Scientific Committee on Consumer Products

SCCP

OPINION ON

HYDROXYPROPYL BIS(N-HYDROXYETHYL-P-PHENYLENEDIAMINE) HCL

COLIPA N° A121

The SCCP adopted this opinion during its 10th plenary of 19 December 2006

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

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

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

SCCP
Questions concerning the safety of consumer products (non-food products intended for the consumer).

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

Scientific Committee members

Contact:
European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels

Sanco-Sc6-Secretariat@ec.europa.eu

ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

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

External experts

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

Keywords: SCCP, scientific opinion, hair dye, hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCl, A121, Directive 768/76/EEC, CAS 128729-28-2,128729-30-6

Opinion to be cited as:
Scientific Committee on Consumer Products (SCCP), 19 December 2006, Opinion on hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCl COLIPA N° A121

TABLE OF CONTENTS

1. BACKGROUND

Submission I on 1,3-Bis[(4-aminophenyl)-(2-hydroxyethyl)-amino]-propan-2-ol tetrahydrochloride was submitted by COLIPA (European Cosmetics Toiletry and Perfumery Association) in July 1996. On 28 June 2000, the opinion on the above mentioned substance was adopted by the Scientific Committee on Cosmetic Products and Non-food Products intended for Consumers (SCCNFP).

The above mentioned substance is listed under the reference number 33 in Annex III, Part 2 (List of substances provisionally allowed) of the Cosmetic Directive (76/768/EEC).

Submission II 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.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider 1,3-Bis[(4-aminophenyl)-(2-hydroxyethyl)-amino]-propan-2-ol tetrahydrochloride safe for use in oxidative hair dye formulations up to a concentration of 1.5% on the head taken into account the scientific data provided?*

2. *Does the Scientific Committee on Consumer Products (SCCP) recommend any further restrictions with regard to the use of 1,3-Bis[(4-aminophenyl)-(2-hydroxyethyl)-amino]-propan-2-ol tetrahydrochloride in 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

Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCl

3.1.1.2. Chemical names

1,3-Bis-[(4-Amino-phenyl)-(2-hydroxy-ethyl)-amino]-propan-2-ol, tetrahydrochloride

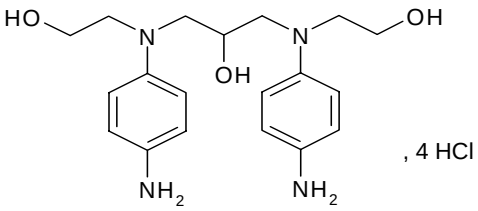
3.1.1.3 Trade names and abbreviations

IMEXINE OAX

3.1.1.4 CAS no.

CAS: 128729-28-2 (4 HCl)
128729-30-6 (Base)
ELINCS: 416-320-2 (IMEXINE OAX)

3.1.1.5	Structural formula



3.1.1.6	Empirical formula

Formula: $C_{19}H_{28}N_4O_3$, 4 HCl

3.1.2	Physical form

A121 is a more or less agglomerated ivory powder, with a strong and irritating odour.

3.1.3	Molecular weight

Molecular weight: 506.30

3.1.4	Purity, composition and substance codes

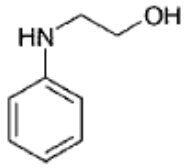
All studies submitted in the present dossier were conducted using test batches that were characterized analytically, i.e.:

- Pil 1 (94.6% pure) for studies conducted in 1990 [3, 6]
- Pil 4X (99.8% pure) for studies conducted in 1994-1996 [1, 2, 4, 5, 7, 8, 10-12, 15-21]
- Op 18 (97.6% pure) for studies conducted in 1997 [9, 13]
- 98218A (>97% pure) and 0500591 (98.8% pure) for studies conducted in 1999 [22, 23]
- CFQ12295 (95.1% pure) and 05046551 (95.3% pure) for the study conducted in 2004 [24]

3.1.5	Impurities / accompanying contaminants

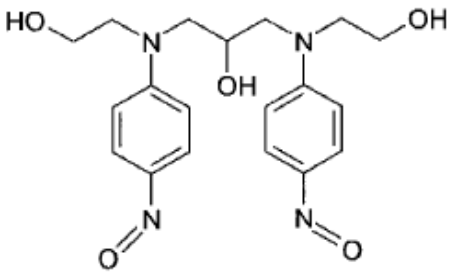
The total impurity content *, studied in batches Pil.4X and Pil.1, is below 0.5 g/100g.

*- 2-Phenylamino-ethanol : Impurity A (Starting material)

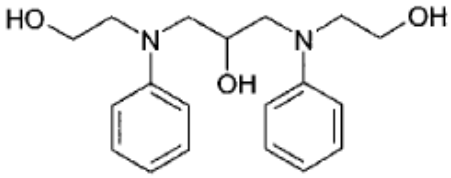


*- 1,3-Bis-[(2-Hydroxy-Ethyl)-(4-Nitroso-Phenyl)-Amino]-Propan-2-ol (Impurity B)

*- 1,3-Bis-[(2-Hydroxy-Ethyl)-Phenyl-Amino]-Propan-2-ol (Impurity C)



Impurity B
Intermediate product of reaction



Impurity C
Intermediate product of reaction

According to analysis data, all these batches are considered to be equivalent.

- 2-Phenylamino-ethanol (impurity A)
  1 mg of A121 batch Pil.4X contains less than 0.2 µg of 2-phenylamino-ethanol (200 µg/g not detected)

- 1,3-Bis-[(2-hydroxy-ethyl)-(4-nitroso-phenyl)-amino]-propan-2-ol (impurity B)
  1 mg of A121 batch Pil.4X contains less than 0.1 µg of 1,3-Bis-[(2-hydroxy-ethyl)-(4-nitroso-phenyl)-amino]-propan-2-ol (100 µg/g not detected)

- 1,3-Bis-[(2-hydroxy-ethyl)-phenyl-amino]-propan-2-ol (impurity C)
  1 mg of A121 batch Pil.4X contains less than 0.1 µg of 1,3-Bis-[(2-hydroxy-ethyl)-(phenyl-amino]-propan-2-ol (100 µg/g not detected)

3.1.6	Solubility

Solubility (g/100ml at 22 °C after 24h)

- water: 760 g/l (according to OECD method A6)
- ethanol: S < 1
- DMSO: S ≥ 20

3.1.7	Partition coefficient (Log P_{ow})

Log $P_{o/w}$: -5 at 20°C

3.1.8	Additional physicochemical specifications

UV light absorption spectrum

The ultra-violet light absorption, in the range of 200 to 400 nm of a 0.01 g/l solution in deionised water exhibits a maximum only at 258 nm. It exhibits a less well-defined maximum at 302 nm.

- the absorbance at 258 nm is about 0.472
- the absorbance at 302 nm is about 0.059

The visible light absorption, in the range 350 to 800 nm of a 10 g/l solution in deionised water exhibits a maximum only at 415.5 nm. It exhibits a less well-defined maximum at 570 nm.

- the absorbance at 415.5 nm is about 0.602
- the absorbance at 570 nm is about 0.093

Infra-red spectroscopy

The infra-red transmission spectrum of the substance to be examined (dispersed in KBr: 1 mg of sample in 200 mg of KBr) is recorded between 4000 and 400 cm^{-1}. The maxima in the spectrum obtained with the substance being examined correspond in position and relative intensity to those in the standard A121 spectrum.

3.1.9.	Stability

No data provided

General Comments on Physico-chemical characterisation

* No data on the stability of the compound itself in the test solutions and in the marketed product were provided.

3.2. Function and uses

Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCl is used in oxidative hair dye formulations at a maximum concentration of 3.0%, which after mixing in a 1:1 ratio with hydrogen peroxide just prior to use, corresponds to a concentration of 1.5% upon application.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/0340/00

Guideline:	OECD 401 (1987)
Species/strain:	Rat, Sprague-Dawley
Group size:	5 male + 5 female
Test substance:	IMEXINE OAX in aqueous solution
Batch:	Pil.4X
Purity:	99.8%
Dose:	females: 1100, 1600, 2000 and 2600 mg/kg bw in a volume of 10 ml/kg, males: 2000 mg/kg bw in a volume of 10 ml/kg
Observation period:	14 days
GLP:	in compliance

Groups of 5 male and 5 female rats received a single dose of test substance by gastric gavage at 2000 mg/kg bw in a limit test. In addition, groups of 5 female rats received doses of 1100, 1600 and 2600 mg/kg bw The animals were observed daily for 14 days. Bodyweights were recorded weekly and macroscopic abnormalities were recorded at autopsy. No histological examinations were performed.

Results
No mortalities were reported in the 1100 and 1600 mg/kg dose groups (females). At 2000 mg/kg bw, the mortality was 40% for females and 60% for males. Four of the five females died after the dose of 2600 mg/kg bw. All deaths occurred within 30 minutes of dosing, except for one male and one female dosed at 2000 mg/kg bw (day 3). Body weight gain of surviving animals was comparable to historical control data.

Table: Mortalities

Dose	Males (dead)	Females (dead)
1100		0/5
1600		0/5
2000	3/5	2/5
2600		4/5

The study authors concluded a LD50 of 2186 (1797 – 2965) mg/kg bw, with comparable toxicity in the males. Clinical signs were reported in some animals of all dose groups from 30 minutes after dosing, and included: hypoactivity, sedation, piloerection and dyspnoea. Lateral decubitis was observed in one male animal. Recovery was complete by day 7 for the females and day 5 for the males. There were no macroscopic abnormalities at autopsy or in the animals found dead during the study.

Conclusion
A121 was found to be moderately toxic to non-toxic via the oral route.

Ref.: 1

3.3.1.2. Acute dermal toxicity

Guideline: OECD 402 (1987)

Species/strain:	Rat, Sprague-Dawley
Group size:	5 males, 5 females
Test substance:	IMEXINE OAX
Batch:	Pil 4x
Purity:	99.8%
Dose:	2000 mg/kg bw (24 h semi-occlusive dressing)
Observation period:	14 days
GLP:	in compliance

A single dose of 2000 mg/kg was applied to 5 male and 5 female rats on a moistened compress for 24 hours. The animals were frequently observed during the hours following treatment and, thereafter, at least daily for a period of 14 days (clinical signs, mortality, body weight gain). A necropsy was performed on each animal killed at the end of the study.

Results
At 2000 mg/kg no mortalities were reported. No changes in body weight gain or clinical signs - except for hypoactivity (in 1 out of 10 animals at 4 and 6 hours after treatment) - were observed. No cutaneous reactions were noted. There were no macroscopic anomalies at autopsy.

Conclusion
A121 was found to be non-toxic via the dermal route.

Ref.: 2

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	/
Species/strain:	New Zealand White rabbit
Group size	3 rabbits
Test substance:	IMEXINE OAX
Batch:	Pil 1
Purity:	not mentioned in the study
Dose:	500 mg (in 0.5 ml distilled water), occluded application for 24 hours
Observation period:	At 1 and 48 h after removal of the patches
GLP:	in compliance

The method used followed that described in the Journal Officiel de la Republique Francaise 21 February 1982. A single dose of the test material (0.5 g) moistened with distilled water (0.5 ml) was loaded on patches and applied to the abraded and intact skin sites of three rabbits (1 females, 2 males) for 24 hours. Thereafter, the patches and any residual test material were removed and after 1 hour and 48 hours (i.e. 24 and 72 hours after application) the test sites were examined for evidence of primary irritation.

Results
The test substance produced well defined erythema and slight to severe oedema (extending the treatment site) after 24 hours. At 72 hours the erythema were scored as very slight to well defined, an incident of very slight oedema was noted at one abraded skin site and slight oedema were observed in one animal (intact and abraded skin site).

Ref.: 6

Guideline:	OECD 404 (1992)
Species/strain:	New Zealand White rabbit, male
Group size:	3 rabbits
Test substance:	IMEXINE OAX
Batch:	Pil 4x
Purity:	99.8%
Dose:	500 mg, semi-occlusive dressing; 3 minutes, 1 or 4 hours
Observation period:	At 1, 24, 48 and 72 h after removal and then daily until day 15
GLP:	Statement included

A single dose of 500 mg prepared on a moistened gauze pad was applied on the clipped skin of male rabbits. The test substance was held in contact with the skin for 3 minutes (1 rabbit), 1 hour (1 rabbit), or 4 hours (3 rabbits) by means of a semi-occlusive dressing. Cutaneous reactions were observed at 1 to 72 hours after removal of the dressing and then daily until day 15.

Results
The test substance produced slight to severe erythema and oedema after 1 and 4 hours of exposure. In the third rabbit after 4 hours exposure and in the rabbit with 3 minutes exposure, only slight oedema was noted (1 hour after removal of the dressing).

Conclusion
The test material was irritant to rabbit skin under the experimental conditions.

Ref.: 7

Local Tolerance

Guideline:	/
Species/strain:	Dunkin-Hartley guinea-pigs
Group size:	6 animals (3 males, 3 females)
Test substance:	IMEXINE OAX
Batch:	OP 18
Purity:	97.6%
Dose:	0.05 ml of a 10% (w/w) solution in water, applied daily for 14 consecutive days (not covered by a dressing)
Observation period:	Before each application and 24 h after the last application
GLP:	Statement included

The test substance (10% w/w) was applied to the clipped skin of the left flank of 3 male and 3 female guinea-pigs once daily for 14 consecutive days; the right flank served as control. Cutaneous reactions were evaluated before exposure and 24 after the last application after removal of the residual test substance.

Results
No clinical signs and no mortality were noted. The cutaneous application of the test substance produced a slight black coloration on day 3 and 4, which could have masked a slight erythema. Very slight erythema was observed on day 9 (all animals), 10 and 15 (2 animals). No significant irritation reaction was observed.

Conclusion
The repeated application of the test material diluted at 10% to the skin of guinea-pigs induced a slight irritation reaction.

Ref.: 9

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405 (1987)
Species/strain:	New Zealand White rabbit
Group size:	1 rabbit
Test substance:	IMEXINE OAX, beige powder
Batch:	Pil 4x
Purity:	99.8%
Dose:	100 mg, administered by ocular route
Observation period:	At 1, 24, 48 and 72 h after treatment
GLP:	Statement included

A single dose of 100mg of the test substance was placed into the conjunctival sac of the left eye of one male rabbit; the right eye remained untreated for control purposes. The eyes were not rinsed after treatment and ocular reactions were observed at 1 to 72 hours after treatment.

Results
Severe ocular reactions were observed in one rabbit following the treatment with the test substance: severe to marked chemosis, slight to moderate conjunctival redness, iris lesions, and moderate to marked corneal opacity. Neovascularisation of the cornea was observed at 72 hours.

Conclusion
The test material is considered as a severe irritant when administered by ocular route to one rabbit.

Ref.: 4

Guideline:	/
Species/strain:	New Zealand White rabbit
Group size:	1 rabbit
Test substance:	IMEXINE OAX
Batch:	Pil 1
Purity:	not mentioned in the study
Dose:	0.1 ml, weighing approx. 54 mg
Observation period:	At 1 and 24 h after treatment
GLP:	in compliance

The method used followed that described in the Journal Officiel de la Republique Francaise 24 October 1984 "Official Method for Evaluation of Eye Irritation". A volume of 0.1 ml of the test material was placed into the right eye of one animal; the left eye remained untreated for control purposes. Assessment of ocular damage/irritation was made at 1 and 24 hours following treatment.

Results
After a single application opalescent corneal opacity, iridial inflammation and severe conjunctival irritation were noted in the treated eye. Other adverse effects were sloughing of the cornea and haemorrhage and pale appearance of the nictitating membrane.

Conclusion
The test material is considered as a strong irritant when administered by ocular route to one rabbit.

Ref.: 3

3.3.3. Skin sensitisation

Guinea pig maximisation test

Guideline:	OECD 406 (1992)
Species/strain:	Dunkin-Hartley guinea-pigs
Group size:	Controls (vehicle) group: 5 males, 5 females, treated group: ten males, ten females
Test substance:	IMEXINE OAX , in isotonic aqueous NaCl solution
Batch:	Pil 4x
Purity:	99.8%
Dose:	Induction: Intradermal injections: at 1% (w/w) Topical application: at 50% (w/w) First challenge: Topical application: at 50% (w/w)
Observation period:	At 24 and 48 h after removal of the dressing
GLP:	Statement included

Following intradermal injections of Freund´s complete adjuvant with or without the test substance (day 1) and topical application of sodium laurylsulfate (10% at day 7) the dorsal region of the animals was treated with the test substance or vehicle (day 8) and was covered by an occlusive dressing for 48 hours. The vehicle used was sterile isotonic saline solution (0.9% NaCl). After further 12 days, all animals were challenged by a topical application of the test substance to the right flank for 24 hours (occlusive dressing); the left flank served as control. Skin reactions were evaluated at 24 and 48 hours later.

Results
In the treated group, very slight, well-defined and marked erythema (grades 1 to 3) and slight (grade 2 in 11 animals) and severe (grade 4 in 1 animal) oedema were observed at 24 hours. At 48 hours very slight to marked erythema (grades 1 to 4) and slight to severe oedema were observed. The cutaneous reactions in 90% of the animals were attributable to the sensitization potential of the test substance at a concentration of 50% (w/w).

Conclusion
The test material is considered to have a strong sensitization potential when administered to skin of guinea-pigs.

Ref.: 12

3.3.4. Dermal / percutaneous absorption

Taken from SCCNFP/0340/00

Penetration in the presence of hydrogen peroxide

Guideline:	none available
Tissue:	Human abdominal or breast epidermis, heat-separated
Method:	Franz diffusion cell (static)
Test substance:	IMEXINE OAX, 1.75% in formulation/H_2O_2 mix
Batch:	PIL 1 (purity not stated in study report)
Dose levels:	c. 40 mg formulation in the presence/absence of 10 mg hair
Replicate cells:	7 without hair, 8 with hair
GLP:	study not in compliance

The skin penetration of COLIPA A121 was evaluated in a static Franz diffusion cell using heat separated human epidermis, with and without addition of finely chopped bleached hair. The test substance was prepared at a concentration of 3.5% in a formulation and then mixed 1:1 with hydrogen peroxide to give a final concentration of 1.75%. Approximately 40 mg of the mixture was applied to 2 cm^2 of epidermal membrane for 30 minutes and then excess washed off with 2% sodium lauryl sulphate solution and dried. Four hours later the levels of substance were measured in the receptor fluid (physiological saline containing 100 µg/ml ascorbic acid) using HPLC. Integrity of the epidermal membrane was checked by

microscopy before the study, and by means of addition of Chinese ink. Any cells showing penetration of the ink were eliminated from the analysis.

Results
The quantity of test substance penetrating through the epidermis to the receptor fluid was close to the limits of detection of the assay used and corresponded to a maximum of 0.004% of applied dose both in the presence and absence of hair. This study did not include determination of recovery of the test substance.

Ref.: 12.1 of submission I

Taken from SCCNFP/0340/00

Penetration in the presence of hydrogen peroxide and p-aminophenol

Guideline:	none available
Tissue:	Human abdominal or breast epidermis, heat-separated
Method:	Franz diffusion cell (static)
Test substance:	IMEXINE OAX, 1.65% in p-aminophenol formulation/ H_2O_2 mix
Batch:	PIL 1
Purity:	not stated in study report
Dose levels:	c. 40 mg formulation in the presence/absence of 10 mg hair
Replicate cells:	7 with and without hair
GLP:	Study not in compliance

The skin penetration of COLIPA A121 was evaluated in a static Franz diffusion cell using heat separated human epidermis, with and without addition of finely chopped bleached hair. The test substance was prepared at a concentration of 3.3% in a formulation containing 0.64% p-aminophenol and then mixed 1:1 with hydrogen peroxide to give a final concentration of 1.65%. Approximately 40 mg of the mixture was applied to 2 cm^2 of epidermal membrane for 30 minutes and then excess washed off with 2% sodium lauryl sulphate solution and dried. Four hours later the levels of substance were measured in the receptor fluid (physiological saline containing 100 µg/ml sodium ascorbate) using HPLC. Integrity of the epidermal membrane was checked by microscopy before the study, and by means of addition of Chinese ink. Any cells showing penetration of the ink were eliminated from the analysis.

Results
The quantity of test substance penetrating through the epidermis to the receptor fluid was close to the limits of detection of the assay used and corresponded to a maximum of 0.004% of applied dose in the presence of hair and 0.005% of applied dose in the absence of hair.
This study did not include determination of recovery of the test substance.

Ref.: 12.2 of submission I

New study

Guideline:	OECD Draft guideline (2000)
Species/strain:	Human dermatomed skin from abdominal plastic surgery
Group size:	4 donors (2 samples/donor)
Test substance	^{14}C-IMEXINE OAX, [U-Ring-14C]
Batch:	CFQ12295
Purity:	95.1%
Dose:	Hair dye mixtures at 20 mg/cm^2 (corresponding to 378.4 $\pm$ 36.0 µg/cm^2 of the dye after mixing)

Observation period: 24 h after application
GLP: Statement included

Two typical hair dye formulations with 3.67 ± 0.25 % (w/w) IMEXINE OAX (175339 containing the coupler m-aminophenol) or 175338 (without m-aminophenol) were applied on the skin surface in vitro after mixing (1/1, w/w) with H_2O_2 or water. After 30 minutes the mixtures were removed by a standardized washing procedure. Twenty four hours after application, the concentration of ^{14}C-IMEXINE OAX and ^{14}C-by-products was determined as radioactivity in skin excess, stratum corneum, epidermis plus dermis and receptor fluid.

Results
Most of the hair dye applied to skin was removed by washing: 93.9 % of (175339 containing the coupler + H_2O_2) and 98.2% of (175338 + water).
The data on skin distribution showed that in the presence of the coupler + H_2O_2 the production of high molecular weight products decreased the absorption of IMEXINE OAX through the skin. Absorbed amounts of IMEXINE OAX and/or by-products (epidermis + dermis + receptor fluid) were significantly lower for 175339 + H_2O_2 (0.6 ± 0.33**%**) than for 175338 + water (2.04 ± 1.76**%**). The highest and the lowest individual values observed for the absorption of 175339 + H_2O_2 were 3.39 **µg/cm²** and 0.55 **µg/cm²**, respectively (Ref. 24, Appendix 7). The highest value is taken into account for the calculation of the margin of exposure.

Table: Cutaneous distribution of ^{14}C-IMEXINE OAX and ^{14}C by-products
Results (mean ± SD) are expressed as $\mu g_{eq}/cm^2$ and % of the applied dose.

Hair dye mixture	**175339 + H_2O_2 (n=8)**	**175338 + water (n=8)**
Skin excess $\mu g_{eq}/cm^2$ (CV%) % of the applied dose (CV%)	 345.1 ± 38.3 (11%) **93.9 ± 2.7 (3%)**	 385.3 ± 35.2 (9%) **98.2 ± 4.0 (4%)**
Stratum corneum (SC) $\mu g_{eq}/cm^2$ (CV%) % of the applied dose (CV%)	 6.29 ± 2.29 (36%) **1.78 ± 0.87 (49%)**	 5.17 ± 3.55 (69%) **1.32 ± 0.96 (72%)**
Epidermis + dermis $\mu g_{eq}/cm^2$ (CV%) % of the applied dose (CV%)	 1.97 ± 1.12 (57%) **0.55 ± 0.33 (60%)**	 7.11 ± 6.24 (88%) **1.85 ± 1.68 (91%)**
Receptor fluid (RF) $\mu g_{eq}/cm^2$ (CV%) % of the applied dose (CV%)	 0.19 ± 0.10 (55%) **0.05 ± 0.03 (57%)**	 0.75 ± 0.56 (75%) **0.19 ± 0.14 (74%)**
Total recovery % of the applied dose (CV%)	 **96.3 ± 3.0 (3%)**	 **101.5 ± 3.5 (3%)**

Ref.: 24

3.3.5. Repeated dose toxicity

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

No data submitted

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

Taken from SCCNFP/0340/00

Guideline: OECD 408 (1981)
Species/strain: Sprague-Dawley rat, Crl CD (SD) BR strain
Group size: 10 male + 10 female
Test substance: IMEXINE OAX in aqueous solution

Batch: Pil.4X (purity 99.8%)
Dose levels: 0, 25, 100 and 400 mg/kg bw/day, 7 days/week by gavage
Exposure period: 13 weeks
GLP: Quality Assurance statement included

Groups of 10 male and 10 female rats were dosed with the test substance by gavage 0, 25, 100 and 400 mg/kg bw/day, 7 days/week for 92 or 93 days. These dose groups were based on a 2-week preliminary study in which a decrease in body weight gain, glucose levels and total proteins was seen at 800 mg/kg bw/day. During the study, the animals were observed for daily for clinical signs and mortality, and bodyweight and food consumption were recorded weekly. At the end of the study, the animals were subjected to orbital bleeding for haematology and blood biochemistry analyses and urine collection for approximately 18 hours. A full autopsy was conducted with recording of weights and macroscopic and microscopic examination of major organs. Opthalmoscopy was conducted before the start and at the end of the study on control and high dose group animals.

Results
There were no clinical signs in animals receiving 25 mg/kg bw/day. Hypersalivation was noted in some animals at 100 mg/kg bw/day, and in 10/10 males and 7/10 females at 400 mg/kg bw/day from week 4. Loud breathing was reported in one male animal at 100 mg/kg bw/day from week 6 for 13 days, and in 3/10 males at 400 mg/kg bw/day from week 6. Regurgitation was recorded in one male at 100 mg/kg bw/day and in 3/10 males at 400 mg/kg bw/day on day 90. In addition, dose-related coloration of the urine, tail and faeces of most animals treated with 100 mg/kg bw/day and all animals at 400 mg/kg bw/day. One male animal died from each of the three treatment groups (day 78, 90 and 91 for the 25, 100 and 400 mg/kg bw dose groups, respectively). Histological examination of the tissues revealed marked to moderate aspiration pneumonia. In the animals treated at 100 and 400 mg/kg bw/day, this was considered to be related to regurgitation and possibly treatment-related. Because regurgitation was not noted in any of the animals treated at 25 mg/kg bw/day, the low dose-group animal that died was thought to have been mis-dosed. Food consumption was decreased in male animals by 8% of control at 100 mg/kg bw and by 9% of control at 400 mg/kg bw in week 9, but was comparable for all other dose groups and times. Bodyweights and efficiency of food utilisation were comparable for all dose groups and therefore the food consumption change at week 9 was not considered to be of toxicological significance. A bilateral partial opacification of the lens was observed in one female given the substance at 400 mg/kg bw/day and was considered to be possibly treatment-related. A bilateral partial opacification of the cornea was also observed in one male at 25 mg/kg bw, but because there were no similar findings in other dose groups, and it is known to occur spontaneously in the test species, it was not considered to be treatment-related.
A slightly raised activated partial thromboplastin time was noted in females given 400 mg/kg bw/day. The value was close to the upper limit of the historical control range and considered to be of minor toxicological significance. Slightly higher urea and creatinine levels were observed in female rats of the upper two dose groups (urea: +25% and +30%; creatinine: +16% and +22%, at 100 and 400 mg/kg bw/day, respectively). As these were not associated with any relevant microscopic findings, they were considered to be of minor toxicological significance. Changes in blood glucose levels were also noted in the upper two dose groups (males: -8% and –11%; females: -10% and –9%, at 100 and 400 mg/kg bw/day, respectively). All individual values were close to or within the historical control range and the change was not considered to be of toxicological importance. Some other haematological and biochemical parameters showed individual minor statistical differences, but these were not dose-related and were within the historical control range. There were no treatment-related changes in urinalysis. The absolute kidney weight was lower (87% of control) for males treated with 100 mg/kg bw/day, but not at the higher dose and it was therefore not considered to be relevant. There were no other significant differences in the organ weights. Microscopic examination revealed minimal to slight brownish pigment accumulation in many organs and tissues (kidneys, alimentary tract, liver and /or

mesenteric lymph nodes in animals given 100 or 400 mg/kg bw/day. Tubular basophilia was noted in some animals of all male dose groups, including controls, but was more severe in males given 400 mg/kg bw/day. Minimal to moderate subacute to chronic aspiration pneumonia was reported in 1/10 females at 25 mg/kg bw/day, 5/10 males and 6/10 females at 100 mg/kg bw/day and 5/10 males and 5/10 females at 400 mg/kg bw/day. Brownish pigment-laden macrophages were present within the lesion for the animals of the two upper dose groups, but not for the female treated at 25 mg/kg bw/day. No other relevant changes were reported. The authors concluded that the substance caused chronic aspiration pneumonia, probably due to irritation of the respiratory tract, and that the dose level of 25 mg/kg bw/day was the No Observed Effect Level.

Ref.: 5 of submission I

Remark
The aspiration pneumonia seen in one female rat at 25 mg/kg bw/day was disregarded as nonsignificant by the study authors, because of the absence of pigmentation. However, on the basis of the information provided, it is not appropriate to discount the possibility that the observation was treatment-related. This would lead to the conclusion that 25 mg/kg bw/day is a LOEL, rather than a NOEL. Tubular basophilia is normally associated with regeneration following kidney damage. Since it was also seen in controls in this study, the significance is unclear but may be related to exacerbation of a pre-existing condition.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Taken from SCCNFP/0340/00

Bacterial gene mutation assay

Guideline:	OECD 471 (1992)
Species/strain:	*Salmonella typhimurium*, TA1535, TA1537, TA98, TA100, *Escherichia coli*, WP2uvrA
Replicates:	Triplicate plates, 2 independent tests (3 with TA100 plus S9)
Test substance:	IMEXINE OAX in aqueous solution
Batch:	Pil.4X
Purity:	99.8%
Concentrations:	62.5-2000 µg/plate with *S. typhimurium* without metabolic activation 312.5-5000 µg/plate with *S. typhimurium* with metabolic activation 312.5-5000 µg/plate with *E. coli* with and without metabolic activation
Positive controls:	without metabolic activation: Sodium azide, 9-Aminoacridine, 2-Nitrofluorene, N-ethyl-N-nitro-nitrosoguanidine with metabolic activation : 2-Anthramine
GLP:	Quality Assurance statement included

COLIPA A121 has been investigated for gene mutation in *Salmonella typhimurium* and *Escherichia coli* using a plate incorporation protocol. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. The second and third studies with S9 included a 1-hour preincubation step. Appropriate negative and positive controls were used. The concentration range was selected on the basis of a preliminary toxicity study indicating that concentrations above 1000 µg/plate were cytotoxic to TA98 and TA100 in the absence of S9 mix.

Results
The test substance did not increase numbers of revertant colonies in the absence of S9. In the presence of S9, a weak positive response (maximum of 2.0-2.2-fold increase, poor concentration response relationship) was seen in the first test with TA1537 and in the second test with TA1535 and TA100. The third test used a narrower concentration range with TA100, but failed to confirm a positive response. Overall, the results did not meet the requirements for a positive response. The positive control agents gave the expected results.
Ref.: 6 of submission I

Cytogenetic assay in CHO cells

Guideline:	/
Species/strain:	Chinese Hamster Ovary Cells
Replicates:	Duplicate cultures, 2 independent tests
Test substance:	IMEXINE OAX in aqueous solution
Batch:	Pil.4X
Purity:	99.8%
Concentrations scored:	25-100 µg/ml without metabolic activation 100-1000 µg/ml with metabolic activation
Positive controls:	Methylmethane sulfonate, Cyclophosphaminde
GLP:	Quality Assurance statement included

COLIPA A121 has been investigated for induction of chromosomal aberrations in CHO cells with a 20 hour harvest time and a range of 3 concentrations. The repeat study included only two harvest times (20 hours and 44 hours) and only one concentration was scored. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. Appropriate negative and positive controls were used in the first test, but not for the second test.

Results
In the first test, the substance induced a significant increase in frequency of aberrations at 100 µg/ml in the absence of S9 (17.9% vs 3.5%, gaps excluded), and in the presence of S9 at 1000 µg/ml (5.0% vs 1.0%, gaps excluded). The latter result was at the upper extreme of the historical control data range. In the second test, the positive result in the absence of S9 was confirmed at the concentration of 75 µg/ml at both harvest times. The result in the second test in the presence of S9 was statistically different from study control and slightly higher than historical controls (5.2% aberrations, gaps excluded). The positive control agent gave the expected result.

Remark
The repeat study was conducted approximately one year after the first, and no concurrent control data are presented. It should be concluded that the study does not meet acceptable standards, but indicates that the test substance is clastogenic.
Ref.: 7 of submission I

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mouse bone marrow cell micronucleus test

Guideline:	OECD 474 (1983)
Species/strain:	Swiss mouse, OF1/ICO: OF1 (IOPS Caw) strain
Group size:	5 male + 5 female
Test substance:	IMEXINE OAX in aqueous solution
Batch:	Pil.4X
Purity:	99.8%
Dose levels:	0, 375, 750 and 1500 mg/kg bw/day for 2 days, by oral gavage

Sacrifice times: 24 hours after final administration
Positive control: Cyclophosphamide
GLP: Quality Assurance statement included

COLIPA A121 has been investigated for induction of micronuclei in the bone marrow cells of mice. A preliminary toxicity study showed a small proportion of mortalities at 1500 and 2000 mg/kg bw and no clinical signs of toxicity at 1000 mg/kg bw Appropriate negative and positive controls were used.

Results
One female mouse died shortly after the first administration at both 375 and 750 mg/kg bw/day. There were no significant increases in the frequency of micronucleated erythrocytes in mice treated with the test substance at any of the three doses compared with concurrent vehicle control groups. The ratio of polychromatic to normochromatic erythrocytes was significantly decreased in the mice treated with 1500 mg/kg bw/day. The positive control agent gave the expected result. The study was conducted adequately and gave no evidence of mutagenicity under the test conditions. The change in ratio of polychromatic to normochromatic erythrocytes demonstrates exposure to the bone marrow.

Ref.: 8 of submission I

Rat bone marrow cell micronucleus test

Guideline: OECD 474 (1983)
Species/strain: Sprague Dawley rat, ICO: OFA-SD (IOPS Caw) strain
Group size: 5 male + 5 female
Test substance: IMEXINE OAX in aqueous solution
Batch: Pil.4X
Purity: 99.8%
Dose levels: 0, 500, 1500 and 2000 mg/kg bw/day for 2 days, by oral gavage
Sacrifice times: 24 hours after final administration
Positive control: Cyclophosphamide
GLP: Quality Assurance statement included

COLIPA A121 has been investigated for induction of micronuclei in the bone marrow cells of rats. A preliminary toxicity study showed no clinical signs of toxicity at 2000 mg/kg bw and therefore this was used as the top dose, in accordance with guidelines. Appropriate negative and positive controls were used.

Results
There were no signs of toxicity and no significant increases in the frequency of micronucleated erythrocytes or in the ratio of polychromatic to normochromatic erythrocytes in rats treated with the test substance at any of the three doses compared with concurrent vehicle control groups. The positive control agent gave the expected result. The study was conducted adequately and gave no evidence of mutagenicity under the test conditions.

Ref.: 9 of submission I

Rat liver *in vivo/in vitro* UDS study

Guideline: study pre-dates OECD guideline 486
Species/strain: Wistar rat, HanIbm: WIST (SPF) strain
Group size: 4-6 males
Test substance: IMEXINE OAX in aqueous solution
Batch: Pil.4X
Purity: 99.8%

Dose levels: 0, 150 and 1500 mg/kg bw, by oral gavage
Sacrifice times: 16 hours (also 2 hours at 1500 mg/kg bw)
Positive control: 2-AAF dissolved in dimethyl sulfoxide/polyethylene glycol 400
GLP: Quality Assurance statement included

COLIPA A121 has been investigated for induction of unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* following *in vivo* dosing. The study pre-dated the OECD guideline, but accords with its requirements. In a preliminary toxicity study, one of two animals died following a dose of 2000 mg/kg bw, and clinical signs of toxicity were observed at 1500 mg/kg bw Appropriate negative and positive controls were used. Animals were sacrificed after 16 hours and 2 hours (1500 mg/kg bw only) and hepatocytes were isolated and treated with 3H-thymidine *in vitro*. Incorporation of radiolabel was assessed using autoradiography.

Results
The results met all the pre-defined criteria for a negative response and therefore the test substance was not found to induce UDS. The positive control agents gave the expected results.

Ref.: 10 of submission I

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Taken from SCCNFP/0340/00

Guideline: OECD 414 (1981)
Species/strain: Sprague-Dawley rat, Crl CD (SD) BR strain
Group size: 25 females (mated)
Test substance: IMEXINE OAX in aqueous solution
Batch: Pil 4X
Purity: 99.8%
Dose levels: 0, 50, 200 and 800 mg/kg bw/day
Treatment period: Days 6 to 15 of pregnancy, inclusive
GLP: Quality Assurance statement included

Groups of 25 female rats were dosed with the test substance at 0, 50, 200 and 800 mg/kg bw/day by gavage on days 6 to 15 after mating. These dose levels were based on a 2-week preliminary study in which a decrease in body weight gain, glucose levels and total proteins was seen at 800 mg/kg bw/day. The dams were observed daily for clinical signs and mortality, and for bodyweight and food consumption on days 2, 6, 9, 12, 15 and 20. They were sacrificed on day 20 of pregnancy, and examined for number of corpora lutea, number and distribution of live and dead foetuses, of early or late resorptions and of implantation sites, and for macroscopic observations. The foetuses were examined for bodyweight, sex and macroscopic external observations, and for skeletal and visceral abnormalities (half for each endpoint). The concentrations, homogeneity and stability of the dosing formulations were verified analytically.

Results
Reddish coloured urine was observed throughout the dosing period in 1 female at 50 mg/kg bw/day and in all females of the two higher dose groups. This was related to excretion of the dye and/or metabolites. There were no clinical signs of toxicity; one high dose female exhibited locomotor difficulties and bent head on day 9 and was sacrificed prematurely on day 10. The condition of this animal was not considered to be treatment-related. There were no other deaths and no abortions occurred. Food consumption and bodyweight gain for females with completed pregnancy were similar in control and treated groups. There were no treatment-related macroscopic changes in the dams. The only observation in the prematurely sacrificed animal was of enlarged mandibular glands. The mean numbers of corpora lutea, implantation sites and live foetuses were higher in the 50 and 800 mg/kg bw/day dose groups. These were not considered to be treatment-related because dosing commenced after implantation.
Other measures of reproductive performance were similar in control and treated groups. A very low incidence of foetal anomalies or malformations was observed, affecting all dose groups, and not considered to be treatment-related.

The authors concluded that the test substance was well tolerated by the pregnant female rat at all dose levels and was not embryotoxic or teratogenic.

Ref.: 11 of submission I

3.3.9. Toxicokinetics

Toxicokinetics after oral application

Guideline:	/
Species/strain:	Rat, Wistar Han
Group size:	Group 1, Plasma kinetics: 9 males, 9 females Group 2, Excretion: 3 males, 3 females
Test substance:	^{14}C-IMEXINE OAX, C 6666 AG [U-Ring-14C]
Batch:	Lot 98218A, purity >97%
Dose:	100 mg/kg bw (1.85 MBq/kg), gavage
Observation period:	Group 1: 1 – 72 h post-gavage Group 2: daily until 168 h post-gavage
GLP:	Statement included

A single dose of the test substance (100 mg/kg bw; gavage) was applied to 9 male and 9 female rats in group 1 (plasma pharmacokinetics) and to 3 males and 3 females in group 2 (excretion balance). In group 1 blood samples were collected at 1, 2, 4, 6, 8, 24, and 72 hours post-gavage. In group 2 urine, faeces and cage-wash were collected pre-dose, and then daily until 168 hours post-gavage; organs/tissues were not analysed as the radioactivity was almost completely eliminated within test period.

Results
Only a small fraction of the test substance was absorbed; absorption based on data for urine excretion plus cage wash was approx. 5 % of the applied dose (2.6 – 14.7 %). The test substance, however, was rapidly absorbed (plasma C_{max} was reached after 1-2 h).
The excretion was rapid and almost complete ($t_{1/2}$ 1.9 h and 2.3 h in males and females, respectively). The radioactivity was mainly eliminated via faeces (>70% within 24 h). The total excretion for urine and faeces were 2.5 and 95.4%, respectively, for males and 3.7 and 88.6 %, respectively, for females.

Ref.: 22

Toxicokinetics after cutaneous application

Guideline: /

Species/strain:	Rat, Wistar Han
Group size:	Group 1, Plasma kinetics: 9 males, 9 females Group 2, Excretion: 3 males, 3 females
Test substance:	^{14}C-IMEXINE OAX, C 6666 AG [U-Ring-14C]
Batch:	Lot 98218A
Purity:	98.4%
Dose:	25 mg/kg bw (2.2 MBq/kg), 30 min, over 10% of body surface area
Observation period:	Group 1: 1 – 72 h post-application Group 2: daily until 168 h post-application
GLP:	Statement included

A single dose of the test substance (100 mg/kg bw; gavage) was applied to 9 male and 9 female rats in group 1 (plasma pharmacokinetics) and to 3 males and 3 females in group 2 (excretion balance). In group 1 blood samples were collected at 1, 2, 4, 6, 8, 24, and 72 hours post-gavage. In group 2 urine, faeces and cage-wash were collected pre-dose, and then daily until 168 hours post-gavage; organs/tissues were not analysed as the radioactivity was almost completely eliminated within test period.

Results
Following topical application (25 mg/kg bw, 30 min) the radioactivity in all plasma samples was below quantifiable limits (<18.2 mg-eq/g). The test substance was very poorly absorbed; based on data for urine and faecal excretion plus skin site and stripping data the mean absorbed dose was 0.63 % (0.48-0.82%). The absorbed radioactivity was mainly eliminated via faeces (>80% within 72 h). While the recovered radioactivity in the site of dressing/wash was high (94-97% of the dose), the values for the skin site were very low (0.07 – 0.15%). A local irritant effect in one female at the application site (small blister) was observed. No other test substance-related clinical signs, morbidity or mortality were observed.

Ref.: 23

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Guideline:	Method established by Unkovic (1983)
Species/strain:	Dunkin-Hartley guinea-pigs
Group size:	Irradiated controls (5 animals), treated group (5 animals), treated + irradiated group (10 animals), irradiated vehicle control group (5 animals)
Test substance:	IMEXINE OAX , in purified water
Batch:	OP 18 (purity 97.6 %)
Dose:	0.2 ml at 10 % (w/w)
Observation period:	Phototoxic effects: at 1, 6 and 24 h after treatment Photoallergic effects: during 8 days
GLP:	Statement included

The phototoxic potential was evaluated after 1 to 24 hours treatment with a single dose. The photoallergenic potential was assessed after several topical applications during an induction period of 8 days and a challenge application of the right (UVA) and left (UVB) flanks of the animals at day 29. Skin reactions were evaluated at day 29, 30 and 31.

Results
The test substance did induce slight cutaneous reactions and, therefore, is not considered to have any phototoxic or photoallergenic potential.

Ref.: 13

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

The concentration of 3.53 % of Hydroxypropyl bis (N-hydroxyethyl-p-phenylenediamine), HCl is mixed before use with H_2O_2 (1/1, w/w). Thus the usage volume of 100 ml contains 1.76%

Maximum absorption through the skin	**=**	**3.39 µg/cm^2**
Maximum absorption per treatment and person (700 cm^2)	**=**	**2.373 mg**
Typical body weight of human	**=**	**60 kg**
Systemic exposure dose (SED)	**=**	**0.04 mg/kg**
LOAEL (mg/kg) (rat, 13 weeks, gavage)	**=**	**25 mg/kg bw**
Lowest internal systemic effective dose derived from toxicokinetics	**=**	**1.25 mg/kg bw**
MOS	**=**	**31**

3.3.14. Discussion

Physico-chemical specification
Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCl is used in oxidative hair dye formulations at a maximum concentration of 3.0%, which after mixing in a 1:1 ratio with hydrogen peroxide just prior to use, corresponds to a concentration of 1.5% upon application.
No data on the stability of the compound itself in the test solutions and in the marketed product were provided.

General toxicity
Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) has a low acute toxicity when administered via the oral or dermal route. In a 13-week oral (gavage) toxicity study in rats chronic aspiration pneumonia was observed at all dose levels (0, 25, 100 and 400 mg/kg bw/day). Therefore, a LOEL of 25 mg/kg bw/day was established. But, given the low oral absorption rate of 5%, the lowest internal (systemic) dose with an adverse effect was considered 1.25 mg/kg bw/day which is used for the calculation of the margin of safety. The test substance was neither embryotoxic nor teratogenic.

Toxicokinetics

Only a small fraction of the test substance was absorbed; absorption based on data for urine excretion plus cage wash was approx. 5 % of the applied dose (2.6 – 14.7 %). The test substance, however, was rapidly absorbed (plasma C_{max} was reached after 1-2 h). Following topical application (25 mg/kg bw, 30 min) the test substance was very poorly absorbed (0.63 %). The excretion was rapid - mainly via faeces (>70% within 24 h).

Considering the lowest internal (systemic) effective dose level from the rat study (1.25 mg/kg bw/day) and the estimated systemic exposure dose in humans (0.04 mg/kg bw) the margin of safety is 31.

Irritation, sensitisation
The substance is skin irritating and severely eye-irritating; however, a dilution of 10% (w/w) in water did not induce skin irritation after repeated application to the skin of guinea-pigs. The test material is considered to have a strong sensitization potential in the Guinea pig.

Dermal absorption
Based on an absorption study with human skin using a typical hair dye formulation with the test substance (i.e., 3.67 $\pm$ 0.25 % (w/w), containing the coupler m-aminophenol after mixing (1/1, w/w) with H_2O_2) the maximum absorption value was 3.39 µg/cm^2. Using the usual calculation factors (absorption area of 700 cm^2/treatment and a body weight of 60 kg per person) the systemic exposure dose is 0.04 mg/kg bw.

Mutagenicity
The test substance was non-mutagenic in bacteria but clastogenic in Chinese Hamster Ovary cells *in vitro*. Genotoxicity was not expressed *in vivo* (bone marrow micronucleus tests, UDS test). In the mouse study, exposure of the bone marrow was demonstrated by the reduction of the PCE/NCE ration. In the rat study, systemic exposure might be assumed derived from the subchronic study in the same species rat where higher dosages induced systemic effects. Therefore, the substance is considered to have no mutagenic potential *in vivo*.

Carcinogenicity
No data was submitted.

4. CONCLUSION

Based on the toxicity of Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) in a sub-chronic oral rat study (at the lowest systemic dose level of 1.25 mg/kg bw/day derived form toxicokinetic data) and the estimated human exposure (highest value calculated from in vitro-experiments on percutaneous absorption: 0.04 mg/kg bw), the margin of exposure is considered too low for a safe use of this substance in hair dye formulations.

The substance is a strong sensitiser in the Guinea pig.

5. MINORITY OPINION

Not applicable

6. REFERENCES

References in italics, consisting in preliminary toxicity studies [*14*] or inadequate studies [*25-27*], are not submitted as full reports in the present dossier.

1. S. de Jouffrey. Acute Oral Toxicity in Rats. CIT Study No. 12041 TAR, 1994
2. S. de Jouffrey. Acute Dermal Toxicity in Rats. CIT Study No. 12042 TAR, 1994
3. R. Guest. Eye Irritation Test in the Rabbit. Safepharm Study No. 109/408, 1990
4. S. de Jouffrey. Acute Eye Irritation in Rabbits. CIT Study No. 12044 TAL, 1995
5. S. de Jouffrey. Acute Eye Irritation in Rabbits. CIT Study No. 12909 TAL, 1995
6. R. Guest. Skin Irritation Test in the Rabbit. Safepharm Study No. 109/409, 1990
7. S. de Jouffrey. Acute Dermal Irritation in Rabbits. CIT Study No. 12043 TAL, 1995
8. S. de Jouffrey. Acute Dermal Irritation in Rabbits. CIT Study No. 12908 TAL, 1995
9. S. de Jouffrey. Local Tolerance Study After Repeated Topical Application for 2 Weeks in Guinea Pigs. CIT Study No. 14933 TSG, 1997
10. S. de Jouffrey. Skin Sensitization Test in Guinea-Pigs (Buehler Test: 3 applications). CIT Study No. 12910 TSG, 1995
11. S. de Jouffrey. Skin Sensitization Test in Guinea Pigs. (Maximization method of Magnusson, B. and Kligman, A.M.). CIT Study No. 12045 TSG, 1995
12. S. de Jouffrey. Skin Sensitization Test in Guinea Pigs. (Maximization method of Magnusson, B. and Kligman, A.M.). CIT Study No. 13354 TSG, 1996
13. S. de Jouffrey. Phototoxic and Photoallergenic Potential by Dermal Route in Guinea Pigs (according to the method of Unkovic.J., 1983). CIT Study No. 14932 TSG, 1997
14. *C. Fabreguettes. Preliminary 2-Week Toxicity Study by Oral Route in Rats. CIT Study No. 12068 TSR, 1995*
15. C. Fabreguettes. 13-Week Toxicity Study by Oral Route in Rats. CIT Study No. 12069 TCR, 1995
16. B. Molinier. Reverse Mutation Assay on Bacteria *Salmonella typhimurium* and *Escherichia coli*. CIT Study No. 12089 MMJ, 1995
17. B. Molinier. *In Vitro* Mammalian Cytogenetic Test in CHO Chinese Hamster Ovary Cells. CIT Study No. 12090 MIC, 1995
18. B. Molinier. Micronucleus Test by Oral Route in Mice. CIT Study No. 12287 MAS, 1995
19. B. Molinier. Micronucleus Test by Oral Route in Rats. CIT Study No. 12931 MAR, 1995
20. W. Völkner. *In Vivo / In Vitro* Unscheduled DNA Synthesis in Rat Hepatocytes with Imexine OAX. CCR Study No. 508800, 1995
21. M.-H. Savary. Embryotoxicity/Teratogenicity Study by Oral Route (Gavage) in Rats. CIT Study No. 12357 RSR, 1995
22. T. Appelqvist. Pharmacokinetics Study in Plasma, Excretion Balance and Tissue Distribution after Single Administration by Oral Route (Gavage) to Rats. CIT Study No. 16434 PAR, 1999
23. T. Appelqvist. Pharmacokinetics Study in Plasma, Excretion Balance and Tissue Distribution after Single Administration by Cutaneous Route to Rats. CIT Study No. 16432 PAR, 1999
24. F. Hueber-Becker. *In Vitro* Percutaneous Absorption of Hydroxypropyl-bis-(N-hydroxyethyl-p-Phenylene Diamine) HCl. L'Oréal Study No. 16056, 2004
25. *S. Clément. Test to Evaluate the Sensitizing Potential by Topical Applications, in the Guinea Pig. Hazleton Study No. 809431, 1988*
26. *Schaefer. Pénétration du colorant IMEXINE OAX à travers l'épiderme humain monté sur cellules de diffusion type Franz. L'Oréal Study No. 92/07/27 – 92/07/29, 1992*
27. *Schaefer. Pénétration du colorant IMEXINE OAX, étudié en présence de 1-hydroxy-3-aminobenzène, à travers l'épiderme humain monté sur cellules de diffusion type Franz. L'Oréal Study No. 92/08/03 – 92/08/06, 1992*

References from opinion SCCNFP/0340/00

5. 13-Week toxicity study by oral route in rats. Centre International de Toxicology (CIT), Evreux, France. Report n° 12069 TCR, 21 July 1995
6. Reverse mutation assay on bacteria Salmonella typhimurium and Escherichia coli. Centre International de Toxicology (CIT), Evreux, France. Report n° 12089 MMJ, 27 February 1995

7. In vitro mammalian cytogenetic test in CHO Chinese hamster ovary cells. IMEXINE OAX. Centre International de Toxicology (CIT), Evreux, France. Report n° 12090 MIC, 30 November 1995
8. Micronucleus test by oral route in mice. IMEXINE AOX. Centre International de Toxicology (CIT), Evreux, France. Report n° 12287 MAS, 9 August 1995
9. Micronucleus test by oral route in rats. IMEXINE AOX. Centre International de Toxicology (CIT), Evreux, France. Report n° 12931 MAR, 25 October 1995
10. In vitro/in vivo unscheduled DNA in rat hepatocytes with IMEXINE AOX. Cytotest Cell Research GmbH and Co.KG (CCR), D-64380 Rossdorf, Federal Republic of Germany. Report n° 508800, 10 August 1995
11. Embryotoxicity/teratogenicity study by oral route (gavage) in rats. IMEXINE AOX. Centre International de Toxicology (CIT), Evreux, France. Report n° 12357 RSR, 17 May 1995
12-1. Pénétration du colorant IMEXINE OAX à travers l'épiderme humain monté sur cellules de diffusion type Franz. Contrôles Biologiques et Méthodes Alternatives – Chimie Analytique – L'OREAL, Aulnay-sous-Bois, France. Report n° 92/07/27 – 92/07/29, 5th October 1992
12-2. Pénétration du colorant IMEXINE OAX. Etudié en présence de 1-hydroxy-3-amino-benzene à travers l'épiderme humain monté sur cellules de diffusion type Franz. Contrôles Biologiques et Méthodes Alternatives – Chimie Analytique – L'OREAL, Aulnay-sous-Bois, France. Report n° 92/08/03 – 92/08/06, 28th October 1992