OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

BASIC BROWN 16

COLIPA n° C9

adopted by the SCCNFP during the 23rd plenary meeting
of 18 March 2003

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is Basic Brown 16 safe for use in cosmetic products?

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

1.3. Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

Basic Brown 16 (INCI)

2.1.2. Chemical names

8-((p-Aminophenyl)azo)-7-hydroxy-2-naphthyl)trimethyl-ammoniumchloride
2-Naphthalenaminium, 8-((4-aminophenyl)azo)-7-hydroxy-N,N,N-trimethyl-, chloride

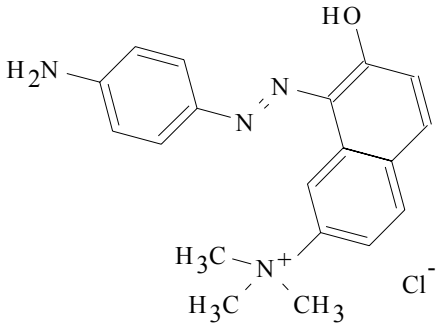
2.1.3. Trade names and abbreviations

Arianor Mahogany
CI 12250

2.1.4. CAS n° / EINECS n°

CAS n° : 26381-41-9
EINECS n° : 247-640-9

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{19}H_{21}N_4OCl$
Mol weight : 356.5 (as chloride)

2.1.7. Purity, composition and substance codes

Composition:	Dye (as chloride)	73.1%
	Sugar	15.1%
	volatile matter/water of crystallisation	6.1%
	inorganic salts (chloride, sulphate, etc.)	to 100%

Purity of the dye (batch no.: Lot 7): >94 area% (HPLC)

2.1.8. Physical properties

Appearance	:	greenish black powder, odourless
Melting point	:	160-170 °C (decomposition)
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	/

2.1.9. Solubility

Soluble in water and ethanol

General comments on analytical and physico-chemical characterisation

* Density and Log Pow not given;
* Nature of sugar not characterised;
* Solubility information is not quantitative;
* Impurities in the dye have not been characterised;
* Inorganic salts (5.7 %) in the dye formulation have not been specified.

2.2. Function and uses

Basic Brown 16 is used in direct hair dye formulations in concentrations up to 2.0%. It is common practice for 35 ml of undiluted formulation to be applied for a period of 30 minutes before washing. It is assumed that application may be repeated weekly.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	study pre-dates OECD Guideline 401
Species	:	CFY rat
Group size	:	2 male + 2 female
Material	:	Basic Brown 16 in 1% aqueous methylcellulose
Batch no	:	not stated
Dose	:	0, 0.1, 1.0, 2.0 and 4.0 g/kg bw in volumes of 1.0 to 40 ml/kg
Observ. Period	:	14 days
GLP	:	not in compliance

Groups of 2 male and 2 female rats received a single oral dose of 0.1, 1.0, 2.0 and 4.0 g/kg bw. Control animals received 1% aqueous methylcellulose in a volume of 40 ml/kg. The animals were observed daily for 14 days for mortality and clinical abnormalities. Body weights and macroscopic observations were recorded, but histological examinations were not performed.

Results
Within one week of dosing, all animals treated at 4.0 g/kg bw died, one female died after a dose of 1.0 g/kg and one after a dose of 2.0 g/kg bw; no male rats died at doses of 1 or 2 g/kg bw. There were no mortalities at 0.1 g/kg. Signs of reaction to treatment, observed shortly after dosing, included piloerection and abnormal body carriage (hunched posture).
Bodyweight gain of surviving treated animals were similar to controls and no abnormalities were recorded at autopsy.

The LD50 was reported to be between 2 and 4 g/kg bw.

Ref. : 1

Guideline	:	/
Species	:	CF1 mouse
Group size	:	10 male
Material	:	Basic Brown 16 in distilled water
Batch no	:	not stated
Dose	:	5.01, 6.31, 7.94 and 10.0 g/kg bw in a volume of 20 ml/kg
Observ. Period	:	7 days
GLP	:	not in compliance

Basic Brown 16 was administered to groups of 10 male CF1 mice at dose levels from 5.01 to 10.0 g/kg bw. Animals were observed for a period of 7 days.

Results
Signs of reaction to treatment were decreased activity, increased respiratory rate and tremors. Death occurred within 24 hours of treatment. One animal died at a dose of 6.31 g/kg, 5 animals at 7.94 g/kg and 9 at 10 g/kg bw. The LD50 was reported to be 7.8 g/kg bw.

Ref. : 2

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

No data

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

Guideline	:	OECD 408
Species	:	Sprague Dawley CD rat
Route	:	oral
Group sizes	:	10 male and 10 female
Material	:	commercial grade Basic Brown 16 in aqueous solution
Batch no	:	KS6024 (purity not stated)
Dose levels	:	0, 50, 150 and 450 mg/kg bw in a volume of 10 ml/kg
Exposure	:	5 days per week for 13 weeks
GLP	:	in compliance

Basic Brown 16, in aqueous solution, was administered by oral gavage 5 days per week to groups of 10 male and 10 female rats at doses of 50, 150 and 450mg/kg bw for 13 weeks. An additional 5 males and 5 females were treated at the same doses then maintained without treatment for observation in a 4-week recovery period. Controls received the vehicle only. The following investigations were performed : daily observations, bodyweights, food consumption, ophthalmoscopy, haematology and clinical chemistry, urinalysis, gross pathological examination, organ weight determination and histopathology.

Results
The dose of 450mg/kg bw resulted in a marked decrease in body weight gain for both male and female animals. The decrease in males was significant from the first week of treatment and resulted in a mean bodyweight of 87% of controls at the termination. In females, the decrease was significant from week 6, with a mean bodyweight of 93% of controls at termination. Additional signs of toxicity at the high dose were abnormal gait, abdominal position and

neurotoxic symptoms. Macroscopic and histological evaluation revealed discoloration of the inner organs. At 150mg/kg bw there was a decrease in body weight gain in male animals (again from week 1, with a terminal mean bodyweight of 93% of control), but no other toxicological effects were reported. Coloured urine was excreted by animals treated with 150 and 450 mg/kg, throughout the treatment period.

The dose of 50mg/kg body weight was tolerated without any signs of adverse effects and is regarded to be the NOAEL.

Ref. : 7

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Test on undiluted material

Guideline	:	/
Species	:	New Zealand white rabbit
Route	:	skin
Group sizes	:	3 male and 3 female
Material	:	undiluted Basic Brown 16
Batch no	:	not stated
Dose	:	0.5 g/in^2
GLP	:	not in compliance

Undiluted Basic Brown 16 was applied at a dose of 0.5 g/in^2 to the backs of 3 rabbits of each sex with shorn intact or scarified skin. The sample was occlusively covered and left in place for 24 hours. Readings were made according to Draize upon removal of the test material and daily for 14 days post administration.

Results
There were no observable reactions to the dye. Basic Brown 16 was considered “not irritant” to rabbit skin.

Ref. : 4

Test on diluted material

Guideline	:	/
Species	:	New Zealand white rabbit
Route	:	skin
Group sizes	:	3 (sex not specified)
Material	:	Basic Brown 16 moistened 1:1 with distilled water
Batch no	:	9-1370F (purity not stated)
Dose	:	0.5 g
GLP	:	not in compliance

0.5 g of the test material was dampened with 0.5 ml distilled water and applied to an area of 1 in^2 on the backs of 3 rabbits each with shorn intact or scarified skin. The sample was covered by an impervious material and left in place for 24 hours. Skin reactions were recorded after 24 and 72 hours.

Results
There were no observable reactions to the dye. Basic Brown 16 was considered “not irritant” to rabbit skin.

Ref. : 5

2.4.2. Irritation (mucous membranes)

Guideline	:	/
Species	:	New Zealand white rabbit
Route	:	eye
Group sizes	:	3 (sex not specified)
Material	:	0.5% Basic Brown 16 solution in physiological saline
Batch no	:	not stated
Dose	:	0.1 ml
GLP	:	not in compliance

0.1 ml of 0.5% solution Basic Brown 16 was instilled into the conjunctival sac of the left eye of three rabbits. The right eye was treated with 0.1 ml of the vehicle and served as a control. Eye reactions were recorded at 30 and 60 minutes and 1 and 2 days following and evaluated by the Draize method.

Results
The treatment provoked no effects on the cornea or iris in any of the test animals. However, there was a discoloration of the conjunctivae.

Ref. : 3

2.5. Sensitisation

Guinea pig assay

Guideline	:	/		
Species	:	Pirbright white guinea pigs		
Group sizes	:	15 test and 10 controls females		
Material	:	Basic Brown 16 in 6.2% aqueous cetyltrimethylammonium chloride		
Batch no	:	not stated		
Concentrations used	:	intradermal induction	:	1.5%
		topical induction	:	1.5%
		first challenge	:	1.5% (topical)
		second challenge	:	0.015, 0.003 and 0.0015% (intradermal)
GLP	:	not in compliance		

The test material was prepared as a 1.5% w/v solution in an aqueous solution of 6.2% of cetyltrimethylammonium chloride. The primary induction was made through intradermal injections of 0.1ml of the prepared test material solution. 0.1ml of the test material solution was then topically applied for three weeks (five times per week) on 3 cm^2 of the skin of the animals. The animals were challenged topically two weeks after the second induction period using 0.01ml of the test material solution. Two weeks later an intradermal injection of the test material solution diluted 1:100, 1:500 and 1:1000 with physiological NaCl solution was made. Skin reactions were recorded after the challenge phases.

The test material did not produce any evidence of skin sensitisation. The sensitisation data in the dossier was generated with a method not conforming with OECD n° 406.

Ref. : 6

2.6. Teratogenicity

Guideline	:	/
Species	:	Sprague Dawley rat, CD strain
Route	:	oral
Group sizes	:	Control group: 26; test group: 28
Material	:	Basic Brown 16 in distilled water
Batch no	:	KS6024
Dose levels	:	0 and 50 mg/kg bw/day in a volume of 10 ml/kg
Administration	:	days 6-15 of gestation
GLP	:	in compliance

Basic Brown 16 was administered by gavage daily to 26 pregnant rats at a dose 50 mg/kg bw/day from days 6 to 15 of gestation. Twenty eight control animals were given the vehicle alone (distilled water). The dams were observed and weighed daily. On day 20 post coitum the dams were sacrificed and Caesarean sections were performed. The number of implantation sites, resorptions, living foetuses and the number of corpora lutea were counted in each litter. The weight of placenta, uterus, foetuses and the sex of the foetuses were recorded. About one-third of each litter was prepared and examined for soft tissue anomalies. The remaining foetuses were examined for skeletal abnormalities after staining with alizarin red S.

Results
Dams : there were no mortalities, abortions or changes in mean body weight gain in dams treated with Basic Brown 16.
Foetuses : there were no treatment related effects on reproduction data or malformations of the foetuses. The level of skeletal variation or ossification in the test and control group were regarded to be similar.

The test material produced no indications of maternal toxicity, embryotoxicity or teratogenicity under the test conditions employed at the dose of 50 mg/kg bw/day.

Ref. : 8

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Skin absorption study in human volunteers

Guideline	:	/
Group size	:	10 males
Material	:	1 mM Basic Brown 16 in 40% aqueous isopropanol
Batch no	:	not stated
Application levels:		20 µl on 5.3 cm^2 skin of the inner forearm
GLP	:	not in compliance

20 µl of a 1 mM solution of the test material, in 40% aqueous isopropanol, were applied to five separate skin areas (5.3 cm^2 s/c) of the inner forearm. After 10 minutes, and 24, 48 and 72 hours, the dye stains of one treatment area after the other were removed by ten repeated strippings with Tesafilm-Spezial^R tape. During the intervals between sampling the skin areas were protected by a special non-occlusive mould. The stripping-tapes were glued on to a white cardboard and kept in the dark until they were used for densitometry. The amounts of the dye that possibly penetrated the skin were estimated from the recovery rates.

Results
It was reported that the dye diffused only to a minor degree into the horny layer; according to the corrected recovery rates. It was concluded that Basic Brown 16 was not absorbed by the skin.

Ref. : 13, 14

***In vivo* study of percutaneous absorption in rats**

Guideline	:	/
Species	:	Sprague Dawley rat
Group sizes	:	3 (sex not specified)
Route	:	topical
Material	:	^{14}C-Basic Brown 16 in a setting lotion formulation
Batch no	:	not stated

Dose levels : 200 mg formulation
GLP : not in compliance

200 mg of a hair setting lotion formulation containing 0.1% ^{14}C-labelled Basic Brown 16, was applied to the clipped dorsal skin of the rats. The animals were lightly anaesthetised for the first hour, after which they were fitted with collars to prevent licking of the application site. Excretion of radioactivity via urine and faeces was measured for 24 hours after application.

Results
The recoveries of radioactivity was less than 0.2% in urine and also in the faeces.

Ref. : 15

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guidelines : OECD 471
Species/strain : *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, TA 1538
Substance : C9
Batch no : not given
Purity : not given
GLP : not in compliance

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

Results
Basic Brown 16 has induced mutation in the strain TA1537 (with and without metabolic activation), in the strain 1538 (in the presence and in the absence of metabolic activation) and in the strain TA98 in the presence of S9. The compound is considered mutagenic

Ref. : 9

***In Vitro* Mammalian Cell Gene Mutations Test**

Guideline : OECD 476
Species/strain : Chinese hamster lung cells V79
Replicates : 2 independent experiments
Substance : Basic Brown 16
Batch no : not given
Purity : not given
GLP : not in compliance
Liver S9 fraction from male Wistar rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system.

Results
Basic Brown 16 did not induce gene mutation in the V79 cells.

Ref. : 10

DNA Damage and Repair-Unscheduled DNA Synthesis-Mammalian Cells *In Vitro*

Guideline	:	OECD 482
Species/strain	:	HeLa cells
Replicates	:	No
Test substance	:	Basic Brown 16, purity not given
GLP	:	not in compliance

Results
Basic Brown 16 did not induce significant increase in the incorporation of 3H-thymidine into the hepatocytes. The method adopted (scintillation counting) is the less sensitive to be used in this type of test, according to the scientific literature). The study is considered inadequate.

Ref. : 14

***In Vitro Mammalian* Chromosome Aberrations test**

Guideline	:	OECD 473
Species/strain	:	Chinese hamster lung cells V79
Replicates	:	1 independent experiment
Substance	:	Basic Brown 16
Batch no	:	not given
Purity	:	not given
Treatment	:	4 h
Doses	:	375-750-1500-3000 µg/ml (-S9 mix) 250-500-1000-2000 µg/ml (+S9 mix)
GLP	:	in compliance

The test has been carried out in the absence and in the presence of S-9 mix (Wistar male rats treated with phenobarbital and beta naphthoflavone).

Results
Basic Brown 16 has clearly induced chromosome aberrations with a frequency higher than the control in all conditions
The compound is considered clastogenic.

Ref. : 16

2.8.2. Mutagenicity/Genotoxicity *in vivo*

***In Vivo* Mammalian Erythrocytes Micronucleus Test**

Guideline	:	OECD 474
Species/strain	:	Mice, CFW 1

Group size	:	5 male + 5 female per group/harvest time
Test substance	:	C9
Batch no	:	not given
Dose levels	:	3000 mg/kg bw
Sacrifice times	:	24, 48 and 72 h
Administration	:	gavage
GLP	:	in compliance

Results
Basic Brown 16 did not induce micronuclei at all conditions. The ratio of normochromatic to polychromatic erythrocytes was not different from the control.
The study is considered inadequate, because only one dose was tested.

Ref. : 12

2.11. Safety evaluation

NOT APPLICABLE

CALCULATION OF THE MARGIN OF SAFETY

(Basic Brown 16)
(semipermanent)

Based on a usage volume of 35 ml, containing at maximum … %

Maximum absorption through the skin	A ($\mu g/cm^2$)	=	$\mu g/cm^2$
Typical body weight of human		=	60 kg
Skin Area surface	SAS (cm^2)	=	cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	mg
Systemic exposure dose (SED)	SAS x A x 0.001/60	=	mg/kg
No observed adverse effect level (mg/kg) (species, study)	NOAEL	=	mg/kg

Margin of Safety	NOAEL / SED	=

2.12. Conclusions

Commercial grade dye of different batches have been used for various tests, but purity (>94%) of the dye has been described only in one case. The impurities in the dye should be described. The test substance is an azo-dye, therefore, free aromatic amine content in the dye (in all batches) is required. The dye formulation contains approximately 15 % sugar and 6% inorganic salts. A complete description of the sugar and salts is required. Following physical properties are also required: density and Log P_{ow}.

The animal sensitisation data in the dossier was generated with a method not conforming with OECD n° 406.

The studies on percutaneous absorption are considered inadequate because of the very low concentration used in the tests.

Basic Brown 16 has been tested adequately only for the induction of gene mutation in bacterial cells and in the mammalian cells, *in vitro* and for chromosome aberrations on V 79 CH cells, *in vitro*.
On the base of these three *in vitro* adequately developed tests, COLIPA C9 is considered to be mutagenic and clastogenic *in vitro*.

2.13. References

1. S.R. Kynoch, G.K. Lloyd. Acute oral toxicity to rats of Mahogany. Huntingdon Research Centre, Report No. 8281/D2/77. September 1977.
2. M. Potokar. Basic Brown 16. Prüfung der akuten Toxizität nach einmaliger oraler Applikation an Mäusen. Henkel KGaA. Abschlussbericht, April 1990. Archiv-Nr. TBD 900254
3. F. Leuschner. Irritant Effects of Five Arianor Dyes on Rabbit Eye Mucosa, Laboratorium für Toxikologie und Pharmakologie, Hamburg. 16. 11. 1967
4. F. Leuschner. Irritant Effects of Five Arianor Dyes on Rabbit Skin, Laboratorium für Toxikologie und Pharmakologie, Hamburg. 16. 11. 1967
5. S.R. Kynoch, M.P. Liggett. Irritant Effects of Mahogany 9 - 1370F on Rabbit Skin, Huntingdon Research Centre, Report No. 7906/4D/77. June 1977
6. Sensiblisierungsversuch mit Basic Brown 16. Bericht aus dem Biologischen Laboratorium der Wella AG, Darmstadt. Bericht vom 11. Mai 1977.
7. M. Potokar. Basic Brown 16. 90-Tage-Test nach Wiederholter oraler Verabreichung an Ratten. Henkel KGaA, Abscluβbericht, Januar 1985. Archiv-Nr. 850004.
8. W. Sterzel, W. Pittermann. Basic Brown 16. Embryotoxicity Study (including Teratogenicity) in the Ratt. Henkel KGaA, Report No. TBD 900355. June 1990.
9. S. Wallat. Basic Brown 16 KS 6024, Prüfung auf Mutagenität im Ames-Test. Henkel KGaA, Abschluβbericht vom 04.09.1985. Archiv-Nr. 850370.
10. H. Michalke (Study Director). Mutagenicity study of Basic Brown 16 (Basic Brown 16) in the mammalian cell gene mutation test with V - 79 Chinese hamster cells in vitro (Project No. HG0391V). Freiburger Labor für Mutagenitätsprüfung der King & Harnasch GmbH, Am Fischerrain 4, D-7815 Kirchzarten. Report, dated July 31, 1991.
11. A, Timm (Study Director). CCR Project 135000 Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats in vitro with Basic Brown 16, CCR, Cytotest Cell Research GmbH & Co. KG, Nieder-Ramstädter Str. 146, D-6100 Darmstadt. F.R.G. Report. dated December 20, 1988.
12. N. Banduhn. Basic Brown 16. Prüfung auf Mutagenität im Mikrokern-Test in vivo. Henkel KGaA. Abschluβbericht vom 05.10.1987. Archivnr. 870593.
13. F. Wingen. Basic Brown 16, Basic Brown 16, CI 12250, Resorptionsscreening an Humanhaut in vivo, Henkel KGaA, Abschluβbericht vom 25.06.1980.

14. F. Wingen, Ch. Gloxhuber and W. Holtmann. Screening- Untersuchungen zur Fage der Hautresorption von Kosmetikfarbstoffen: Parts 1 and 2. J. Soc. Cosmet. Chem. 34, 47-72 (1983).
15. L.J. Wolfram. Inter-office correspondence. Clairol. January, 1984.
16. Czich, A. In vitro chromosome aberration test in Chinese Hamster V79 cells with Arianor Mahogany. RCC Cytotest Cell research GmbH, Rossdorf, project 680904. 2001

3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the information submitted is insufficient to allow an adequate risk assessment to be carried out. Accordingly, the SCCNFP considers that it is not possible to assess the safe use of the substance.

Before any further consideration, the following information is required :

* A study on percutaneous absorption according to the Notes of Guidance (SCCNFP/0321/00);

* data on the genotoxicity/mutagenicity following the SCCNFP-opinion “Proposal for a Strategy for Testing Hair Dye Cosmetic Ingredients for their Potential of Genotoxicity / Mutagenicity”, doc. n° SCCNFP/0566/02 of 4 June 2002, and in accordance with the Notes of Guidance, regularly updated by the SCCNFP (doc. n° SCCNFP/0321/00).

4. Other considerations

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5. Minority opinions

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