Scientific Committee on Consumer Products

SCCP

OPINION ON

Basic Brown 17

COLIPA n° B7

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

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

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

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

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

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Opinion to be cited as: SCCP (Scientific Committee on Consumer Products), Opinion on Basic Brown 17, 30 September 2008

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1. BACKGROUND

Submission I for Basic Brown 17, with the chemical name 8-[(4-Amino-3-nitrophenyl)azo]-7-hydroxy-N,N,N-trimethyl-2-naphthalenaminium chloride, (CAS 68391-32-2) and the free base with the CAS 3070871-30-0 was submitted in August 1992 by COLIPA [1].

Submission II was submitted in January 2002.

The Scientific Committee on Consumer Products (SCCP) adopted at the 2nd plenary meeting of 7 December 2004 the opinion (SCCP/0683/04) with the conclusion, that "*the information submitted is inadequate to assess the safe use of the substance. Before any further consideration, the following information is required:*

* *complete physico-chemical characterisation of the test substances used, including data on stability*
* *data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance"*

According to the current submission III, submitted by COLIPA in July 2005, Basic Brown 17 is used in non-oxidative hair dye formulations in a concentration up to 2%.

Submission III 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

Does the Scientific Committee on Consumer Products (SCCP) consider Basic Brown 17 safe for use as a non-oxidative hair dye with a concentration of maximum 2.0% taking into account the scientific data provided?

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Basic Brown 17 (INCI)

3.1.1.2. Chemical names

2-Naphthalenaminium, 8-[(4-amino-3-nitrophenyl)azo]-7-hydroxy-N,N,N-trimethyl-, chloride
8-[(4-amino-3-nitrophenyl)diazenyl]-7-hydroxy-N,N,N-trimethylnaphthalen-2-aminium chloride
8-[(4-Amino-3-nitrophenyl)azo]-7-hydroxy-N,N,N-trimethyl-2-naphthalenaminium chloride
[8-[(4-amino-3-nitrophenyl)azo]-7-hydroxy-2-naphthyl]trimethylammonium chloride.
1-[(3-Nitro-4-aminophenyl)azo]-2-hydroxy-7-trimethylammoniumchloride, naphthalene-
1-(3'-Nitro-4'-amino)-phenyl-azo-2-hydroxy-7-trimethylammonium chloride, naphthalene-

Error in the Inventory of Cosmetic Ingredients
The chemical name that had been included is [8-[(4-amino-2-nitrophenyl)azo]-7-hydroxy-2-naphthyl]trimethylammonium chloride instead of the correct [8-[(4-amino-3-nitrophenyl)azo]-7-hydroxy-2-naphthyl]trimethylammonium chloride. See note below, in section 3.1.1.4.)

3.1.1.3. Trade names and abbreviations

Trade names:	Arianor Sienna Brown, Jaracol Sienna, Brown SAT 000918 Sienna Brown
Colour Index:	CI 12251
COLIPA number:	B 007

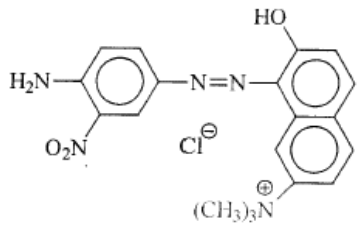
3.1.1.4. CAS / EINECS number

CAS:	68391-32-2 (chloride)
EINECS:	269-944-0 (chloride)

Note
The CAS n° 68391-32-2 and EINECS n° 269-944-0 are the correct ones for Basic Brown 17. The CAS n° 71134-97-9 and EINECS n° 275-216-3 correspond to Basic Red 118 (8-[(4-Amino-2-nitrophenyl)azo]-7-hydroxy-2-naphthyl)trimethylammonium chloride 2.

The latter numbers and the respective name were erroneously given in the EU Cosmetic Inventory as corresponding to Basic Brown 17, and these incorrect designations were used in the two previous COLIPA submissions for Basic Brown 17 (Submission I 1993 and Submission II 2002). The error in the inventory has since been corrected.

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{19}H_{20}N_5O_3^+ Cl^-$ (Chloride content: 8.82%)

3.1.2. Physical form

Dark brown fine powder

3.1.3. Molecular weight

Molecular weight: 401.85 (as chloride)

3.1.4. Purity, composition and substance codes

Purity and Composition of material used in the market

Purity by titration:	> 70% (w/w)
Purity by HPLC assay:	> 92% (area)
Methylsulfate	< 14% (w/w)
Chloride	< 4.5% (w/w)
Solvent content (water):	< 8.0% (w/w)
Impurities*:	
2-nitrobenzene-1,4-diamine	< 250 ppm (MAK classification 3B; IARC: limited evidence for carcinogenicity in animals)
Basic Red 118 (2-nitro isomer)	< 6% (w/w)
NBTRI	< 1% (w/w)

Ref.: 1

* Reported names and structures of the Impurities

Basic Red 118, (CAS 71134-97-9)	8-[(4-amino-2-nitrophenyl)diazenyl]-7-hydroxy-N,N,N-trimethylnaphthalen-2-aminium chloride or 1-[(2'-Nitro-4'-aminophenyl)-azo]-2-hydroxy-7-trimethyl-ammoniumchloride naphthalene
NBTRI	7-Hydroxy-N,N,N-trimethylnaphthalen-2-aminium chloride

Analytical description of Batches used in Toxicity studies

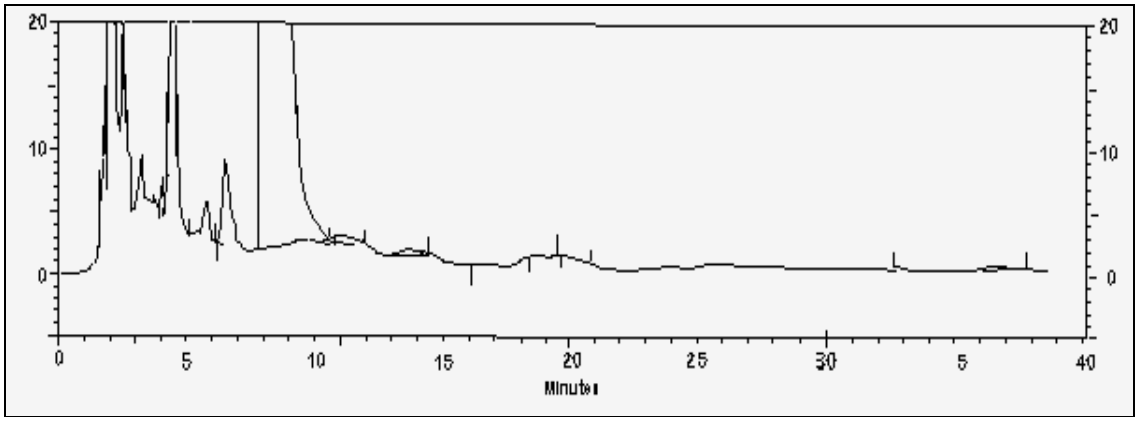
Batch	64960101 = SAT 050019	NDKS 1944 = SAT 000918 = SAT 040270
Reference	(2)	(3) and (4)
Identity	verified by ^{1}H-, ^{13}C-NMR-spectroscopy, IR-spectrometry and UV-spectrometry	
Purity by NMR	77.4% (w/w)	33% (w/w)
Purity by HPLC assay	94.2% (area) (230 nm) 98% (area) (460 nm)	96.3% (area)
Methylsulfate	11.6% (w/w)	8.2% (w/w)
Chloride	3.3% (w/w)	3.6% (w/w)
Sulfate	--	1.1% (w/w)
Sodium:	--	0.5% (w/w)
Saccharose *	--	15.1% (w/w)
Solvent content	7.0% (w/w)	6.5% (w/w)
water:	6.5% (w/w)	6.0% (w/w)
methanol:	0.5% (w/w)	0.5% (w/w)
Impurities:		
2-nitrobenzene-1,4-diamine	150 ppm	120 ppm
Basic Red 118 ** (2-nitro isomer)	4.5% (w/w)	2.3% (area)
NBTRI	0.65% (w/w)	N.D.

* Note: batch NDKS 1944 represents an actual market material it contains the extender saccharose which has been used to adjust the colour strength to a certain predefined value.

** Basic Red 118 is banned as no safety file was submitted; however, an exception is included in entry 1291 of Annex II as a the contaminant in Basic brown 17 when used as a substance in hair dye products

Declaration of the applicant
"The batch of Basic Brown 17 used in the acute oral toxicity test is not fully analytically described. According to information from the laboratories that have synthesized this batch concerning the identity and purity of the material produced at that time, it can be concluded that the former not fully described batch is representative and its specification is quite similar to the fully characterized batch 64960101."

3.1.5. Impurities / accompanying contaminants



Several unidentified HPLC peaks were found in batch NDKS 1944.

3.1.6. Solubility

water:	10 – 100 g/l	room temperature
ethanol:	< 1 g/l	room temperature
DMSO:	1 – 10 g/l	room temperature

3.1.7. Partition coefficient (Log P_{ow})

Log Po/w: 1.82 (calculated)

3.1.8. Additional physical and chemical specifications

Organoleptic properties: slight odour
Melting point: 200 – 202 °C
Boiling point:
Flash point:
Vapour pressure:
Density:
Viscosity:
pKa:
Refractive index:
pH:
UV_Vis spectrum (200-800 nm):

3.1.9. Homogeneity and Stability

No data

General Comments to physico-chemical characterisation

The following properties do not or poorly comply with the basic requirements for proper characterisation:

- The dye content and the purity of the different batches were not fully described;
- Information on impurities is incomplete with the exception of batch 64960101;
- Log P_{ow}: calculated values cannot be accepted as an estimate of the true physical constant without justification
- No experimental data are given on stability;

3.2. Function and uses

Basic Brown 17 is used as a direct dye for hair colouring products. The final concentration on head of Basic Brown 17 can be up to 2.0%.

3.3. Toxicological Evaluation

The batches have varying dye contents due to the addition of an extender. The results from the toxicity tests should to be re-calculated according to dye content of the test substance.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCP/0683/03

Guideline:	/
Species:	CFY rat
Group size:	4 male + 4 female
Substance:	Basic Brown 17 in 1% aqueous methylcellulose
Batch:	not stated
Dose:	0, 0.1, 1.0, 4.0, 8.0 and 16.0 g/kg bw in a volume of 1 to 40 ml/kg
Observation period:	14 days
GLP:	not in compliance
Study period:	June – July 1977

The rats (CFY strain) were treated with Basic Brown 17 with a range of dose levels from 0.1 to 16 g/kg body weight. The test compound was prepared as 10 and 40 % (w/v) suspensions in 1% aqueous methylcellulose. Rats dosed with the vehicle alone served as controls. All animals were observed for a period of 14 days.

Signs or reaction to treatment, observed shortly after dosing, included lethargy, piloerection, decreased respiratory rate and abnormal body carriage (hunched posture). Two male rats from the highest dose group died and one female rat from the highest dose group died. After 14 days observation, the LD50 was reported to be between 8 and 16 g/kg bw.

Ref.: 5

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline:	OECD 404 (2002)
Species:	Albino rabbit, New Zealand White, (SPF-quality)
Group:	3 males
Substance:	B007 (Basic Brown 17)
Batch:	NDKS 1944
Purity:	96.3% (HPLC)
Dose:	0.5 g B007, moistened with water
Vehicle:	water
GLP:	in compliance

Study period: 29 June to 16 July 2004

Three rabbits were exposed to 0.5 grams of the test substance, applied onto clipped skin for 4 hours using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours after exposure.

Results
The exposure resulted in very slight erythema and/or very slight to slight oedema in the treated skin-areas of the rabbits, which had resolved within 48 hours.
Yellow-brown staining of the treated skin by the test substance was observed throughout the observation period, which did not hamper the scoring of the skin reactions. No remnants of the test substance were present on the skin.

Animal number	Mean 24 – 72 hours	
	Erythema	Oedema
35	0.3	0.0
84	0.3	0.0
82	0.3	0.3

Conclusion
The study authors concluded that the test substance was not irritant to the rabbit skin under the conditions of the study.

Ref.: 6

Comment
In contrast to the conclusions drawn by the study authors, the data illustrate that the test substance could cause mild and transient irritation to the rabbit skin.

3.3.2.2. Mucous membrane irritation

Guideline: OECD 405 (2002)
Species: Albino rabbit, New Zealand White, (SPF-quality)
Group: 3 males
Substance: B007 (Basic Brown 17)
Batch: NDKS 1944
Purity: 96.3% (HPLC)
Dose: 45 mg B007 (approximately 1 ml)
GLP: in compliance
Study period: 6 to 19 July 2004

Single samples of approximately 45 mg of B 007 (a volume of approximately 0.1 ml) were instilled into one eye of each of three rabbits. Observations were made 1, 24, 48 and 72 hours and 7 days after instillation.

Results
Instillation of the test substance resulted in effects on the iris in two animals and on the conjunctivae in all animals. Iridial irritation grade 1 was observed and had resolved within 24 or 72 hours. The irritation of the conjunctivae consisted of redness, chemosis and discharge and had completely resolved within 7 days in all animals. No corneal opacity was observed, and treatment of the eyes with 2% fluorescein, 24 hours after test substance instillation revealed no corneal epithelial damage.
Remnants of the test substance were present in the eye 1 hour after instillation and on the outside of the eyelids 24 and 48 hours after instillation in one animal.

Animal number	Mean 24 – 72 hours			
	Corneal opacity	Iris	Conjunctivae	
			Redness	Chemosis
39	0.0	0.0	1.7	0.3
64	0.0	0.3	2.0	1.0
65	0.0	0.0	1.7	0.3

Conclusion
The study authors considered B007 not to be irritant to rabbit eyes.

Ref.: 7

Comment
In contrast to the conclusions drawn by the authors, the data illustrate that the test substance could cause mild and transient irritation to the rabbit eye.

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2002)
Species:	Mice, CBA/CaHsdRcc (SPF)
Group:	28 females (5 test groups, 2 negative control groups) 12 females (3 positive control groups)
Substance:	B007
Batch:	64960101
Purity:	77.4% (NMR)
Concentration:	0.2, 0.5, 1, 3 and 6% (w/v) B007 in ethanol/water (7/3, v/v)
Dose:	25 µl
Vehicle:	ethanol/water (7/3, v/v)
Control:	5, 10 and 25% (w/v) α-hexylcinnamaldehyde in acetone:olive oil (4:1, v/v)
Method of Analysis:	β-scintillation counter
GLP:	in compliance
Study period:	23 March – 6 July 2005

Five groups of four female mice were treated daily with the test item at concentrations of 0.2, 0.5, 1, 3 and 6 % (w/v) in ethanol/water (7/3, v/v) by topical application to the dorsum of each ear lobe (left and right) for three consecutive days. 6% was the highest technically applicable concentration in the vehicle. Two control groups of four mice each were treated with the vehicle (ethanol/water (7/3, v/v)) only. Three positive control groups of four mice each were treated with 5, 10 and 25% (w/v) α-hexylcinnamaldehyde in acetone:olive oil (4:1, v/v) in a separate study.

Five days after the first topical application the mice were injected intravenously into a tail vein with radio-labelled thymidine (^{3}H-methyl thymidine). Approximately five hours after intravenous injection, the mice were sacrificed, the draining auricular lymph nodes excised and pooled per group. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes which were subsequently washed and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was determined by the incorporation of ^{3}H-methyl thymidine measured in a ß-scintillation counter.

No clinical signs were observed in any animals of the control groups. On the third application day, a slight erythema was observed at both dosing sites in all mice of the 3%-group. Since the second application day, a moderate or slight erythema was observed at both dosing sites in all mice of 6%-group, persisting for the remainder of the in-life phase of the study.

The results obtained (Stimulation Index (S.I.)) are reported in the following table.

Concentration (%)	S.I.
Test item	
0.2	1.0
0.5	1.0
1	1.3
3	0.9
6	1.3
α-hexylcinnamaldehyde	
5	2.4
10	3.6
25	11.2

No test item related findings, such as significant body weight loss or local/systemic findings were observed up to the concentration of 1 %. At the higher concentrations tested, i.e. 3 and 6%, some test item related signs, such as slight to moderate ear erythema, were observed at the local dosing sites but no clear change of dpm/LN was caused by this local irritant effect. All treated animals survived the scheduled study period.

Conclusion
The study authors concluded that the test item did not show an allergenic potential when tested up to the concentration of 6 % (w/v) in ethanol/water (7/3, v/v).

Ref.: 8

Comment
The vehicle used is not a recommended vehicle for LLNA. The maximum test concentration is too low. A conclusion on sensitisation cannot be drawn.

3.3.4. Dermal / percutaneous absorption

Taken from SCCP/0683/03, re-evaluated

Guideline:	OECD 428 (2000)
Test substance:	Basic Brown 17
Batch:	NDKS 1944, SAT000918 and SAT010439
Purity:	33% (NMR)
Tissue:	pig (6-8 weeks old) dermatomed skin (trunk)
Skin integrity:	skin electrical resistance measurement
Method:	Static diffusion cell 2.54 cm²
Receptor fluid:	physiological saline 75 / ethanol 25
Formulation tested:	aqueous-methanol solution (50/50) and standard commercial type formulation
Dose formulation applied:	10 µl/cm² and 10 mg/cm²
Concentration ingredient:	2% w/w
Solubility receptor fluid:	/
Replicate cells:	6 cells mounted and interpreted for each formulation
Duration of the contact:	30 minutes non occluded
Duration of the diffusion:	48 hours (sampling time: 0.5, 2, 6, 12, 24, 30 and 48 hours)
Analytical method:	HPLC with visible detection
Validation:	limit of quantitation (0.1 µg/ml)
GLP:	in compliance
Study period:	19 June – 23 July 2001

The skin penetration of Basic Brown 17 was evaluated in a static diffusion cell system. Fresh pig skin removed from the trunk was dermatomed to a constant thickness 400 µm, it was then stored frozen until use. The integrity of the skin was evaluated by the measurement of its electrical resistance. The skin surface temperature was monitored (32 ± 1 °C). Because of the low solubility of Basic Brown 17, the receptor fluid was saline/ethanol 75/25. This

alcohol concentration is acceptable according to OECD (Guidance Document for the conduct of skin absorption studies, 1999) for which the amount of ethanol can reach 50 %. The test substance was prepared at a concentration of 2 % in a "reference vehicle" (solution in methanol) and in a "commercial type" formulation. Approximately 10 µl or mg/cm² of the formulation (exactly measured) was applied to 2.54 cm² for 30 minutes. The excess from the skin surface removed with a cotton swab soaked in water/ethanol, 50/50. The substance was measured using HPLC in the receptor fluid during the 48 hours of the diffusion. At the end, Basic Brown 17 was assayed by HPLC, in the horny layer collected by tape stripping (up to 21 strips), in the epidermis and dermis altogether. After assay of Basic Brown 17 in the washing material (skin excess) the mass balance of the study was calculated (88.0 ± 4.53% of the applied dose for the alcoholic solution, 91.3 ± 5.22% of the applied dose for the standard formulation)

Results
For the two formulations tested, most of the hair dye applied was recovered at the skin surface in the washing liquids (84% for the aqueous-alcoholic formulation and 78% for the standard formulation). The quantity of test substance penetrating, from both excipients, through the skin to the receptor fluid was lower than the quantitation limit (< 0.094 % of the dose for each product). No material was recovered in the horny layer whatever the formulation. The total amount absorbed calculated from the epidermis, the dermis and the receptor fluid contents was 0.106 ± 0.106% of the applied dose for the aqueous-alcoholic solution, and 0.066 ± 0.024% (range: 0.033 – 0.093) of the applied dose for the standard formulation at the end of 48 hours of diffusion after a contact with the skin of 30 minutes.
Ref.: 14

Comment
The dye content of the test substance was only 33%. Too few chambers were used. The solubility in the receptor fluid was not mentioned. The dose applied was 10 mg/cm² instead of the required 20 mg/cm². Due to these shortcomings, the study is not adequate for the calculation of the Margin of Safety.

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

Guideline:	OECD 408
Species/strain:	Rat, Wistar Hannover (HsdBrlHan:Wist)
Group size:	20 (10 per sex) + recovery group (control/high dose 5 per sex)
Test substance:	B 007
Batch:	64960101
Purity:	77.4% (NMR)
Dose:	0, 60, 120 and 180 mg/kg/day
Vehicle:	distilled water
Route:	gavage at a dose volume of 10 ml/kg body weight.
Exposure:	13 weeks + 4 week recovery control/high dose groups
GLP:	in compliance
Date:	12 Dec 2005

During the study, the clinical signs, death, body weight and food consumption were recorded. The recovery groups were additionally examined during the 4-week treatment-free period. At the end of the study, the animals were killed and routine pathological investigations performed.

Results
Two control males and 2 males and 1 female receiving 180 mg/kg/day B 007 were found dead during the treatment period. A control male died of accidental trauma on Day 18, while a recovery group control male died on Day 34. The high dose female died on Day 21, the two high dose males on Days 57 and 87 of dosing.
Body weight and food consumption were unaffected by treatment. No ophthalmological changes occurred during treatment.
During the daily pre- and post-dose observations, no clinical signs were detected. At the weekly clinical signs and functional observation tests, no significant differences between control and test substance-treated animals were seen.
There were no changes in motor activity in animals receiving the test substance compared with controls. Tail pinch response was slightly increased in high dose animals at the end of the treatment period but not at the end of recovery.
There were no significant haematological changes noted during the study. Two mid-dose females and six high dose (three females and three males) showed moderate to marked alteration in aspartate aminotransferase. Since only one of these high dose female also had raised alanine aminotransferase, liver injury was excluded. In the high dose group, significantly raised gamma-glutamyl transferase, cholesterol, triglycerides, glucose levels and lowered sodium were observed. At the end of the recovery phase, these were similar to the control values.
Some of high dose animals showed bilirubinuria, but as no hyperbilirubinaemia was observed, it was thought that this could be due to the presence of the test substance and/or its metabolites in the urine.

Post mortem results unscheduled deaths
The post mortem examination of both the control males and the high dose females showed incomplete collapse in the lungs. Both the lungs and thymus were a dark/red colour. In the other high dose male deaths, the lungs, thymus, spleen and thyroid were also dark/red colour. In one, other changes noted were firm consistency of the heart, irregular surface and swelling of the liver and oedematous consistency of the prostate.

Post mortem results treatment groups
The high dose rats showed dark coloration in the brain, heart, kidneys, ovaries, skeletal muscle, spleen and thyroid with the females more effected than males. In the mid dose, both sexes showed dark coloration of the spleen and thyroid. In addition, some females had dark coloration in the heart and skeletal muscle.

Post mortem results recovery group
Dark coloration was still present after 4 weeks of recovery in the brain, heart, skeletal muscle, spleen and thyroid of treated males and females, when compared with the controls.

Histopathology unscheduled deaths
Moderate to marked myocarditis, centrilobular hepatocytic necrosis with instances of acute inflammation and moderate to marked lymphoid depletion of the thymus were described in both males dosed at 180 mg/kg/day and were seen as the possible cause of death.
The most important changes, observed in the treated female were pulmonary congestion, lymphocytolysis of the mesenteric lymph nodes and thymus and lymphoid depletion of the spleen. Accidental damage was considered the cause of moderate haemorrhage in the lungs and thymus in 1 of the 2 control males.

Histopathology treatment group (90 day)
Yellow/brown pigmentation was the most relevant treatment-related change observed in the heart, kidneys, liver, spleen, thyroids, Peyer's patches (ileum) and skeletal muscle of animals, both sexes, receiving ≥ 120 mg/kg/day, when compared with controls.
Yellow/brown pigmented macrophages were also seen in the lungs of females receiving ≥ 120 mg/kg/day. Treated males were similar to the controls.

In addition, males and females dosed at 180 mg/kg/day showed yellow/brown pigmentation in the adrenals, ovaries, uterus, mesenteric/cervical lymph nodes and thymus.
An increased incidence of extramedullary haemopoiesis in the spleen in all treated groups was described.
The remaining lesions, including the statistically significant increased incidence of inflammatory cell foci described in the heart of males dosed at 60 mg/kg/day were considered either incidental in origin or an expression of spontaneous pathology, commonly seen in this species under the experimental conditions used.

Histopathology recovery group
Yellow/brown pigmentation was still seen in the adrenals, heart, kidneys, liver, ovaries, spleen, thyroid, skeletal muscle and mesenteric/cervical lymph nodes in the high dose group.
Absolute and relative thyroid weights were lower than controls in females dosed at 180 mg/kg/day at the end of the recovery period; in the same animals the relative liver weights were higher than controls.
Changes in clinical chemistry parameters that could be correlated with the histopathological changes detected in the heart and skeletal muscle were a moderate to marked increase in aspartate aminotransferase detected in some animals dosed at 120 and 180 mg/kg/day at the end of the treatment period.

Conclusion
On the basis of this study, the low dose level of 60 mg/kg/day may be considered the No Observed Adverse Effect Level (NOAEL). As the dye content of the test substance was only 77.4%, the NOAEL is recalculated to 46 mg/kg/day (60 x 0.774)

Ref.: 12

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537.
Replicates:	triplicates in only one experiment both in the presence and absence of S9- mix.
Test substance:	B 007
Solvent:	deionised water
Batch:	64960101
Purity:	77.4% (NMR)
Concentrations:	3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate without and with S9-mix
Treatment:	direct plate incorporation with 48 h incubation without and with S9-mix
GLP:	in compliance
Study period:	January - April 2007

B 007 was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone-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 and TA100 both without and with S9-mix. Toxicity was evaluated for 8 concentrations up to the prescribed maximum

concentration of 5000 µg/plate on the basis of a reduction in the number of spontaneous revertant colonies and/or clearing of the bacterial background lawn. Since in this pre-experiment evaluable plates were obtained for five concentrations or more in the strains used, the pre-experiment is reported as part of the main experiment. Both the pre and main experiment were performed with the direct plate incorporation method. Negative and positive controls were in accordance with the OECD guideline.

Results
Precipitation of B 007 was observed from 2500 µg/plate and above in the pre-experiment and from 333 µg/plate and above in the overlay agar of the main experiment. The undissolved particles of B 007 had no influence on the data recording. Both without and with S9-mix toxic effects were observed at 333 µg/plate for TA100 and TA102 and at 2500 µg/plate for TA98 and TA1537. Toxic effects were not observed for TA1535.
B 007 treatment resulted in a biologically relevant and dose dependent increase in the number of revertant colonies in strains TA98 and TA 1537. At the two highest concentrations, the number of revertant colonies was reduced due to overlapping toxic effects.

Conclusion
Under the experimental conditions used B 007 was mutagenic in this gene mutation tests in bacteria.

Ref.: 9

Comment
Since a positive result was obtained, a second experiment was not performed.

In Vitro Mouse Lymphoma assay (*tk* locus)

Guideline:	OECD 476 (1998)
Cells:	L5178Y Mouse lymphoma cells
Replicates:	two parallel cultures in 2 independent experiments
Test substance:	B 007
Solvent:	deionised water
Batch no.:	64960101
Purity:	77.4% (NMR)
Concentrations:	Experiment I: 8.1, 16.3, 32.5, 65.0 and 97.5 µg/ml without S9-mix 16.3, 32.5, 65.0, 130.0 and 195.0 µg/ml with S9-mix Experiment II: 8.0, 16.0, 32.9, 64.0, 128.0 and 192.0 µg/ml without S9-mix
Treatment	Experiment I: 4 h treatment without and with S9-mix; expression period 72 h and selection period of 10-15 days Experiment II: 24 h treatment without S9-mix; expression period 48 h and selection period of 10-15 days
GLP:	In compliance
Study period:	January - June 2005

B 007 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Test concentrations were based on the results of a pre-test on toxicity measuring relative suspension growth. In the main test, cells were treated for 4 h or 24 h (without S9 in experiment II only) followed by an expression period of 72 or 48 h to fix the DNA damage into a stable *tk* mutation. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Toxicity was measured in the main experiments as percentage relative total growth of the treated cultures relative to the total growth of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
In experiment I, precipitation was noted at 97.5 and 130.0 µg/ml without S9-mix and at 195.0 and 260.0 µg/ml with S9-mix; in experiment II precipitation occurred at 128.0 and 192.0 µg/ml.
The appropriate level of toxicity (10-20% survival after the highest dose) was not reached in experiment with S9-mix pointing to insufficient exposure of the cells.
Both in experiment I and II no biological relevant and dose dependent increase in the number mutant colonies was observed independent of the presence or absence of S9-mix.

Conclusion
Under the experimental conditions used, B 007 was not mutagenic in this mouse lymphoma assay using the *tk* locus as reporter gene.

Ref.: 10

Comment
The appropriate level of toxicity (10-20% survival after the highest dose) was not reached in experiment with S9-mix which may point to insufficient exposure of the cells. Historical control data were only reported for the total number of mutant colonies; historical data for "small" and "large" colonies were not available.

In vitro micronucleus test

Guideline:	draft OECD 487 and OECD 473 (*in vitro* chromosomal aberration test)	
Cells:	Chinese hamster V79 cells	
Replicates:	duplicate cultures in 2 independent experiments	
Test substance:	B 007	
Solvent:	deionised water	
Batch:	64960101	
Purity:	77.4% (NMR)	
Concentrations:	experiment IA:	128.1, 256.3, 2050.0 and 4100.0 µg/ml without S9-mix
	experiment IA:	128.1, 256.3, 1025.0 and 2050.0 µg/ml with S9-mix
	experiment IB:	31.3, 62.5, and 125.0 without S9-mix
	experiment IIA:	128.1, 256.3 and 512.5 µg/ml without S9-mix
	experiment IIA:	128.1, 256.3, 512.5 and 1025.0 µg/ml with S9-mix
	experiment IIB:	100.0, 150.0, 200.0, 250.0, 300.0 and 350.0 µg/ml without S9-mix
	experiment IIB:	100.0, 200.0, 400.0, 600.0 and 800.0 µg/ml with S9-mix
Treatment	experiment IA and IB:	4 h treatment; harvest time 24 hours after the beginning of treatment, without S9-mix
	experiment IA:	4 h treatment; harvest time 24 hours after the beginning of treatment, with S9-mix
	experiment IIA and IIB:	20 h treatment; harvest time 24 hours after the beginning of treatment without S9-mix
	experiment IIA and IIB:	4 h treatment; harvest time 48 hours after the beginning of treatment with S9-mix
GLP:	In compliance	
Study period:	February - September 2005	

B 007 has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in V79 cells. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. A pre-test on cell growth inhibition (XTT assay) with 4 h treatment was performed in order to determine the toxicity of B 007, the solubility during exposure and thus the test concentrations for the main micronucleus test. The highest concentration should produce clear toxicity with reduced cell growth. Considering the toxicity data of the pre-test and the

occurrence of precipitation of B 007, 4100 µg/ml (≈ 10 mM the prescribed maximum concentration) was chosen as top concentration in experiment IA. To corroborate the data of this experiment in the absence of S9-mix, a confirmatory experiment (experiment IB) was performed with a top dose of 500 µg/ml. Dose selection in experiment IIA was influenced by Basic Brown 17 toxicity and precipitation observed in experiment I. Due to the steep dose toxicity curve, a repeat experiment (experiment IIB) was performed with narrower dilution steps to proof if genotoxicity observed at highly toxic concentrations far below the 40% of control level was an artificial finding.
The treatment period in the main test was 4 h in experiment I (without and with S9-mix) and in experiment II (with S9-mix) or 20 h in experiment II (without S9-mix). Harvest time was 24 h or 48 h (experiment II with S9-mix only) after the beginning of culture. For assessment of cytotoxicity a XTT test was additionally carried out in parallel to the main micronucleus test. Negative and positive controls were in accordance with the draft guideline.

Results
In all experiments clear toxic effects indicated by reduced cell numbers below 40% of control were observed at least at the highest concentrations scored after treatment with B 007 except in experiment IB in the absence of S9-mix.
In experiment IA, in the absence of S9-mix, a statistically significant but non-dose-related increase in the rate of micronucleated cells was observed at the lowest and highest dose. The values of highest dose were at the laboratory's control data range (0.0 – 1.8% micronucleated cells). Concerning the lowest dose, in the confirmatory experiment IB this finding was not confirmed. Consequently, the positive finding was considered not biologically relevant. In experiment IA, in the presence of S9-mix no biologically relevant increase in the percentage of micronucleated cells was observed after treatment with the test item.
In experiment IIA, in the absence and the presence of S9-mix, a statistically significant increase in the number of micronucleated cells exceeding the range of the historical control data was observed at the highest doses (512.5 and 1025 µg/ml, respectively). These concentrations were strongly cytotoxic indicated as by cell numbers of 7.9% and 12.9% of control, respectively.
Due to the steep dose-toxicity curve a repeat experiment, designated experiment IIB, was performed with narrower dilution steps to prove if the genotoxicity observed could have been an artefact induced by general test item toxicity. In the absence of S9-mix, at a cytotoxic level of about 40% of control the number of micronucleated cells (2.05% and 2.00%) slightly exceeded the historical control data range (0.0 – 1.8% micronucleated cells). Therefore, the test item was regarded as non-genotoxic in the absence of metabolic activation.
In the presence of S9-mix, at cytotoxic test item levels and associated with precipitation from doses equal or exceeding 200 µg/ml, the number of micronucleated cells (2.68% and 2.33%) slightly exceeded the range of the historical control data (0.0 – 1.8% micronucleated cells). Due to the high value of the respective solvent control (1.80% micronucleated cells), these two slight increases have to be regarded as biologically irrelevant.
The observations of experiment IIA in the absence and the presence of metabolic activation were not confirmed in the repeat experiment IIB with narrower dilution steps. Therefore, it has to be considered that the findings in both parts of experiment IIA were artefacts induced by general test item toxicity.

Conclusion
Under the experimental conditions used, the test substance did not induce an increase in micronucleated cells and, consequently, is not genotoxic (clastogenic and/or aneugenic) in V79 cells.

Ref.:11

Comment

The authors of the report consider the findings of experiment IIB without S9-mix (2.05% and 2.00%) and with S9-mix (2.68% and 2.33%) close enough to the range of the historical control data (0.0 – 1.8% micronucleated cells) to conclude that these finding are not biologically relevant. However, in the report the historical control data for aqueous solvents is 0.7 (a range is not reported because it is based on only 4000 cells scored). If this range of historical control data is considered as the most appropriate one than the findings can be concluded as positive. However, in these cases also the solvent control data are outside the range of historical control data.
In experiment IIB the authors conclude that due to the high value of the respective solvent control (1.80% micronucleated cells), the two slight increases at the highest doses have to be regarded as biologically irrelevant. The SCCP considers a high value of a control not as a reason for regarding a positive result as not biologically relevant but merely as an indication for a poor experiment.

The SCCP considers the results of this *in vitro* micronucleus test as equivocal.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

No data submitted

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline:	OECD 414
Species/strain:	Rat, Wistar HsdBrlHan: female
Group size:	25
Test substance:	B-007
Batch:	64960101
Purity:	77.4% (NMR)
Dose:	0, 60, 120, 240 mg/kg/d in distilled water
Route:	Gavage, 10 ml/kg
Exposure:	Gestation Day (GD) 5-19
GLP:	in compliance
Date:	7 November 2005

Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed in situ were considered to be at GD 0 and assigned to individual housing. Animals were also examined for clinical observations, general appearance, abortions, premature deliveries and death daily. Food consumption and body weight were recorded on GD 0, 5, 9, 12, 15 and 20. On GD 20, the animals were killed and examined macroscopically. Foetuses were removed by Caesarean section.

Results

Maternal

During the study, there was one unscheduled high dose death on GD 11. Macroscopic examination showed enlarged adrenals, abnormal swollen intestinal tract content, liver and spleen were a dark colour.
A total of nine females were not pregnant at termination: two in the control group, five in the low dose group and one each in the mid- and high dose groups. Unilateral implantation was present in one high dose female.
The number of females with live foetuses on GD 20 was 23 controls, 20 low dose group, 24 mid-dose and 23 in the high dose.
Scabs and hairloss were the principal clinical signs observed in the treated females during the treatment period, but also occasionally occurred in the control group.
Abrasion and aggressive behaviour were noted in two different high dose females on GD 19 and 20, respectively. Dyspnoea was recorded in one low dose female on GD 7. No other signs of reaction to treatment were recorded at the daily pre- and post-dose observations. Light to dark brown staining was observed on the cage floor in the high dose group, considered to be caused by the test substance, probably eliminated in the urine.
When compared with the controls, statistically significant reductions in body weight gain (GD 9) and food consumption (GD 9 and 12) were noted in the high dose group. Aligned with this, was a statistically significant lower terminal body weight and consequently absolute weight gain in the high dose group compared with the control. Gravid uterus weight was not affected by treatment. At *post mortem*, the spleen was dark and occasionally swollen in the high dose females. This was considered related to the colour of the test item. Other findings were not dose related and considered to be incidental or spontaneous.
At all dose levels, the litter means for corpora lutea, implantations, litter sizes, live foetuses, early and late resorptions, foetal body weights, percent resorbed conceptuses, and percent live male foetuses were similar to the controls. No dams had litters of only resorbed conceptuses, and there were no dead foetuses.

Foetal parameters
13 small foetuses were found (control 4, low dose 3, mid-dose 5 and high dose 1). In one mid-dose foetus, brain ventricles were enlarged. This was considered incidental. There were no other dose-dependent, significant differences in the litter or foetal incidences of any gross external, soft tissue or skeletal alterations.

Conclusion
The maternal No Observed Adverse Effect Level (NOAEL) was considered to be 120 mg/kg/day. The foetal No Observed Adverse Effect Level (NOAEL) was higher than 240 mg/kg/day. As the dye content of the test substance was only 77.4%, the NOAELs were recalculated to 93 mg/kg/day (maternal) and 186 (foetal).

Ref.: 13

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

3.3.14. Discussion

Physico-chemical properties
Basic Brown 17 is used as a direct dye for hair colouring products. It is used without mixing with an oxidising agent (e.g. hydrogen peroxide). The final concentration on head of Basic Brown 17 can be up to 2.0%.
The dye content and the purity of the different batches were not fully described. Information on impurities is incomplete with the exception of batch 64960101. Calculated values for Log P_{ow} cannot be accepted as an estimate of the true physical constant without justification.
No experimental data are given on stability.

General toxicity
After 14 days observation, the LD_{50} was reported to be between 8 and 16 g/kg bw.
On the basis of a 90-day study, the No Observed Adverse Effect Level (NOAEL) was 46 mg/kg/day (corrected for dye content).
The NOAEL for maternal toxicity was considered to be 93 mg/kg/day. The NOAEL for foetal toxicity was higher than 186 mg/kg/day (both values corrected for dye content).

Irritation / sensitisation
The data illustrate that the test substance could cause mild and transient irritation to the rabbit skin and eye.
The test item did not show an allergenic potential when tested up to the concentration of 6% (w/v) in ethanol/water (7/3, v/v). As the test was not properly performed, a sensitising potential of Basic Brown 17 cannot be excluded.

Dermal absorption
The dye content of the test substance was only 33%. Too few chambers were used. The solubility in the receptor fluid was not mentioned. The dose applied was 10 mg/cm² instead of the required 20 mg/cm². Due to these shortcomings, the study is not adequate for the calculation of the Margin of Safety.

Mutagenicity / genotoxicity
The *in vitro* genotoxicity of Basic Brown 17 is investigated in valid genotoxicity tests for the three types of genotoxic endpoints: gene mutation, structural and numerical chromosome aberration. B 007 induced gene mutations in bacteria. Basic Brown 17 did not induce mutations in an *in vitro* gene mutation test in mammalian cells. Basic Brown 17 was not

considered genotoxic in an *in vitro* micronucleus test (V79 cells) by the study authors. However, SCCP considers the findings of this test as equivocal.
To reach a definitive conclusion, the equivocal findings in the *in vitro* micronucleus test have to be clarified to exclude a genotoxic potential of Basic Brown 17.

Carcinogenicity
No data submitted

4. CONCLUSION

The SCCP is of the opinion that the information submitted is insufficient to allow a final risk assessment to be carried out.

Before any further consideration:

- complete physico-chemical data must be submitted
- an *in vitro* percutaneous absorption study has to be performed following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance;
- the equivocal findings in the *in vitro* micronucleus test have to be clarified to exclude a genotoxic potential of Basic Brown 17.

A skin sensitising potential of Basic Brown 17 cannot be excluded.

5. MINORITY OPINION

Not applicable

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