatment. The induction procedure consisted of intra-dermal injections and one topical application, 7-day apart. On a day designated as day 1, the animals received 3 pairs of intra-dermal injections in the scapular area, consisting of:

- 50% (v/v) Freund's Complete Adjuvant in physiological saline (FCA, control and treated animals)
- hydroxybenzomorpholine at 0% or 1% (w/w) in paraffin oil (control and treated animals, respectively)
- a 50/50 (w/v) mixture of FCA and 0% or 1% hydroxybenzomorpholine (w/w) in paraffin oil (control and treated animals, respectively)

As hydroxybenzomorpholine at 25% (w/w) in paraffin oil (maximum practicable concentration) was non-irritating, the animals were treated on day 7 with 0.5 ml sodium lauryl sulphate in petrolatum (10%) to elicit local irritation. On day 8, 0.5 ml of hydroxybenzomorpholine at 0 or 25% (w/w) in paraffin oil was prepared on a dry compress and applied over the intra-dermal injection sites in control and treated animals, respectively. The compress was held under occlusion for 48 hours by means of surgical tape and elastic adhesive bandage (topical induction). Two weeks after the topical induction (day 22), 0.5 ml of hydroxybenzomorpholine at 0 or 25% (w/w) in paraffin oil was prepared on a dry compress and applied to a 4 cm^2 area to all control and treated animals on their left and right flanks, respectively (challenge topical application). These patches were kept for 24 hours under occlusion. The animals were examined and cutaneous reactions were assessed 24 and 48 hours after dressing removal.

Results
No skin reactions were observed at the vehicle (paraffin oil) or hydroxybenzomorpholine challenge application sites in control or treated animals, at any examination time-point.

Conclusion
Under the conditions of the present study, hydroxybenzomorpholine was non-sensitising.

Ref.: 5

3.3.4. Dermal / percutaneous absorption

***In vitro* Percutaneous Absorption Study using dermatomed human Skin**

Guideline:	OECD 428 draft
Species:	Human female
Group:	8 female donors; abdominal or breast skin
Substance:	IMEXINE OV
Batch:	0508918 (and CFQ13914 Batch 1 of 6-Hydroxy[benzene ring-U-^{14}C]-benzomorpholine)
Purity:	98.3% (98.5% for the radiochemical label)
Dose:	1% in a formulation
GLP:	in compliance

Human abdominal or breast skin samples were obtained from eight different female donors subjected to plastic surgery. The skin samples were transferred on ice and kept frozen at -20°C until use.

Skin samples were dermatomed (380-400 µm in thickness) and mounted in flow-through diffusion cells, using calcium and magnesium-free phosphate-buffered saline as the receptor fluid. The integrity of the skin was checked by determining the permeability coefficient for tritiated water (<2.5 x 10^{-3} cm/h for all selected membranes), and skin was maintained at approximately 32°C. Twenty-four diffusion cells were used in two separate experiments.

In a first experiment (oxidative conditions), a typical oxidative hair dye formulation containing 2% w/w hydroxybenzomorpholine associated with the primary intermediate p-phenylenediamine (PPD) was mixed with the developer (1:1, w/w) to yield a final concentration of 1% w/w hydroxybenzomorpholine. Twenty (20) mg/cm² of this mixture was applied to the skin surface (corresponding to exactly 212.1 µg/cm² of hydroxybenzomorpholine). After 30 minutes, the remaining formulation on the skin surface was removed using standardized washing procedures, simulating use conditions. Twenty-four (24) hours after application, the percutaneous absorption of [^{14}C]-Hydroxybenzomorpholine was estimated by measuring its concentration by liquid scintillation counting in the following compartments/samples: skin washes (dislodgeable dose), *stratum corneum* (isolated by tape strippings), living epidermis/dermis and receptor fluid.

In a separate experiment, a similar experimental procedure was applied to evaluate the percutaneous absorption of hydroxybenzomorpholine in non-oxidative conditions, using a formulation devoid of PPD and mixed with water (1:1, w/w) to yield a final concentration of 1% hydroxybenzomorpholine (about 20 mg/cm^2 were applied, corresponding exactly to 208.1 µg/cm^2).

Results

A summary of the mean results is provided in the table below.

Formulation / Test preparation	**Oxidative**	**Non Oxidative**
Target Hydroxybenzomorpholine concentration in formulation (% w/w)	2.00	2.00
Actual Hydroxybenzomorpholine concentration in formulation (% w/w)	2.03	2.04
Target Hydroxybenzomorpholine concentration in test preparation (% w/w)	1.00	1.00
Actual Hydroxybenzomorpholine concentration in test preparation (% w/w)	1.01	1.02
Target application rate of test preparation (mg/cm²)	20.0	20.0
Actual application rate of test preparation (mg/cm²)	21.0	20.4
Hydroxybenzomorpholine (% applied dose)	(Mean ± SD)	
Dislodgeable dose	94.30 ± 2.33	94.99 ± 5.70
Unabsorbed dose *	96.18 ± 2.58	96.08 ± 5.07
Absorbed dose **	0.16 ± 0.12	1.77 ± 1.03
Dermal delivery ***	0.25 ± 0.15	2.20 ± 1.24
Mass balance	96.43 ± 2.54	98.28 ± 4.69
Hydroxybenzomorpholine (µg equiv/cm²)	(Mean ± SD)	
Dislodgeable dose	194.91 ± 6.36	193.46 ± 6.51
Unabsorbed dose *	198.78 ± 6.08	195.70 ± 4.79
Absorbed dose **	0.32 ± 0.26	3.62 ± 2.14
Dermal delivery ***	0.52 ± 0.33	4.50 ± 2.58
Mass balance	199.30 ± 6.12	200.20 ± 3.51

* Unabsorbed dose = dislodgeable dose + stratum corneum + unexposed skin

** Absorbed dose = receptor fluid + receptor rinse

*** Dermal delivery = exposed skin (except stratum corneum) + absorbed dose

A total of 22 samples of human skin yielded data that could be analysed (10 in oxidative conditions and 12 in non-oxidative conditions). Most of the hydroxybenzomorpholine applied on the skin surface was removed with the skin washes (dislodgeable dose, about 94% and 95% of the applied dose in oxidative and non-oxidative conditions, respectively), and the total recovery rate was about 96% and 98% in oxidative and non-oxidative conditions, respectively.

The mean amounts of hydroxybenzomorpholine considered as absorbed (dermal delivery) were estimated as follows (sum of the amounts measured in living epidermis/dermis and receptor fluid): 0.52 ± 0.33 µg equiv/cm^2 (0.25 ± 0.15% of the applied dose) and 4.50 ± 2.58 µg equiv/cm^2 (2.20 ± 1.24% of the applied dose) in oxidative and non-oxidative conditions, respectively.

	Oxidative conditions		**Non-oxidative conditions**	
Cutaneous distribution	**µg equiv/cm²**	**% applied dose**	**µg equiv/cm²**	**% applied dose**
Dislodgeable dose	194.91 ± 6.36	94.30 ± 2.33	193.46 ± 6.51	94.99 ± 5.70
Receptor fluid	0.32 ± 0.26	0.16 ± 0.12	3.62 ± 2.14	1.77 ± 1.03
Dermal delivery *	**0.52 ± 0.33**	0.25 ± 0.15	4.50 ± 2.58	2.20 ± 1.24

* Dermal delivery = exposed skin (except stratum corneum) + absorbed dose (= receptor fluid + receptor rinse)

Conclusion
The amounts of hydroxybenzomorpholine considered as absorbed from a typical oxidative hair colouring formulation containing hydroxybenzomorpholine at a final concentration of 1% were estimated to be 0.52 ± 0.33 µg/cm^2 (0.11 – 1.04 µg/cm^2) and 4.50 ± 2.58 µg/cm^2 (0.84 – 8.53 µg/cm^2) in oxidative and non-oxidative conditions, respectively.

Ref.: 16

3.3.5. Repeated dose toxicity

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

Guideline:	OECD 407
Species:	Rat, Sprague Dawley OFA
Route:	oral
Group sizes:	10 male and 10 female
Substance:	Imexine OV suspended 2 % Polysorbate 80 sterile water hydrogel (containing dimethicone)
Batch:	Op. 90
Purity:	99.2%, but not specified in study report
Dose:	0, 10, 100 and 1000 mg/kg bw/day in a volume of 10 mg/kg bw
Exposure:	30 days - male, 31 days – female
GLP:	in compliance

The test substance, suspended in a hydrogel containing 2% polysorbate 80 in sterile water (containing dimethicone as an anti-foaming agent), was administered by gavage once daily to groups of Sprague Dawley rats (10/sex) for 30 days (males) and 31 days (females). The dose levels were 10, 100 and 1000 mg/kg bw. The control group (10/sex) received the vehicle alone. All animals were killed at the end of the study.

All animals were observed twice daily for mortality and clinical signs. Body weights and food consumption were recorded individually at weekly intervals. Ophthalmoscopic examination was performed on day 0 and at termination in the control and high dose group animals only. Blood samples were taken from all animals at day 0 and at termination for haematological and clinical chemistry investigations. Urine samples were collected on day 0 and during week 4. Organ weights were recorded. Macroscopic and histopathologic examination of tissues was undertaken.

Results
No treatment related mortalities were reported. Abnormalities reported in animals treated with 1000 mg/kg bw included; lethargy and excessive salivation, decreased body weight in males, decreased food consumption in the first three days of treatment in both sexes, a slight increase in the number of neutrophils in males, a 65 % increase in triglycerides in females, dark discolouration and slight acidification of the urine and an increase in urinary proteins.
The high dose group also showed an increase in both relative and absolute liver, kidney and testis weights.
There were no overt or biochemical signs of toxicity in animals treated with either 10 or 100 mg/kg bw.
Histological examination of the kidneys showed dose-related epithelial necrosis, basophilia and tubular dilation in the cortical tubules in male rats treated with 100 and 1000 mg/kg bw. No effects were seen in animals treated with 10 mg/kg bw per day. No histopathological effects were seen in female rats.

Conclusion
In this study, daily oral administration of hydroxybenzomorpholine to rats for 4 weeks did not produce any signs of toxicity at 10 mg/kg/day. The main changes were microscopic findings in the kidneys observed with a dose-related incidence and severity at 100 and 1000 mg/kg/day. The No Observed Adverse Effect Level (NOAEL) was considered to be 10 mg/kg/day.

Ref.: 6

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

Guideline:	OECD 408
Species:	Rat, Sprague Dawley Crl:CD (SD)BR (VAF plus)
Route:	oral
Group sizes:	10 male and 10 female
Substance:	Imexine OV
Batch:	T 142
Purity:	99.4%
Dose:	0, 5, 25 and 125 mg/kg bw/day in a volume of 10 mg/kg bw
Vehicle:	Suspension in 0.5% aqueous methylcellulose with Tween 80 at 0.5% of final formulation
Exposure:	90 days
GLP:	in compliance

The test solutions were made daily and used within 5 h of preparation. Evaluations and measurements included daily clinical observations and deaths and weekly body weight and food intake, and ophthalmological examination in acclimation period (all animals) and in week 13 (control and high dose animals). Haematological, blood clinical chemistry investigations (week 13) and urinalysis (week 12) were performed at the end of the dosing period.

At the end of the treatment period, animals were killed and subjected to macroscopic examination. Selected organs were weighed, and a wide range of organs/tissues were preserved. Histopathology was performed for specified tissues/organs from control, high dose group and the sole low dose female that died, together with lungs and gross lesions from the low and mid dose groups.

Results
The chemical analysis of the dose formulations given showed that achieved concentrations were close to the intended values.
One low dose female died in week 8 without showing any prior clinical sign. Cause of death could not be determined, and in the absence of adverse clinical signs and deaths at the mid and high dose, this isolated death was not attributed to hydroxybenzomorpholine.
There were no adverse clinical signs. The only sign observed was brown staining of tail and skin at 125 mg/kg/day. There were no changes in body weight gain or food intake. No changes attributed to hydroxybenzomorpholine were observed at ophthalmological, haematology, blood biochemical or urinary examinations, apart from brown-discoloured urine at 125 mg/kg/day. This was considered to indicate systemic exposure following oral administration of hydroxybenzomorpholine.
There were no significant changes in organs weight and no macroscopic or microscopic findings that could be attributed to the administration of hydroxybenzomorpholine.
In this study, the No Observed Adverse Effect Level (NOAEL) was considered to be 125 mg/kg/day. The only changes observed were the staining properties of hydroxybenzomorpholine causing brown staining of the skin, tail and urine at 125 mg/kg/day.

Ref.: 7

Comment
According to the OECD 408, a toxic dose should have been used.

3.3.5.3. Chronic (> 12 months) toxicity

/

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537
Replicates:	3 replicates in 2 individual experiments both in the presence and absence of S9
Test substance:	6-hydroxybenzomorpholine
Solvent:	DMSO
Batch:	0508918
Purity:	98.3%
Concentrations:	Experiment 1: 312.5 - 5000 µg/plate without and with S9 Experiment 2: 312.5 - 5000 µg/plate without and with S9
Treatment:	Experiment 1: direct plate incorporation with 48 – 72 h incubation time without and with S9

	Experiment 2: direct plate incorporation with 48 - 72 h incubation time without S9 pre-incubation method was used with 60 minutes pre-incubation and 48 -72 h incubation time with S9
GLP:	In compliance

Hydroxybenzomorpholine was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a preliminary toxicity test with strains TA98, TA100 and TA102. Toxicity was evaluated on the basis of a reduction in the number of revertant colonies and/or thinning of the bacterial background lawn. Since hydroxybenzomorpholine was freely soluble and non toxic in this preliminary toxicity test, it was tested up to the prescribed maximum concentration of 5000 µg/plate. The preliminary toxicity test, experiment 1 and experiment 2 without S9 were performed with the direct plate incorporation method, experiment 2 with S9 according the preincubation method. Negative and positive controls were in accordance with the OECD guideline.

Results
Precipitation of hydroxybenzomorpholine was not observed. Marked toxicity was only seen in the TA98 strain without S9 at 5000 µg/plate. Toxicity was not noted towards all the other strains used without or with S9. A coloration of agar was noted at dose levels of 2500 µg/plate and above without S9 and at 5000 µg/plate with S9.
In experiment 1 without S9 a slight increase in the number of revertants was seen in the TA1535 strain. This increase was considered not biologically relevant since the number of revertants remained within the range of the historical control and the increase could not be confirmed in the second experiment.
A more or less dose related and reproducible increase was found in the number of revertants in strain TA 98 with S9.

Conclusion
Under the experimental conditions used hydroxybenzomorpholine was genotoxic (mutagenic) in the gene mutation tests in bacteria in strain TA98 in the presence of S9 metabolic activation.

Ref.: 8

In vitro Mammalian Cell Gene Mutation Test (hprt locus)

Guideline:	OECD 476	
Cells:	L5178Y Mouse lymphoma cells	
Replicates:	2 replicates in 2 independent experiments	
Test substance:	6-hydroxybenzomorpholine	
Solvent:	DMSO	
Batch:	0508918	
Purity:	98.3%	
Concentrations:	Experiment 1:	25 - 350 µg/ml (without S9)
		50 - 600 µg/ml (with S9)
	Experiment 2:	50 - 200 µg/ml (without S9)
		50 - 300 µg/ml (with S9)
Treatment	3 h both without and with S9; expression period 7 days	

GLP: In compliance

Hydroxybenzomorpholine was assayed for gene mutations at the *hprt* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Test concentrations were based on the results of a cytotoxicity range-finding experiment measuring relative survival. In the main test, cells were treated for 3 h followed by an expression period of 7 days to fix the DNA damage into a stable *hprt* mutation. Liver S9 fraction from Arachlor 1254-induced rats was used as exogenous metabolic activation system. Toxicity was measured as percentage relative survival of the treated cultures relative to the survival of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
The test meets to all acceptance criteria and, consequently, the study was considered valid.
In both experiments in the absence and presence of S9 the appropriate level of toxicity (10-20% survival after the highest dose) was reached pointing to sufficient exposure of the cells.
In the absence of S9 no increase in mutant frequency was seen in the first experiment. In the second experiment a statistical significant increase was found at an intermediate concentration. As this result appeared not reproducible and remained within the historical control values this increase was considered not biological relevant.
In the presence of S9 in the first experiment a statistical significant increase in mutant frequency was found at the highest dose evaluated. This mutant frequency was similar to the upper limit of the historical control value, indicating that the increase was minimal whereas significance may be due to a low concurrent control value. In the second experiment no increases in mutant frequencies were found. At the highest dose (which is lower than the highest dose in experiment 1) an almost similar level of toxicity was found but no increase in mutant frequency. The increase in mutant frequency at the highest dose in experiment 1 was, therefore, considered as not biological relevant.

Conclusion
Under the experimental conditions used, hydroxybenzomorpholine was considered not mutagenic in the gene mutation test with mouse lymphoma cells at the *hprt* locus.

Ref.: 9

In vitro chromosome aberration test

Guideline:	OECD 473
Replicates:	2 replicates
Cells:	CHO (K_1-BH_4)
Test substance:	Imexine OV
Solvent:	DMSO
Batch:	Op. 90
Purity:	99.9 %
Concentrations:	9.8 – 39.1 µg/ml in the absence of S9 39.1 – 313 µg/ml in the presence of S9
Treatment:	21 h followed immediately by harvest in the absence of S9. 4 h treatment and harvest time 21 h after start of treatment in the presence of S9
GLP:	In compliance

Hydroxybenzomorpholine has been investigated in the absence and presence of metabolic activation for the induction of chromosomal aberrations in CHO cells. From a range of concentrations up to prescribed maximum concentration of 5000 µg/ml, the dose level causing a decrease in mitotic index of 50% of the solvent control value was used as the highest dose level. In the absence of S9 cells were treated for 21 h and immediately harvested; in the presence of S9 cells were treated for 4 h and harvested 21 h after the start of treatment. Two hours before harvest, each culture was treated with colchicine solution (final concentration 0.25 µg/ml) to block cells at metaphase of mitosis. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Toxicity was determined by measuring the decrease in the mitotic index. Chromosome (metaphase) preparations were stained with 10% Giemsa and examined microscopically for chromosomal aberrations. Negative and positive controls were in accordance with the OECD draft guideline.

Results
Hydroxybenzomorpholine induced sufficient toxicity as the mitotic index after the highest dose was reduced with about 50% compared to the negative control both in the absence or presence of S9.
In both the absence and the presence of a metabolic activation, hydroxybenzomorpholine (Imexine OV) did not cause a biological relevant and dose dependent increase in cells with chromosome aberrations.

Conclusion
Under the experimental conditions used hydroxybenzomorpholine did not show evidence for a genotoxic (clastogenic) activity in CHO cells *in vitro*.

Ref.: 10

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mouse bone marrow micronucleus test in mice

Guideline:	OECD 474
Species/strain:	CD1 mice
Group size:	5 mice/sex/group
Test substance:	Imexine OV
Batch:	E70 CXB
Purity:	not given
Dose level:	400 mg/kg bw
Route:	gavage, once
Vehicle:	arachis oil BP
Sacrifice times:	24, 48 and 72 h after the treatment.
GLP:	In compliance

Hydroxybenzomorpholine has been investigated for the induction of micronuclei in bone marrow cells of mice. The test concentration was based on the result of a range finding toxicity study in which mice were exposed to a range of concentrations; 400 mg/kg was selected as the maximum tolerated dose level. In the main experiment mice were exposed by gavage to a single dose of 400 mg/kg bw. Bone marrow cells were collected 24, 48 and 72 h after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between normochromatic to polychromatic erythrocytes (PCE/NCE ratio). Moreover, all animals were

observed daily for signs of overt toxicity and death. Bone marrow preparations were stained and examined microscopically for the PCE/NCE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
One female mouse from the 48 h group died. The ratio PCE/NCE substantially changed in the 72 h group as compared to the untreated controls at 72 h indicating that hydroxybenzomorpholine did have cytotoxic properties in the bone marrow and consequently must have been biologically available. Moreover, many of the treated mice showed lethargic signs immediately after dosing and ptosis at 24, 48 and 72 h after dosing indicating to systemic toxicity and confirming exposure to hydroxybenzomorpholine.
Biological relevant increases in the number of micronucleated PCEs compared to the concurrent vehicle controls were not found following treatment with hydroxybenzomorpholine at any time point.

Conclusion
Under the experimental conditions used hydroxybenzomorpholine did not induce micronuclei in bone marrow cells of treated mice and, consequently, hydroxybenzomorpholine was not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 11

Mouse bone marrow micronucleus test in rats

Guideline:	OECD 474
Species/strain:	Sprague-Dawley rats
Group size:	5 rats/sex/group
Test substance:	hydroxybenzomorpholine
Batch:	0508918
Purity:	98.3 %
Dose level:	500, 1000 and 2000 mg/kg bw
Route:	experiment 1: gavage, once experiment 2: gavage, twice 24 h apart
Vehicle:	carboxymethylcellulose 0.5%
Sacrifice times:	experiment 1: 24 h for all dose levels, vehicle control and positive control, 48 h for the highest dose and vehicle control. experiment 2: 24 h for all dose levels, vehicle control and positive control.
GLP:	In compliance

Hydroxybenzomorpholine has been investigated for the induction of micronuclei in bone marrow cells of rats.
Test concentrations were based on a preliminary toxicity test in which clinical signs and mortality was recorded for a period of 48 h. In the first experiment rats were exposed by gavage to single doses of 0, 500, 1000 and 2000 mg/kg bw hydroxybenzomorpholine. Bone marrow cells were collected 24 h or 48 h (highest dose and vehicle control only) after dosing. Additional rats were used in a confirmatory test (highest dose, vehicle control and positive control). Satellite rats allocated for determination of plasma level of hydroxybenzomorpholine (determined 0.5, 1 and 4 h after treatment) were incorporated.
In the second experiment rats were exposed by gavage to two doses 24 h apart of 0, 500, 1000 and 2000 mg/kg bw hydroxybenzomorpholine. Bone marrow cells were only collected 24 h after

the second dose. Again satellite rats allocated for determination of plasma level of hydroxybenzomorpholine (determined 0.5, 1 and 4 h after treatment) were incorporated.
Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE ratio). Moreover, all animals were observed daily for clinical signs and mortality. Bone marrow preparations were stained and examined microscopically for the PCE/NCE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
The results of the measurement of plasma levels in experiment 1 clearly demonstrated that animals were systemically exposed to hydroxybenzomorpholine. However, the PCE/NCE ratios of the treated groups were equivalent with those from the untreated controls. No mortality and no clinical signs were observed in the rats of either sex. Exclusively, in the female high dose group a slightly higher (but not statistically significant) frequency of micronucleated erythrocytes was found. In the additional confirmatory groups again a slightly higher frequency in micronucleated erythrocytes (again not statistically significant but slightly above historical data) was found.
In experiment 2 measurement of plasma levels again demonstrated the systemic exposure of the animals. The PCE/NCE ratios of all treated groups were equivalent with those from the untreated controls. No mortality and no clinical signs were observed in the rats of the low dose group. Rats receiving the mid dose showed piloerection after the second treatment. At the high dose 1 of the 5 females was found dead 2 h after the second treatment and 1 out of 3 of the supplementary (back up) females 24 h after the second treatment. All rats showed hypoactivity after the first treatment and pilo-erection was observed in all surviving rats after the second treatment. A biological relevant increase in micronucleated erythrocytes was not found in any of the groups treated with hydroxybenzomorpholine.
Since the findings in the high dosed females in the first could not be confirmed in the second experiment, the slightly higher frequency in micronucleated erythrocytes in the high dose females was not considered biologically relevant.

Conclusion
Under the experimental conditions used hydroxybenzomorpholine did not induce micronuclei in bone marrow cells of treated rats and, consequently, hydroxybenzomorpholine was not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of rats.

Ref.: 12

In vivo unscheduled DNA synthesis (UDS) test

Guideline:	OECD 486
Species/strain:	Crl:CD® (SD)IGS BR rats
Group size:	4 female rats/group
Test substance:	6-hydroxybenzomorpholine
Batch:	0508918
Purity:	98.3 %
Dose level:	375, 750 and 1500 mg/kg bw
Route:	gavage, once
Vehicle:	carboxymethylcellulose 0.5%
Sacrifice times:	2-4 h and 14-16.5 h after dosing
GLP:	In compliance

Hydroxybenzomorpholine was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Rats were treated in vivo. The highest dose selected for this UDS assay was 1500 mg/kg bw based on the results of a dose range finding study. In this dose range finding study 2 out of 3 females died about 8 h after dosing in the 2000 mg/kg bw group whereas 1 out of 3 females treated with 1500 mg/kg bw died one day after dosing. Because females were found to be more sensitive to acute toxicity than the males, only females were used in the main experiment. Next to 1500 mg/kg bw, 2 additional dose levels were selected using dilutions of the highest dose.
Hepatocytes for UDS analysis were collected at 2 - 4 h and 14 - 16.5 h after administration of hydroxybenzomorpholine. All animals from each group were perfused for the collection of hepatocytes and establishment of cultures. After attachment of the cultures they were labelled for 4 h with 10 μCi/ml ^{3}H-thymidine. Evaluation of autoradiography was done after 6-10 days exposure.
UDS was measured by counting nuclear grains and substracting the average number of grains in 3 nuclear sized areas adjacent to each nucleus; this value is referred to as nuclear grain count.
The nuclear labelling, measured as the mean net nuclear grain count or the percent of nuclei with five or more net nuclear grains, is used to determine if a response has occurred
Unscheduled synthesis was determined in 50 randomly selected hepatocytes per dose. Negative and positive controls were in accordance with the OECD guideline.

Results
At the 2-4 h time point clinical signs observed in the high dose group prior top perfusion were squinted eyes, lacrimation, hypoactivity, sensitivity to touch and brown urine. At the 14-16.5 time point all animals were normal after dosing with the exception of hypoactivity in the low dose group and slight hypoactivity in the high dose group. Prior to perfusion animals treated with the high dose (1500 mg/kg bw) were observed with (slight) hypoactivity, brown urine, laboured breathing, ataxia and/or chromodacryorrhea.
Both for the 2-4 h and the 14-16.5 time point after treatment none of the individual groups showed an increased mean net nuclear grain count as compared with the untreated control. Also the number of cells with 5 or more nuclear grains per cells never reached the necessary criterion of 10% above the percentage found for the untreated control. Thus, no evidence for UDS was obtained in any treatment group at both time points.

Conclusion
Under the experimental conditions used hydroxybenzomorpholine did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in the in vivo UDS test.

Ref.: 13

3.3.7. Carcinogenicity

Topical application, mice

Guideline:	/
Species/strain:	Swiss-Webster mice
Group size:	50 animals per sex and dose
Test substance:	One hair dye formulation containing 1.1% hydroxybenzomorpholine
Batch:	/
Purity:	not stated
Dose level:	0.05 ml of a solution containing 1.1% hydroxybenzomorpholine (dye formulation P-25) prior to mixing with an equal volume of 6% hydrogen peroxide. The mixture was used within 15 minutes after mixing
Route:	Topical, 1 application weekly
Exposure:	21 months
GLP:	not in compliance

The experiment involved 12 different dye formulations, but hydroxybenzomorpholine was in just one of these, and 3 negative control groups.

Dye applied topically to a 1 cm^2 area on a clipped (24 hours prior to application) site in the interscapular region. Mice received a dose of 0.05 ml topically without occlusion once weekly from 8 – 10 weeks of age for 21 months. The animals were observed daily for mortality and signs of toxicity, and were weighed monthly. A continuous weekly record was maintained for any skin lesions noted. After 9 months of treatment, 10 males and 10 females per group were necropsied and the study was terminated after 21 months. Skin and internal organs were evaluated histologically.

Six males and 8 females survived to 21 months in the group receiving the oxidative formulation containing 0.55% hydroxybenzomorpholine. At 21 months, there were 8, 9 and 12 males and 11, 14 and 14 females surviving in the three control groups. There were no significant differences in absolute or relative liver or kidney weights in groups of 10 male and 10 female mice necropsied after 7 and 9 months. There were no statistically significant differences in the distribution of tumours among treated and control groups.

Comment
2,4-Diaminoanisole (EU, carcinogen, Category 2) was tested in the same experiment and no response was found. No conclusion with regard to carcinogenicity can be made from the study.

Ref.: 14

The rat study is not mentioned in the submission

Topical application, rats

Guideline:	/
Species/strain:	Male and female weanling Sprague Dawley rats
Group size:	60 animals per sex and dose

Test substance:	One hair dye formulations containing 1.1% hydroxybenzomorpholine
Batch:	/
Purity:	not stated
Dose level:	0.5 ml of a solution containing 1.1% hydroxybenzomorpholine (dye formulation P-25) prior to mixing with an equal volume of 6% hydrogen peroxide. The mixture was used within 15 minutes after mixing
Route:	Topical. 1 application twice weekly
Exposure:	114 weeks
GLP:	in compliance

The experiment involved 10 different dye formulations but A25 was in just one of these and 3 negative control groups.

Groups of 60 male and 60 female were obtained from the first mating (F_{1a}) of a multi-generation reproduction study in rats treated with a hair dye formulation containing 0.55% hydroxybenzomorpholine. The F_0 parents had received topical application of the hair dye formulation from the time of their weaning to the weaning of their offspring. The dye formulation was administered topically to the shaved (24 hours prior to application) neck and back area twice weekly. An initial dosage level of 0.2 ml/rat was increased incrementally by 0.1 ml per week until 0.5 ml was achieved. There were three independent control groups each containing 60 males and 60 females, which received no treatment.

The rats were observed daily for overt signs of toxicity and for mortality. Detailed observations were recorded weekly. Individual body weights were recorded weekly for the first 14 weeks and monthly thereafter. Group food consumption was recorded weekly. Haematological, biochemical and urinalysis studies were done on 5 males and 5 females per group at 3, 12, 18, and 24 months of study. After 12 months of treatment, 5 males and 5 females from each group were sacrificed and necropsied and all rats of a sex group were sacrificed and necropsied when survival reached 20%. Histopathological evaluations were performed on 18 tissues (plus tumour masses) including treated skin.

Several male and female rats from the treated group had hyperkerathosis and/or acanthosis involving the stomach mucosa. These conditions were not reported in any rats from the three control groups nor in any rats from the P-25 group and was considered possible compound related.

Hepatocellular hypertrophy/hyperplasia or hyperplastic/hypertrophic nodules were observed in the livers of several rats, especially males, from the group containing hydroxybenzomorpholine. The increase in incidence of these lesions when compared to rats from the three control groups was considered possibly compound related.

Survival just prior to terminal sacrifice at week 117, 118 or 119 was 12 males and 14 females for the formulation group. Survival in the control groups were 15 males and 14 – 18 females for the control groups. The mean body weights at week 114 in the treated group were 701 g in males and 458 g in females. Control group values ranged from 682 to 759 g in males and 477 to 513 g in females.

There were no significant changes in haematological values in the treated groups at 18 and 24 months. No significant differences considered to be treatment related were observed in the biochemical studies or in the urinalysis. Non-neoplastic lesions were those commonly found in

ageing rats and were considered to be spontaneous. No increased tumour incidences were found in any of the tissues examined.

Ref.: A

Comment
2,4-Diaminoanisole (EU, carcinogen, Category 2) was tested in the same experiment and no response was found.
No conclusion with regard to carcinogenicity can be made from the study.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

3.3.8.2. Teratogenicity

Guideline:	OECD 414 (2001)
Species:	Rat, Sprague Dawley Crl:CD (IGS)BR (COBS-VAF)
Route:	oral by gavage
Group sizes:	24
Substance:	Hydroxybenzomorpholine
Batch:	0508918
Purity:	98.3%
Dose:	0, 20, 125 and 500 mg/kg bw/day in volume of 5 ml/kg day
Vehicle:	Suspension in 0.5% aqueous methylcellulose
Exposure:	Gestation Days (GD) 6 -19
GLP:	in compliance

The test solutions were prepared under nitrogen and stored for up to 9 days at + 4 °C and under nitrogen until used. Hydroxybenzomorpholine was suspended in 0.5% aqueous carboxymethylcellulose and given at 5 ml/kg. These dose levels were selected on the basis of the results from a preliminary study where dose-related maternal toxicity was observed at 400 and 550 mg/kg/day [32].
Each animal was checked at least twice on dosing days and once on other days for clinical signs and death. Body weight was recorded on GD 0, 3, 6, 9 12, 15 18 and 20. Food intake, was recorded over intervals; GD 0-3, 3-6, 6–9, 9-12, 12–15, 15–18, and 18-20.
Post-mortems of all animals were conducted on GD 20. Adrenals, brain, heart, kidneys, liver, Ovaries and intact uterus removed and examined for corpora lutea, implantation sites, the presence of resorption sites (early and late) and foetuses (live, dead and position). Live foetuses were weighed sexed and checked for gross malformations. Approximately half of the live foetuses where examined for visceral anomalies, and the remaining foetuses were examined for skeletal anomalies.

Results
There were no deaths. Clinical signs were limited to orange-coloured urine in all females at 500 mg/kg/day and most females at 125 mg/kg/day. Presence of coloured urine was considered to be evidence of systemic exposure following oral administration of hydroxybenzomorpholine.
Mean body weight gain was statistically significantly decreased at 500 mg/kg/day (-19%) compared with controls, mainly due to a transient body weight loss at the onset of dosing.

Consequently, mean body weight was lower than for controls over the entire dosing period, and carcass weight (body weight minus gravid uterine weight) was also lower at 500 mg/kg/day. Group mean food intake at 500 mg/kg/day was slightly but statistically significantly decreased in comparison with controls over the entire dosing period.
Litter parameters including group mean foetal weight were similar in all groups. The incidences of foetuses with a ventricular septal defect, (2/165, 3/159 and 1/161 at 20, 125 and 500 mg/kg, respectively), were not considered to be treatment-related in the absence of any dose-response. Higher foetal and litter incidences of short supplementary ribs were observed at 500 mg/kg/day. In comparison with controls, these incidences were not very high, but fell outside the laboratory historical control range.

Ref.: 15

Comment
These short supplementary ribs were not considered an adverse effect. In the absence of full supplementary ribs, abnormal numbers of pre-sacral vertebrae and short supernumerary ribs are reversible and not considered to be adverse foetal effects. Accordingly, the No Observed Adverse Effect Level for this study was considered to be 125 mg/kg/day for maternal toxicity and at 500 mg/kg/day for developmental toxicity.

3.3.9. Toxicokinetics

/

3.3.10. Photo-induced toxicity

/

3.3.11. Human data

/

3.3.12. Special investigations

/

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(Hydroxybenzomorpholine)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**1.04 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.728 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.012 mg/kg**
No observed effect level (mg/kg) (rat, oral, 28 day study)	**NOAEL**	**=**	**10 mg/kg**

Margin of Safety	**NOAEL / SED**	**=**	**833**

3.3.14. Discussion

Physico-chemical specifications
Hydroxybenzomorpholine is used in oxidative hair dye formulations at a maximum on-head concentration of 1%, after mixing with the developer containing hydrogen peroxide.
Hydroxybenzomorpholine is a secondary amine, and thus, it is prone to nitrosation. Total nitrosamine content is 10-50 times higher than permitted in the Cosmetics Directive. Stability of hydroxybenzomorpholine in marketed products is not provided.
Adequate chemical identification /characterisation has been performed only for two batches (Op.59 and 0508918) of hydroxybenzomorpholine. Chemical identification and characterisation of batch Op.45 as hydroxybenzomorpholine is based only on HPTLC.

General toxicity
Hydroxybenzomorpholine has a low order of acute oral toxicity. In a 90 day study, clinical signs of toxicity were minimal. The NOAEL for general toxicity in the 28-day repeat-dose study in rats was considered to be 10 mg/kg bw/day.
In the developmental toxicity study the NOAEL was considered to be 125 mg/kg/day for maternal toxicity and 500 mg/kg/day for developmental toxicity.

Irritation, sensitisation
Hydroxybenzomorpholine was non-irritating to rabbit skin and moderately irritating to rabbit eyes when tested undiluted. At 2% in 0.5% aqueous carboxymethylcellulose, it was transiently irritating to rabbit eyes.
It was non-sensitising under the conditions of the submitted study.

Dermal absorption
The amounts of hydroxybenzomorpholine considered as absorbed from a typical oxidative hair colouring formulation containing hydroxybenzomorpholine at a final concentration of 1% were estimated to be 0.52 ± 0.33 µg/cm^2 (0.11 – 1.04 µg/cm^2) and 4.50 ± 2.58 µg/cm^2 (0.84 – 8.53 µg/cm^2) in oxidative and non-oxidative conditions, respectively.

Mutagenicity
Hydroxybenzomorpholine produced gene mutations in bacteria in the presence of S9 metabolic activation but not in mammalian cells on the *hprt* locus of mouse lymphoma cells. Negative results were also found in an *in vitro* chromosome aberration test (CHO cells) up to the limit of cytotoxicity. In two in vivo bone marrow micronucleus tests performed up to lethal doses in mice and in rats, hydroxybenzomorpholine did not produce any increased micronucleus frequency. Finally, hydroxybenzomorpholine was non-genotoxic in an *in vivo* test for the evaluation of DNA damage and repair (UDS test in rats) that was conducted up to the MTD.
Overall, the genotoxicity program on hydroxybenzomorpholine investigated both endpoints of genotoxicity: gene mutations and chromosome aberrations. As hydroxybenzomorpholine did not produce gene mutations and chromosome aberrations in mammalian cells in vitro and in vivo, and as the positive result in bacteria was covered by a negative in vivo UDS test, hydroxybenzomorpholine is considered not genotoxic.

Carcinogenicity
No conclusion with regard to carcinogenicity can be made from the studies submitted.

4. CONCLUSION

Based on the information provided and assuming that the nitrosamine content is less than 50 ppb as required by Directive 76/768/EEC on cosmetic products, the SCCP is of the opinion that the use of hydroxybenzomorpholine itself as an oxidative hair dye substance at a maximum concentration of 1.0% in the finished cosmetic product (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer.

Hydroxybenzomorpholine 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.

Based on the previous submission, the nitrosamine content is 10-50 times higher than permitted in Directive 76/768/EEC on cosmetic products. The nitrosamine content in ten different batches of hydroxybenzomorpholine is reported to be 492-2616 ppb. More recent data about Nitrosamine content should be provided.

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

References in italics are not submitted as full reports in the present dossier [18-37]. They consist of reports for studies which were considered to be inadequate for submission, reports for preliminary toxicity studies or publications and can be provided upon request. Appropriate data bases were searched for relevant safety data on A025. 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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