



Scientific Committee on Consumer Safety

SCCS

OPINION ON

Basic Brown 17

COLIPA n° B7

The SCCS adopted this opinion at its 14th plenary meeting
of 27 March 2012

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 Safety (SCCS), 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 Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

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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 (EC 269-944-0 and CAS 68391-32-2) and with the CAS 3070871-30-0 for the free base, was submitted in August 1992 by COLIPA¹.

The first scientific opinion (SCCP/0683/03) was adopted by the Scientific Committee on Consumer Products (SCCP) at the 2nd plenary meeting of 7th December 2004 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".*

The second scientific opinion (SCCP/1173/08) was adopted on 30 September 2008 by the SCCP with the following conclusion: *"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."

The current submission IV on the substance Basic Brown 17 is a response to the above mentioned opinion. Basic Brown 17 is used in non-oxidative hair dye formulations in a concentration up to 2.0%.

2. TERMS OF REFERENCE

1. *Does SCCS consider Basic Brown 17 safe for use in non-oxidative hair dyes with a concentration of maximum 2.0% taken into account the scientific data provided?*
2. *And/or does the SCCS has any further scientific concerns with regard to the use of Basic Brown 17 in non-oxidative hair dye formulations?*

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-

3.1.1.3. Trade names and abbreviations

Synonym:	Arianor Sienna Brown, Jaracol Sienna, Sienna Brown
Sample codes of the applicant:	SAT 000918 SAT 040270 SAT 050019
Colour Index:	CI 12251
COLIPA number:	B 007

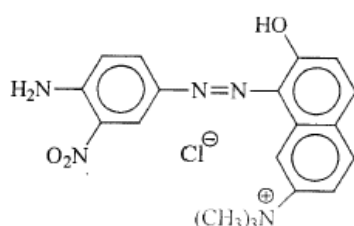
3.1.1.4. CAS / EC number

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

Note

CAS n° 71134-97-9 and EC n° 275-216-3, which correspond to Basic Red 118 (8-[(4-Amino-2-nitrophenyl)azo]-7-hydroxy-2-naphthyl)trimethylammonium chloride, were used in the two previous COLIPA submissions for Basic Brown 17 (Submission I 1992 and Submission II 2001). This appeared to be due to an error in the EU cosmetic inventory, which has been corrected now.

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**Analytical description of Batches used in Toxicity studies**

Batch	64960101 = SAT 050019	NDKS 1944 = SAT 000918 = SAT 040270
Reference	(5)	(6, 7, 8)
Identity	verified by 1H -, ^{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)	96.3% (area)
	98% (area) (460 nm)	64.4% (w/w) *
Methylsulfate	11.6% (w/w)	8.2% (w/w)
Chloride	3.3% (w/w)	3.6% (w/w)
Sulfate	0.1% (w/w)	1.1% (w/w)
Sodium:	--0.2% (w/w)	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)	
Impurities:		
2-nitrobenzene-1,4-diamine	150 ppm	120 ppm
Basic Red 118 *** (2-nitro isomer of B17)	4.5% (w/w)	2.3% (w/w)
NBTRI	0.65% (w/w)	N.D.

* Batch 64960101 (NMR content 77.4%) was used as reference standard

** 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 (CAS 71134-97-9) is banned as no safety file was submitted; however, an exception is included in entry 1291 of Annex II as the contaminant in Basic Brown 17 when used as a substance in hair dye products

NBTRI: 7-Hydroxy-N,N,N-trimethylnaphthalene-2-aminium chloride

Declaration of the applicant

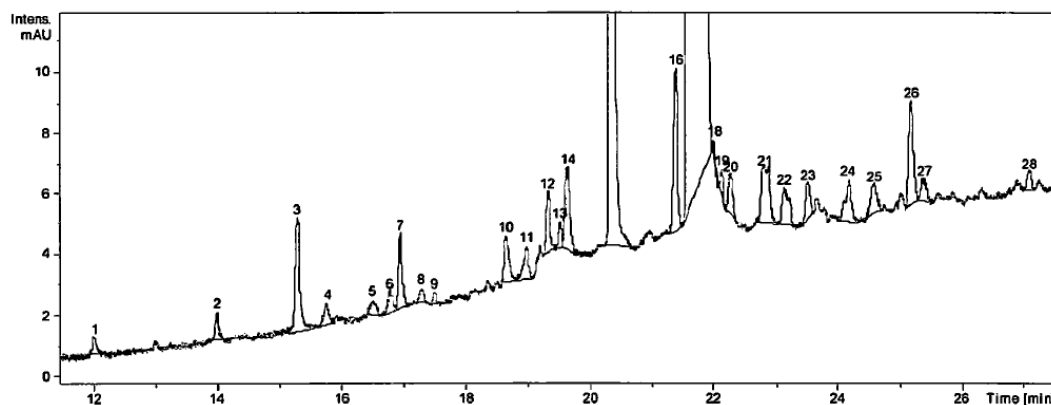
"The batch of COLIPA B 007 used in the acute oral toxicity test is not fully analytically described. However, information is available from the laboratories that have synthesized this batch concerning the identity and purity of the material produced at that time. From this information, it can be concluded that the former not fully described batch is representative and its specification is quite similar to the fully characterized batch 64960101."

Comment of the SCCS

The dye content of batch SAT 050019 based on NMR, should be considered as semi-quantitative.

The dye content of batch NDKS 1944 based on HPLC analysis, should also be considered as semi-quantitative.

3.1.5. Impurities / accompanying contaminants



Besides 2-nitrobenzene-1,4-diamine, Basic Red 118 and NBTRI (described in 3.1.4), several other impurities were found in B7 as shown in the chromatogram above (Basic Brown 17, Batch NDKS 1944).

Employing HPLC-MS, some impurities of Basic Brown 17 were explained by higher or lower methylated species of the starting material 7-hydroxy-N,N,N-trimethylnaphthalen-2-aminium chloride or of the main compound 8-[(4-amino-3-nitrophenyl)azo]-7-hydroxy-N,N,N-trimethylnaphthalene-2-aminium chloride.

Besides this an intermediate substance and some by-products could be found according to the mass spectra.

Taking into account the structures which could be reasonably assigned to numerous peaks detected in the HPLC were:

- For batch NDKS 1944, 15 of the 28 peaks accounting for 98.5 area% are identified.
- For batch 6496010, 13 of the 27 peaks accounting for 98.7 area% are identified.

Peak table with main masses detected (MS)

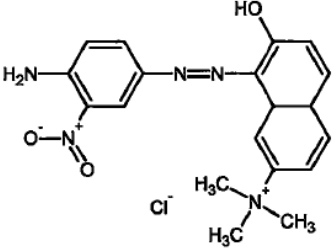
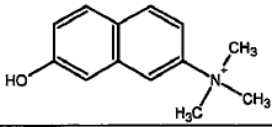
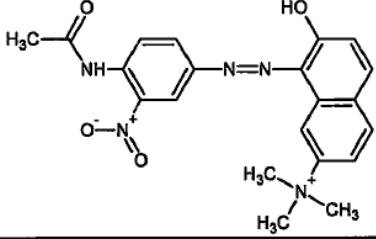
RT* (min)	NDKS 1944		64960101		m/z
	Area	Area Frac, %	Area	Area Frac, %	
12	3	0.1	43	0.6	202.187
13	-	-	7	0.1	347
14	4	0.1	-	-	230.215
15.3	20	0.4	15	0.2	338
15.8	4	0.1	15	0.2	216.201
16.5	2	0	4	0.1	338
16.8	4	0.1	2	0	No MS-signal
17	11	0.2	18	0.2	338.323
17.3	2	0	-	-	352.1
17.5	1	0	3	0	No MS-signal
18.7	9	0.2	43	0.6	474.382
19	7	0.1	11	0.1	382
19.3	9	0.2	5	0.1	474
19.5	3	0.1	-	-	408
-	-	-	5	0.1	474
19.6	13	0.2	-	-	321.306
20.2	-	-	5	0.1	474.382
20.3	131	2.5	306	4	366.351
20.9	-	-	8	0.1	365

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RT* (min)	NDKS 1944		64960101		m/z
	Area	Area Frac, %	Area	Area Frac, %	
21.4	25	0.5	4	0	367.352
21.6	5014	93.9	7150	92.7	366.351
22	1	0	2	0	Covered by main peak
22.1	2	0	-	-	Covered by main peak
22.2	6	0.1	9	0.1	380
-	-	-	4	0.1	518
22.8	16	0.3	5	0.1	502
23.1	9	0.2	19	0.2	379.364
23.5	5	0.1	5	0.1	502.384
24.1	9	0.2	5	0.1	502
24.6	7	0.1	9	0.1	502
25.1	16	0.3	3	0	398
25.3	4	0.1	5	0.1	502
27	3	0	-	-	521
Sum		100.1		100.1	

* Retention Time

The interpretation of some of the m/z in the table above is described in the following table:

m/z	Interpretation
366	Main component or Isomer (Basic Red 118) 
	Higher methylated materials (+CH ₃ , -H)
380	One additional methyl-group
	Lower methylated materials (-CH ₃ , +H)
352	One methyl-group less
338	Two methyl-groups less
202	Starting material 
	Higher methylated materials (+CH ₃ , -H)
216	One additional methyl-group
230	Two additional methyl-groups
408	Intermediate material 
502	Byproducts

3.1.6. Solubility

Water:	145 g/l	at 20 °C, pH 5.6, EC method A6
Ethanol:	< 1 g/l	room temperature
DMSO:	1 – 10 g/l	room temperature

Comment

Water solubility is not determined by EU Method A.6

3.1.7. Partition coefficient (Log P_{ow})

Log Po/w:	-0.1466	Method EU A.8
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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):	λ _{max} 216 nm and 462 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 risk assessment relates to the batches 64960101 (SAT 050019) and NDKS 1944 (SAT 000918 and SAT 040270). The quantification of the Basic Brown 17 content in these batches has been considered semi-quantitative. Both batches contain more than 25 impurities. Approximately half of the impurities have been chemically characterised and only 3 impurities were quantified. The chemical specifications of Basic Brown 17 in marketed hair dye formulations should not be significantly different from those described in section 3.1.4.
- The impurity Basic Red 118 in the two batches of Basic Brown 17 was 4.5 % (w/w) and 2.3% (w/w). According to the Cosmetic Directive, Basic Red 118 is not permitted for use in cosmetics except as an impurity in Basic Brown 17. However, the Cosmetic Directive does not mention the permitted content of Basic Red 118 as an impurity in Basic Brown 17.
- Water solubility is not determined by EU Method A.6
- Stability of Basic Brown 17 in test solutions and in typical hair dye formulations was not reported.

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 be re-calculated according to dye content of the test substance.

The safety assessment relates to the batches as tested, with the indicated impurities (2-nitrobenzene-1,4-diamine 150 ppm, Basic Red 118 (2-nitro isomer of B17) 4.5%, NBTRI (7-Hydroxy-N,N,N-trimethylnaphthalene-2-aminium chloride) 0.65%.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCP/0683/03

Guideline:	/
Species:	CFY rat
Group size:	2 male + 2 female
Substance:	Basic Brown 17 in 1% aqueous methylcellulose
Batch:	/
Dose:	0, 0.1, 1, 4, 8 and 16 g/kg bw in a volume of 1 to 40 ml/kg bw
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 bw. 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 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

Taken from SCCP/1173/08, modified

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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

The SCCS considers that, under the conditions of the study, the test substance showed some irritant potential to the rabbit skin.

3.3.2.2. Mucous membrane irritation

Taken from SCCP/1173/08, modified

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. 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

The SCCS considers that, under the conditions of the study, the test substance showed some irritant potential to the rabbit eye.

3.3.3. Skin sensitisation

Taken from SCCP/1173/08

Local Lymph Node Assay (LLNA)

Guideline: OECD 429 (2002)
 Species: Mice, CBA/CaHsdRcc (SPF)
 Group: 28 females (5 test groups, 2 negative control groups)
 Substance: B007 (Basic Brown 17)
 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 (³H-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 ^3H -methyl thymidine measured in a β -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. From 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

New study, submission IV, 2010

Guideline:	OECD Guideline 428
Test substance:	Basic Brown 17
Batch:	64960101
Formulation batch:	FGS09MK0003/01
Purity:	77.4%
Tissue:	dermatomed pig skin from the back region (2 females, 2 males) (780-800 μm)
Skin integrity:	skin electrical resistance measurement (>4 k Ω)
Method:	static diffusion cell 3.14 cm^2
Receptor fluid:	sodium chloride, 0.9% w/v
Formulation tested:	typical hair dye formulation (2% w/w)
Dose formulation applied:	21.18 mg/cm^2 of the formulation equivalent to 20 mg/cm^2 of test substance
Concentration ingredient:	1.58% active ingredient (2% in formulation)
Solubility receptor fluid:	water: 10 – 100 g/l, room temperature
Replicate cells:	10 (2 of 12 were rejected low mass balance)

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Duration of the contact: 30 min
 Duration of the diffusion: 24 hours (sampling time: 0.5, 1, 2, 4, 6, and 24 h)
 Analytical method: LC-MS/MS
 GLP: in compliance
 Study period: September 2009- February 2010

The dermal bioavailability of Basic Brown 17 has been studied, following topical application of a typical hair dye formulation (2.0%, w/w) to excised dermatomed pig skin.

Previously frozen dermatomed pig skin from 2 males and 2 females was mounted into static diffusion cells (12 replicates in total) containing receptor fluid (sodium chloride, 0.9% w/v) in the receptor chamber. The skin surface temperature was maintained at ca 32°C throughout the experiment. An electrical resistance barrier integrity test was performed and any pig skin sample exhibiting a resistance <4 kΩ was excluded from subsequent dermal bioavailability measurements.

20 mg/cm² of the hair dye formulation was applied to dermatomed pig skin mounted into static diffusion cells *in vitro*.

Dermal bioavailability was assessed by collecting receptor fluid aliquots at 0.5, 1, 2, 4, 6 and 24 h post dose. At 30 min post dose exposure was terminated by washing the skin surface with a dilute shampoo solution and water. The skin surface was dried with tissue paper (tissue swabs). At 24 h post dose, the washing procedure was repeated. The skin was then removed from the static diffusion cells, dried and the *stratum corneum* was removed with 20 successive tape strips. The remaining skin was divided into exposed and unexposed skin.

The receptor fluid in the receptor chamber was removed into a bulk receptor fluid vial and stored at ambient temperature. All samples were analysed by LC-MS/MS.

Results

The dermal bioavailability of Basic Brown 17 following topical application of the compound in hair dye formulation to pig skin *in vitro* was 0.48% (1.62 µg/cm²) of the applied dose. The majority of the dose was removed by washing the skin. The mass balance was complete (102.47%).

Table 1 Distribution of B007 (% Applied Dose) at 24 h Post Dose Following 30 Min Topical Application of B007 in Hair Dye Formulation to Dermatomed Pig Skin (Mean + SD, n = 10)

	Cell Number and Animal Number												Mean	SD
	Cell 1 07M	Cell 2 07M	Cell 3 07M	Cell 4 41F	Cell 5 41F	Cell 6 41F	Cell 7 37F	Cell 8 37F	Cell 9 37F	Cell 10 04M	Cell 11 04M	Cell 12 04M		
Skin Wash 0.5 h	28.72	56.21	33.41	65.97	32.85	63.63	22.24	51.24	13.80	58.37	10.42	89.16	45.94	23.53
Tissue Swab 0.5 h	62.57	22.52	68.30	32.85	65.69	36.24	68.30	47.65	75.08	40.88	58.39	11.00	50.86	20.52
Dislodgeable Dose 0.5 h	91.28	78.74	101.71	98.82	98.34	99.86	90.54	98.89	88.87	99.25	68.81	100.16	96.79	4.65
Skin Wash 24 h	3.45	0.67	2.50	0.97	2.45	0.83	5.60	1.22	9.48	0.98	3.27	0.30	2.78	2.84
Tissue Swab 24 h	2.32	0.56	2.91	0.66	1.65	0.46	1.60	0.66	3.50	0.35	1.90	0.10	1.42	1.18
Donor Chamber Wash	0.46	0.27	0.47	0.30	0.46	0.46	0.28	0.83	0.60	0.00	1.02	0.00	0.39	0.25
Total Dislodgeable Dose	97.52	80.24	107.59	100.75	103.10	101.61	98.02	101.60	102.46	100.58	75.00	100.57	101.38	2.80
Unexposed Skin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Unabsorbed	97.52	80.24	107.59	100.75	103.10	101.61	98.02	101.60	102.46	100.58	75.00	100.57	101.38	2.80
Stratum Corneum 1	0.04	0.07	0.07	0.01	0.04	0.03	0.12	0.17	0.27	0.13	0.10	0.05	0.09	0.08
Stratum Corneum 2	0.10	0.29	0.19	0.02	0.03	0.03	0.04	0.17	0.14	0.09	0.16	0.03	0.08	0.06
Stratum Corneum 3	0.06	0.30	0.06	0.00	0.02	0.03	0.05	0.10	0.11	0.11	0.05	0.02	0.06	0.04
Stratum Corneum 4	0.06	0.11	0.02	0.03	0.05	0.02	0.02	0.05	0.07	0.08	0.02	0.04	0.04	0.02
Stratum Corneum 5	0.09	0.11	0.16	0.01	0.01	0.00	0.05	0.07	0.05	0.04	0.03	0.02	0.05	0.05
Stratum Corneum 6-10	0.10	0.18	0.53	0.06	0.06	0.11	0.12	0.18	0.25	0.16	0.09	0.06	0.16	0.14
Stratum Corneum 11-20	0.07	0.21	0.34	0.07	0.09	0.12	0.12	N.S.	0.18	0.13	0.27	0.09	0.13	0.08
Dermal Adsorption	0.53	1.26	1.36	0.20	0.29	0.34	0.51	0.74	1.06	0.73	0.73	0.31	0.61	0.37
Dermal Absorption (Exposed Skin)	0.67	0.84	0.82	0.67	0.24	0.70	0.30	0.33	0.33	0.58	0.61	0.20	0.48	0.22
Receptor Fluid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Receptor Chamber Wash	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Percutaneous Penetration	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dermal Bioavailability	0.67	0.84	0.82	0.67	0.24	0.70	0.30	0.33	0.33	0.58	0.61	0.20	0.48	0.22
Mass Balance	98.72	82.34	109.77	101.61	103.64	102.65	98.83	102.67	103.86	101.88	76.33	101.08	102.47	3.11

Zero values are <LLOQ

N.S. = no sample

Cells 2 and 11 rejected from Mean and SD due to low mass balance (<90%)

Opinion on Basic Brown 17

Table 2 Distribution of B007 ($\mu\text{g}/\text{cm}^2$) at 24 h Post Dose Following 30 min Topical Application of B007 in Hair Dye Formulation to Dermatomed Pig Skin (Mean + SD, n = 10)

	Cell Number and Animal Number												Mean	SD
	Cell 1 07M	Cell 2 07M	Cell 3 07M	Cell 4 41F	Cell 5 41F	Cell 6 41F	Cell 7 37F	Cell 8 37F	Cell 9 37F	Cell 10 04M	Cell 11 04M	Cell 12 04M		
Skin Wash 0.5 h	96.19	188.30	111.91	220.99	110.03	213.14	74.50	171.64	46.21	195.53	34.89	298.64	153.88	78.83
Tissue Swab 0.5 h	209.57	75.45	228.78	110.03	220.05	121.38	228.78	159.62	251.49	136.92	195.60	36.85	170.35	68.73
Dislodgeable Dose 0.5 h	305.77	263.75	340.69	331.02	330.08	334.51	303.29	331.26	297.70	332.45	230.49	335.49	324.23	15.58
Skin Wash 24 h	11.57	2.25	8.36	3.24	8.20	2.78	18.77	4.09	31.75	3.27	10.94	1.02	9.30	9.50
Tissue Swab 24 h	7.77	1.87	9.76	2.20	5.52	1.54	5.34	2.20	11.74	1.17	6.37	0.35	4.76	3.94
Donor Chamber Wash	1.55	0.91	1.57	1.01	1.55	1.54	0.94	2.77	2.03	0.00	3.41	0.00	1.30	0.85
Total Dislodgeable Dose	326.66	268.77	360.39	337.47	345.35	340.37	328.34	340.32	343.21	336.89	251.22	336.86	339.59	9.39
Unexposed Skin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Unabsorbed	326.66	268.77	360.39	337.47	345.35	340.37	328.34	340.32	343.21	336.89	251.22	336.86	339.59	9.39
Stratum Corneum 1	0.15	0.23	0.22	0.05	0.14	0.12	0.39	0.56	0.89	0.42	0.34	0.18	0.31	0.26
Stratum Corneum 2	0.32	0.98	0.63	0.05	0.11	0.09	0.12	0.57	0.48	0.29	0.54	0.10	0.28	0.22
Stratum Corneum 3	0.19	1.00	0.20	0.00	0.05	0.11	0.17	0.35	0.37	0.36	0.16	0.06	0.19	0.14
Stratum Corneum 4	0.21	0.36	0.05	0.09	0.16	0.05	0.06	0.15	0.24	0.25	0.08	0.13	0.14	0.08
Stratum Corneum 5	0.32	0.36	0.54	0.04	0.04	0.00	0.18	0.24	0.15	0.14	0.09	0.06	0.17	0.16
Stratum Corneum 6-10	0.35	0.59	1.77	0.19	0.19	0.37	0.39	0.60	0.84	0.54	0.30	0.20	0.54	0.48
Stratum Corneum 11-20	0.24	0.70	1.14	0.24	0.29	0.41	0.39	N.S.	0.59	0.45	0.91	0.30	0.45	0.28
Dermal Adsorption	1.77	4.22	4.56	0.67	0.99	1.14	1.71	2.47	3.56	2.45	2.43	1.04	2.03	1.25
Dermal Absorption (Exposed Skin)	2.25	2.80	2.75	2.23	0.81	2.33	1.02	1.12	1.11	1.93	2.04	0.67	1.62	0.75
Receptor Fluid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Receptor Chamber Wash	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Percutaneous Penetration	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dermal Bioavailability	2.25	2.80	2.75	2.23	0.81	2.33	1.02	1.12	1.11	1.93	2.04	0.67	1.62	0.75
Mass Balance	330.67	275.80	367.70	340.37	347.14	343.83	331.06	343.90	347.88	341.28	255.69	338.57	343.24	10.41

Zero values are <LLOQ

N.S. = no sample

Cells 2 and 11 rejected from Mean and SD due to low mass balance (<90%)

Ref.: 16 (subm IV)

Comment

The experiment is well performed. Under non-oxidative conditions, the mean + 1SD (1.62 + 0.75) = 2.37 $\mu\text{g}/\text{cm}^2$ should be used to calculate MoS of Basic Brown 17.

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 SCCP/1173/08**

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 bw/day
 Vehicle: distilled water
 Route: gavage at a dose volume of 10 ml/kg bw
 Exposure: 13 weeks + 4 week recovery control/high dose groups
 GLP: in compliance
 Date: 12 Dec 2005

During the study, 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 bw/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 parameters 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 female, which were found dead, showed incomplete collapse in the lungs. Both the lungs and thymus had a dark/red colour. In the other high dose male deaths, the lungs, thymus, spleen and thyroid had 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 bw/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 bw/day, when compared with controls.

Yellow/brown pigmented macrophages were also seen in the lungs of females receiving ≥ 120 mg/kg bw/day. Treated males were similar to the controls.

In addition, males and females dosed at 180 mg/kg bw/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 bw/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 bw/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 bw/day at the end of the treatment period.

Conclusion

On the basis of this study, the low dose level of 60 mg/kg bw/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 bw/day (60 x 0.774).

Ref.: 12 (2005 submission)/
14 (2010 submission IV)

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:	<i>Salmonella typhimurium</i> 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 in the first experiment, 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-mix 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, respectively.

The appropriate level of toxicity (10-20% survival after the highest dose) was not reached in the experiment with S9-mix pointing to insufficient exposure of the cells.

Both in experiment I and II no biological relevant and concentration 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 concentration) was not reached in the 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 (<i>in vitro</i> 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 (\approx 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 concentration of 500 µg/ml. Concentration selection in experiment IIA was influenced by Basic Brown 17 toxicity and precipitation observed in experiment I. Due to the steep concentration 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-concentration-related increase in the rate of micronucleated cells was observed at the lowest and highest concentration. The values of highest concentration were at the laboratory's control data range (0.0 – 1.8% micronucleated cells). Concerning the lowest concentration, 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 concentrations (512.5 and 1025 µg/ml, respectively). These concentrations were strongly cytotoxic indicated by cell numbers of 7.9% and 12.9% of control, respectively.

Due to the steep concentration-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 concentrations 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

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***Taken from SCCP/1173/08***

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 bw/d in distilled water
Route:	Gavage, 10 ml/kg bw
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, whereas liver and spleen had 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 in the controls, 20 in the low dose group, 24 in the mid-dose group and 23 in the high dose.

Scabs and hair loss were the principal clinical signs observed in the treated females during the treatment period, but also occurred occasionally 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 bw/day. The foetal No Observed Adverse Effect Level (NOAEL) was higher than 240 mg/kg bw/day. As the dye content of the test substance was only 77.4%, the NOAELs were recalculated to 93 mg/kg bw/day (maternal) and 186 mg/kg bw/day (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 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.

The risk assessment relates to the batches 64960101 (SAT 050019) and NDKS 1944 (SAT 000918 and SAT 040270). The content of Basic Brown 17 in these batches has been considered semi-quantitative. Both batches contain more than 25 impurities. Approximately half of the impurities have been chemically characterised and only 3 impurities were quantified. The chemical specifications of Basic Brown 17 in marketed hair dye formulations should not be significantly different from those described in section 3.1.4.

The impurity Basic Red 118 in the two batches of Basic Brown 17 was 4.5 % (w/w) and 2.3% (HPLC peak area). According to the Cosmetic Directive, Basic Red 118 is not permitted for use in cosmetics except as an impurity in Basic Brown 17. However, the Cosmetic Directive does not mention the permitted content of Basic Red 118 impurity in Basic Brown 17.

Water solubility is not determined by EU Method A.6

Stability of Basic Brown 17 in test solutions and in typical hair dye formulations was not reported.

General toxicity

After 14 days observation, the LD₅₀ 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 60 mg/kg bw/day. As the dye content of the test substance was only 77.4%, the NOAEL is recalculated to 46 mg/kg bw/day (60 x 0.774).

The NOAEL for maternal toxicity was considered to be 93 mg/kg bw/day. The NOAEL for foetal toxicity was higher than 186 mg/kg bw/day (both values also corrected for dye content).

Irritation / sensitisation

Under the conditions of the study, the test substance showed some irritant potential to the rabbit skin and rabbit eye.

A conclusion on sensitisation cannot be drawn (vehicle used is not a recommended vehicle for LLNA; the maximum test concentration is too low).

Dermal absorption

0.48% of the applied dose corresponds to 1.62 µg/cm². The mean + 1SD (1.62 + 0.75) = 2.37 µg/cm² should be used to calculated MoS.

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. Basic Brown 17 induced gene mutations in bacteria. Basic Brown 17 did not induce mutations in an *in vitro* gene mutation test in mammalian cells. However, the appropriate level of toxicity (10-20% survival after the highest dose) was not reached in the experiment with S9-mix which may point to insufficient exposure of the cells. Basic Brown 17 was not considered genotoxic in an *in vitro* micronucleus test.

As Basic Brown 17 is positive in the gene mutation test in bacteria, it has to be considered as an *in vitro* genotoxic compound.

Carcinogenicity

No data submitted

4. CONCLUSION

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

Before any further consideration, the following information must be submitted:

- a proper chemical specification of Basic Brown 17 with respect to its purity and impurities;
- data on the stability of Basic Brown 17 in the test solutions and in typical hair dye formulations;
- additional studies to exclude a genotoxic potential of Basic Brown 17.

5. MINORITY OPINION

Not applicable

6. REFERENCES**Additional references received after commenting period**

- i. Frischmann M. (2009). Final Report B 007 Basic Brown 17 – Determination of Water Solubility. Henkel AG & Co KGaA report, internal code: R 1200093
- ii. Meinigke B., Lefev A. (2012): Stability of B007 – 8[(4-amino-3-nitrophenyl)azo]-7-hydroxy-N,N,N-trimethyl-2-naphthalenaminium chloride in aqueous solution. Henkel AG & Co KGaA report, internal code: R 1200096
- iii. Meinigke B., Lefev A. (2012): Stability of B007 – 8[(4-amino-3-nitrophenyl)azo]-7-hydroxy-N,N,N-trimethyl-2-naphthalenaminium chloride in sodium chloride solution. Henkel AG & Co KGaA report, internal code: R 1200097
- iv. Meinigke B. (2012): Comments to "SCCS, Opinion on Basic Brown 17, SCCS/1448/11, 27 March 2012". Henkel AG & Co KGaA report, internal code: R 1200098
- v. Meinigke B. (2012): Raw Material Description B007 (Sodium chloride as extender). Henkel AG & Co KGaA report, internal code: R 1200094

- vi. Meinigke B. (2012): Raw Material Description B007 (Saccharose as extender). Henkel AG & Co KGaA report, internal code: R 1200095
- vii. Ullmann L.G. (2005). B 007: Local Lymph Node Assay (LLNA) in mice (identification of contact allergens). RCC Ltd, Itingen, Switzerland, internal study code: A03003. Henkel AG & Co. KGaA report, internal code: R 0500286
- viii. Wollny H.-E. (2005). Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with B 007, CCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 872601, Archive code at Henkel AG & CO. KGaA, Report No. R 0500186
- ix. 1st Amendment to final Report: Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with B 007, Harlan Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 872601, Archive code at Henkel AG & Co. KGaA, Report No. R 0800303

Submission IV, 2010

1. Wiebel F. (2005). COLIPA B 007 - Basic Brown 17 Submission III. Archive code at Henkel AG & Co. KGaA, Düsseldorf, Report No. R 0500099
2. Frischmann M. (2009). Determination of Partition Coefficient of Basic Brown 17. Archive code at Henkel AG & Co. KGaA, Düsseldorf, Report No. R 100119
3. Meinigke B. (2010). Impurities of Basic Brown 17 Q SCCP 30-09-2008 V01. Archive code at Henkel AG & Co. KGaA, Düsseldorf, Report No. R 100121
4. RMD B 007 V03 (2010). Raw Material Description B 007. Archive code at Henkel AG & Co. KGaA, Düsseldorf, Report No. R 100118
5. Meinigke B. (2005). Dossier of hair dye B 007 – Analysis of batch 64960101 used in toxicological tests. Henkel AG & Co. KGaA Düsseldorf Report R 0500254
6. Bernecker U. (2004). Dossier of hair dye B 007 – analytical tests on identity and purity for dossier. Henkel AG & Co. KGaA Düsseldorf Report R 0400295
7. Meinigke B. (2005). Supplement to dossier of hair dye B 007 – Analysis of batch NDKS 1944 used in toxicological tests. Henkel AG & Co. KGaA Düsseldorf Report R 0500438
8. Meinigke B. and Lefev A. (2009). B 007 #NDKS 1944 - HPLC determination of dye content. Archive code at Henkel AG & Co. KGaA, Düsseldorf, Report No. R 100120
9. Ullmann L.G. (2005). B 007: Local Lymph Node Assay (LLNA) in mice (identification of contact allergens). RCC Ltd, Itingen, Switzerland, internal study code: A03003. Archive code at Henkel AG & Co. KGaA, Düsseldorf, Report No. R 0500286
10. Wollny H.-E. (2005). Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with B 007, CCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 872601. Archive code at Henkel AG & Co. KGaA, Report No. R 0500186
11. 1st Amendment to final Report: Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with B 007, Harlan Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 872601, Archive code at Henkel AG & Co. KGaA, Report No. R 0800303
12. Schulz M. (2005). In vitro micronucleus assay in Chinese hamster V79 cells with B 077. RCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 877102. Archive code at Henkel AG & Co. KGaA, Report No. R 0500329
13. Völkner W. and Poth A. (2010). Statement on SCCP opinion on Basic Brown 17 (COLIPA B 007) (SCCP/1173/08) in particular on the validity of the reported in vitro micronucleus test (CCR 877102) and drawn conclusion, Harlan Cytotest Cell Research GmbH, Rossdorf, Germany. Archive code at Henkel AG & Co. KGaA, Report No. R 1000101
14. Valdoni V. and Manno R.A. (2005). B 007 – 13 week oral toxicity study in rats followed by a 4 week recovery period. Research Toxicology Centre (RTC) Rom, Italy, internal study code: 30990. Archive code at Henkel AG & Co. KGaA, Düsseldorf, Report No. R 0500464

15. Liberati V. (2000). B 007 – prenatal developmental toxicity study in rats. Research Toxicology Centre (RTC), Rom, Italy, internal study code: 31010. Archive code at Henkel AG & Co. KGaA, Düsseldorf, Report No. R 0500382
16. Toner F. (2010). The In vitro Dermal Bioavailability of B 007 Through Pig Skin. Charles River, Tranent Edinburgh, UK, internal study code 787257, report # 30759. Archive code at Henkel AG & Co. KGaA, Düsseldorf, Report No. R 1000056

Submission III, 2005

1. Meinigke, B. (2005). Raw Material Description – B 007. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500253
2. Meinigke, B. (2005). Dossier of hair dye B 007 – Analysis of batch 64960101 used in toxicological tests. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500254
3. Bernecker, U. (2004). Dossier of hair dye B 007 – analytical test on identity and purity for dossier. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400295
4. Meinigke, B. (2005). Supplement to dossier of hair dye B 007 – Analysis of batch NDKS 1944 used in toxicological tests. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500438
5. Kynoch, S.R. and Lloyd, G.K. (1977). Acute oral toxicity to rats of Sienna Brown. Huntingdon Research Centre, Cambridgeshire, England, internal study code: 8280/D2/77. Archive code at Henkel KGaA, Düsseldorf: R 9501186
6. van Huygevoort, A.H.B.M. (2004). Primary skin irritation / corrosion study with B 007 in the rabbit (4-hour semi-occlusive application). NOTOX B.V., s’-Hertogenbosch, The Netherlands, internal study code: 408779. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400865
7. van Huygevoort, A.H.B.M. (2004). Acute eye irritation / corrosion study with B 007 in the rabbit. NOTOX B.V., s’-Hertogenbosch, The Netherlands, internal study code: 408825. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400866
8. Ullmann, L.G. (2005). B 007: Local Lymph Node Assay (LLNA) in mice (identification of contact allergens). RCC Ltd, Itingen, Switzerland, internal study code: A03003. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500286
9. Sokolowski, A. (2005). Salmonella typhimurium reverse mutation assay with B 007. RCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 872602. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500145
10. Wollny, H.-E. (2005). Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with B 007, CCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 872601. Archive code at Henkel KGaA, Report No. R 0500186
11. Schulz, M. (2005). In vitro micronucleus assay in Chinese hamster V79 cells with B 007, RCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 877102. Archive code at Henkel KGaA, Report No. R 0500329
12. Valdoni, V. and Manno R.A. (2005). B 007 – 13 week oral toxicity study in rats followed by a 4 week recovery period. Research Toxicology Centre (RTC) Rom, Italy, internal study code: 30990. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500464
13. Liberati, V. (2000). B 007 – prenatal developmental toxicity study in rats. Research Toxicology Centre (RTC), Rom, Italy, internal study code: 31010. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500382
14. Owen, H.M. (2001). In vitro penetration of Basic Brown 17 through pig skin from an aqueous-alcoholic vehicle and a standard formulation. Central Toxicology Laboratory, Cheshire, UK, internal study code JV1659. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0100857