Scientific Committee on Consumer Safety

SCCS

OPINION ON

Disperse Red 17

COLIPA n° B5

The SCCS adopted this opinion at its 4th plenary meeting

on 12 December 2013

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).

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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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1. BACKGROUND

Submission I for Disperse Red 17 (CAS n. 3179-89-3), B5, with the chemical name 1-(4'-Nitrophenylazo)-2-methyl-4-bis-(ß-hydroxyethyl) aminobenzene, was submitted in December 1998 by COLIPA.
Submission II was submitted by Colipa in November 2001.
Submission III was submitted by Colipa in July 2005.

The Scientific Committee on Consumer Product (SCCP) adopted at its 18th plenary meeting of December 2008 the opinion (SCCP/1161/08) with the following conclusion:

The SCCP is of the opinion that the safety of Disperse Red 17 cannot he assessed based on the data submitted. Before any further consideration, an additional in vivo mutagenicity test should be performed to exclude the gene mutation potential of Disperse Red 17. The stability of Disperse Red 17 in oxidative hair dye formulations has not been demonstrated.

A sensitising potential of Disperse Red 17 could not be excluded.

Submission IV for Disperse Red 17 was submitted by Cosmetics Europe in May 2013. According to the applicant the attached submission IV provides the toxicological study as well as information related to the chemical composition and stability of disperse Red 17, (CAS n. 3179-89-3), B5.

2. TERMS OF REFERENCE

1. *Does the SCCS consider Disperse Red 17 safe for use as an ingredient in non-oxidative hair dye formulations with a concentration on the scalp of maximum 0,2% taken into account the scientific data provided?*

2. *Does the SCCS consider Disperse Red 17 safe for consumers, when used as an ingredient in oxidative hair dye formulations with a concentration on the scalp of maximum 2.0% taken into account the scientific data provided?*

3. *And/or does the SCCS recommend any restrictions with regard to the use of Disperse Red 17 in oxidative and non-oxidative hair dye formulations (e.g. max conc. in the finish cosmetic product, dilution ratio with hydrogen peroxide, warning)?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Disperse Red 17 (INCI name)

3.1.1.2. Chemical names

Ethanol, 2,2'-[[3-methyl-4-[(E)-(4-nitrophenyl)azo]phenyl]imino]bis- (CA Index name, 9CI)
1-(4'-Nitrophenylazo)-2-methyl-4-bis-(beta-hydroxyethyl)aminobenzene
2,2'-[[3-methyl-4-[(E)-(4-nitrophenyl)azo]phenyl]imino]diethanol (IUPAC)
(2,2'-[[3-methyl-4-[(4-nitrophenyl)azo]phenyl]imino]bisethanol)
2-[(2-Hydroxy-ethyl)-[3-methyl-4-(nitro-phenylazo)-phenyl]-amino]-ethanol

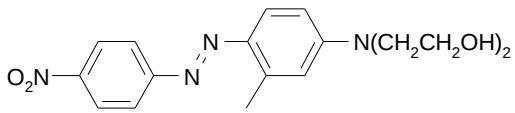
3.1.1.3. Trade names and abbreviations

Sedona Red (Commercial grade)
Intrasperse Red YNB Conc. (Crompton & Knowles)
CI 11210
COLIPA n° B005

3.1.1.4. CAS / EC number

CAS: 3179-89-3 (Disperse Red 17, INCI dictionary)
EC: 221-665-5 (2,2'-[[3-methyl-4-[(4-nitrophenyl)azo]phenyl]imino]bisethanol)

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{17}H_{20}N_4O_4$

3.1.2. Physical form

Dark brown powder

3.1.3. Molecular weight

Molecular weight: 344.37 g/mol

3.1.4. Purity, composition and substance codes

Chemical structure of 2,2'-[[3-methyl-4-[(E)-(4-nitrophenyl)azo]phenyl]imino]diethanol was established by NMR spectroscopy. The chemical characterization of the various batches is described in the table below.

Chemical Characterisation of various batches of Disperse red 17. The references of studies are from Submission IV.

Description of sample	928017	40T60N4520	928017	406T60N4520	40T60N4520	L-801	7213013439	097-09/04-02
Comments	Previous submission		Follow up analysis to quantify impurities and remove lignin sulfate from the area % determinations		Follow up analysis to compare area % of all 4 batches			
References of Analyses	A 2005/350 (Ref. 2)	A 2005/350 (Ref. 2) G 2004/009 (Ref. 3)	G10-A13518 (Ref. 4)	G10-A13518 (Ref. 4)	HDC (Ref. 5)	HDC (Ref. 5) A001699 (Ref. 6)	HDC (Ref. 5) OND35210 (Ref. 7)	*HDC (Ref. 5)*
NMR content / %, w/w	40.3	31.0					30.1	
HPLC purity / area %								
210 nm	70.4	74.6	96.2 *	90.8 *	91.5 *	95.9 *	96.3 *	97.2 *
254 nm	80.5	84.5	96.1 *	90.6 *	91.3 *	96.8 *	96.5 *	98.7 *
510 nm	98.9	97.1	97.6 *	92.0 *	92.0 *	97.2 *	97.1 *	97.8 *
Impurity N-(2-hydroxyethyl)-3-methyl-4-((4-nitrophenyl)azo)-aniline [area% @510nm] / [w/w%]	0.8	0.053	0.23% area 0.097% w/w	0.30% area 0.11% w/w	0.22	0.44	1.6	0.9
p-Nitroaniline / ppm[a]	50	13						
m-Tolyldiethanolamine / ppm	< 200[b]	9						
Impurity N-(2-hydroxyethoxyethyl),N-(2-hydroxyethyl)-3-methyl-4-((4-nitrophenyl)azo)-anilin [area% @510nm] / [w/w%]			1.32% area 0.62% w/w	4.96%area 1.98%w/w	4.9	1.4	1.1	1.2
N-(2-hydroxyethyl),N-methyl-3-methyl-4-((4-nitrophenyl)azo)-aniline (ppm)			ND Not detected LOD 20ppm	ND Not detected LOD 20ppm	ND	ND	ND	ND
N,N-Di(2-hydroxyethoxyethyl)-3-methyl-4-((4-nitrophenyl)azo)-aniline ([area% @510nm] / [w/w%]			0.01% area 0.0055% w/w	0.48% area 0.23% w/w	1.4% °	ND	ND	ND
Impurity 1 [area% @510nm] 19.5min				0.42	1.2% area	ND	ND	ND
Impurity 2 [area% @510nm] 20.1min				0.69	ND	0.66% area	ND	ND
Impurity 3 [area% @510nm] 29.5min				1.16				
Impurity 4 [area% @510nm] 24.0min			0.27					
Impurity 5 [area% @510nm] 34.1min			0.49					
o-Tolyldiethanolamine (ppm)			10	10				
HPLC content	39.1	32.3						
Loss on drying / %, w/w	5.9	6.0						
Water content / %, w/w	6.6	9.2				6.25% w/w		
Sulphated ash / %, w/w	11.7	14.9						
Sodium ligninsulfonate / %, w/w	54	57						
Heavy metals	< 10 ppm with the exception of Fe with 220 ppm	< 10 ppm						

* Sodium ligninsulfonate peaks were not integrated
° signals not separated

ND not detected
[a] classified by German MAK as carcinogenic category 3A
[b] below detection limit; indicated value is the detection limit of the used method

The applicant informed that: "*For hair dye use, the applicant selects material for which the dye content ranges between 30 and 40%. Over the last few years, the applicants have worked with the supplier to reduce the level of impurities in the dye and the current specification requires an area % purity of DISPERSE RED 17 of >95% at 510nm. Other grades and purities of the material are commercially available (eg. for use in the textile business); however, these do not meet the applicant's specification for use as a hair colourant.*"

SCCS comment
Since the last opinion on Disperse Red 17 (SCCP/1161/08), the applicant reanalysed the two old batches of Disperse Red 17. The data on reanalysis, as well as data on additional 4 batches of Disperse Red 17, are presented in the recent submission (submission IV). The chemical characterization of the old batches as requested by SCCP is now complete.

As informed by the applicant, other grades and purities of the material are commercially available (eg. for use in the textile business), which do not meet the applicant's specification for use as a hair colourant. According to the applicant, the Disperse Red 17 for the use as an ingredient in hair dye formulations meets following specifications as described in the table below.

Applicant's description of the specification of Disperse Red 17 for the use as hair colourant:

Assay:	30 – 40 %w/w
Purity:	> 95 % area measured at 510nm
Impurity: N-(2-hydroxyethoxyethyl),N-(2-hydroxyethyl)-3-methyl-4-((4-nitrophenyl)azo)-anilin N-(2-hydroxyethyl)-3-methyl-4-((4-nitrophenyl)azo)-anilin	 < 2 % area measured at 510nm < 1.5 % area measured at 510nm

The SCCS considers that the above specification of Disperse Red 17 for the use as an ingredient in hair dye formulations is in accordance with the chemical characterization of the various batches of Disperse Red 17 described in this Opinion.

3.1.5. Impurities / accompanying contaminants

See point 3.1.4.

3.1.6. Solubility

Water solubility: 0.3 mg/l at 22 °C (EU - A.6) *(Reference: 11)*
Receptor fluid*: 10.68 µg/ml at 32 °C (taken from *SCCNFP/0677/03*)
* receptor fluid used in percutaneous absorption study: PBS buffer w/o Ca^{2+}, Mg^{2+} Instamed® 9.55g/l containing 0.25% of Tween 80

3.1.7. Partition coefficient (Log P_{ow})

Log $P_{o/w}$: 3.575, for Sedona Red purity 31.5% (EU Method A8)

3.1.8. Additional physical and chemical specifications

Melting point:	150-152 °C	(EU – A.1)	*(Ref. 6)*
Boiling point:	decomposition at 175°C	(EU – A.2)	*(Ref. 8)*
Relative density:	1.0923 (20°C)	(EU - A.3)	*(Ref. 9)*
Vapour pressure:	2.8 exp – 6 hPa (20°C)	(EU - A.4)	*(Ref. 10)*
Viscosity:	/		
pH-value:	3.5 (saturated aqueous solution commercial dye, 20°C)		*(Ref. 4)*
pKa:	/		
Refractive index:	/		

UV_Vis spectrum (200-800 nm): /

3.1.9. Homogeneity and Stability

The solutions of Disperse Red 17 (dye content 41% dye) used in 13 weeks oral toxicity testing were shown to be stable for 4 days (variation up to 4%) and 9 days (variation up to 12%).
Disperse Red 17 (dye content 31%) was stable in an approximately 5% solution in DMSO for 7 days (recovery 98.2-100%).
Disperse Red 17 (dye content 31%) was stable in an approximately 1% solution in acetone/water for 7 days (recovery 100-106%).
The stability of Disperse Red 17 in combination with 6% hydrogen peroxide was tested at room temperature for a total time period of 45 min at room temperature. The recovery of Disperse red 17 was found to be 92.4% (t=15 min), 91.5% (t=30 min) and 90.5% (t=45 min).
Disperse Red 17 is stable (> 90%) over a 45 min. period in the presence of hydrogen peroxide and persulfate.

General comments to physico-chemical characterisation

- 2-[3-Methyl-4-(4-nitro-phenylazo)-phenylamine]ethanol) impurity (up to 2%) is a secondary amine and N-(2-hydroxyethoxyethyl), N-(2-hydroxyethyl)-3-methyl-4-((4-nitrophenyl)azo)-anilin impurity (up to 1.5%) is a tertiary amine. These impurities are prone to nitrosation and may generate nitrosamines. Nitrosamine content in Disperse Red 17 is not known.

- The stability of Disperse Red 17 in marketed products is not described.

3.2. Function and uses

a) Oxidative Hair Colorants
Disperse Red 17 is used as a non-reactive hair colouring agent (direct dye) in oxidative hair dye formulations at a maximum on-head concentration of 2%, inclusive dispersant.

b) Semi-permanent Hair Colorants
Disperse Red 17 is used as a non-reactive hair colouring agent (direct dye) in semi-permanent hair dye formulations at a maximum on-head concentration of 0.2%, inclusive dispersant.

The quality of B005 (pure dye + dispersant mixture in a percentage of 31% and 40% of pure dye amount), as described in the dossier and used for the risk assessment, is identical to the marketed consumer products. This means that the "usual maximum levels of up to 0.2% on head in semi-permanent hair dye formulations and of up to 2.0% (up to 0.81% pure dye) on head in oxidative hair dye formulations" in the marketed products refers to the "dye + dispersant".

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/0677/06

Guideline:	OECD 401 (1987)
Species/strain:	Sprague Dawley rat, Crl:CD (SD) BR
Group size:	5 males + 5 females
Test material:	Disperse Red 17 dispersed in water
Batch:	928017/02 (dye content: 41.2%)
Dose:	2000 mg/kg bw
Observ. period:	14 days
GLP:	in compliance
Study period:	December 1994 – February 1995

The dose group was selected on the basis of a preliminary range-finding study in which rats were given the test compound in water at dose levels from 100 to 2000 mg/kg bw. The dose selected for the Limit Test was 2000 mg/kg bw. Groups of 5 male and 5 female rats received a single dose of test substance by gastric gavage. The animals were observed 1, 2 and 4 hours after dosing and thereafter daily for 14 days. Body weights were recorded on days 1, 8 and 15 of the study. Macroscopic examination of main organs was performed after autopsy. No histological examinations were performed.

Results
In the preliminary range-finding study, one death was reported at 1000 mg/kg bw but the only reported clinical signs were dose-related pink skin tone due to the compound and the death was considered to be unrelated to treatment. There were no deaths during the Limit test. Body weight gain was considered normal for the age and strain of rat. The only clinical sign was a pink discoloration of the skin, apparent from 1 hour to 7 days after dosing, and at autopsy an orange coloration of the mammary tissue and /or abdominal fat, attributed to the staining properties of the substance and not considered to be a toxic effect. The distribution and persistence of staining indicates that the substance has the potential to accumulate, at least at the high dose used in this acute study.

Ref.: 16

SCCS comment
Disperse Red 17 has a low acute toxicity potential with LD_{50} values above 2000 mg/kg bw in rats.
Although no analytical data (including possible impurities) of the test batch is available and although the study was performed according to an older, no longer valid OECD guideline, the study was carried out in compliance with GLP and the results are considered sufficiently reliable to evaluate the acute oral toxicity of Disperse Red 17.

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 SCCNFP/0677/06

Guideline:	OECD 404 (1992)
Species/strain:	New Zealand albino rabbits
Group size:	3 females
Test material:	Disperse Red 17
Batch:	928017/02 (dye content: 41.2%)
Dose:	0.5g
GLP:	in compliance
Study period:	29 November - 3 December 1994

The substance (0.5 g moistened with water) was applied to a 6.25 cm² area of intact skin of 3 female rabbits. Semi-occlusive patches were applied and left in place for a 4-hour period. Remaining test substance was removed by swabbing with cotton wool swabs soaked in warm water. The skin was examined for erythema, eschar formation and oedema at 1, 24, 48 and 72 hours after removal of the patches. An index of Cutaneous Primary Irritation was calculated from the mean scores at the sites and at each time point.

Results
No signs of irritation were noted on the skin. Red/orange staining was reported at all-time points.

Ref.: 17

3.3.2.2. Mucous membrane irritation / Eye irritation

Taken from SCCP/1161/08

Guideline:	/
Species:	rabbit
Group:	2 groups of 3 animals
Substance:	dyestuff CI 11210
Batch:	/
Purity:	/
Dose:	1 ml of 0.3 and 3% of CI 11210 in Diethylphthalate (DEP)
GLP:	not in compliance
Date:	1970

In a modified Draize test for eye irritation, 0.1 ml of a 0.3 and 3% concentration of the test substance CI 11210 in DEP was instilled into the one eye of 6 rabbits. The untreated eye served as the control. Observations were made 1, 24, 48, 72 hours and 7 and 14 days after applications. Scoring of the eyes was done by the method of Draize.

Results
The 3% test concentration caused slight short lasting conjunctival irritation of the rabbit eye. No alteration of the cornea was observed macroscopically.

Ref.: 18

3.3.3. Skin sensitisation

Taken from SCCNFP/0677/06

Magnusson and Kligman study

Guideline:	OECD 406 (1992)	
Species/strain:	Dunkin-Hartley guinea pigs	
Group size:	10 test + 5 control, females	
Test material:	Disperse Red 17 dispersed in water	
Batch:	928017/02 (dye content: 41.2%)	
Concentration:	intradermal induction:	0.1ml 50% Freund's complete adjuvant (FCA)
		0.1ml 5% (w/v) test substance
		0.1ml 5% (w/v) test substance/FCA
	induction of irritation:	0.5ml 10% Sodium lauryl sulphate (Day 6)
	topical induction:	0.5ml 2.5% test substance (Day 8)
	challenge:	2.5% test substance for 24 hours, occluded
GLP:	in compliance	
Study period:	30 November 1994 – 7 January 1995	

A preliminary intradermal study indicated that a 5% w/v test substance could be used without provoking an irritant response.
Induction commenced with three pairs of intradermal injections of FCA, test substance (5%) and a mixture of the two. Six days later, 0.5 ml of 10% sodium lauryl sulphate was applied to the injection site to induce a local irritation. The induction process was completed day 8 with a single topical application of 0.5 ml the test substance (2.5%) under occlusive patch to the shoulder region for 48 hours. An interval of two weeks was allowed after induction and then the animals were challenged by a single topical application of the test substance (2.5%) under occlusive patch on the left flank for 24 hours. Appropriate controls were treated with vehicle at all stages and the test substance-induced animals received vehicle alone on the right flank.
The skin was examined 24 hours after administration of the intradermal injection and again after removal of the topical patches for signs of irritation. The skin was examined 24 and 48 hours after removal of the challenge patches.

Results
Skin staining was observed due to the test substance and was reported to preclude accurate assessment of erythema after the induction and the challenge application in 6/10 animals. No adverse reaction was observed in any of the treated guinea pigs. The author concluded that the test substance was not a sensitiser to guinea pig skin.

Ref.: 20

SCCS comment
Excessive staining due to the test substance made assessment "difficult" in 6/10 animals. Therefore the study should be considered as equivocal.

Dendritic cell activation assay (*in vitro*)

Guideline:	/
Species/strain:	peripheral blood monocyte derived dendritic cells (PBMDC), 4 human donors
Group size:	/

Test material:	Disperse Red 17
Batch:	40T60N4520 (R0015361) (dye content: 31.0%)
Purity:	84.5 area% at 254 nm (HPLC) 97.1 area% at 510 nm (HPLC)
Concentration:	10, 20, 30 and 40 µg/ml
Vehicle:	dimethylsulfoxide (DMSO)
Negative control:	dimethylsulfoxide (DMSO)
GLP:	not in compliance
Study period:	13 – 28 October 2005

The test item was examined *in vitro* in a peripheral blood monocyte derived dendric cell (PBMDC) activation assay. PBMDCs were exposed in three independent experiments for 24 and 30 hours to concentrations of 10, 20, 30 and 40 µg/ml of the test substance. The activation of immature DCs pooled from four different human donors was evaluated by flow cytometric analysis of DC86 positive cells and quantitative measurement of interleukin-1β, interleukin-8 and Aquaporin P3 gene expression.

Results
Among the four activation markers tested, CD86 protein expression and Aquaporin 3 (AQP3) gene expression were significantly altered, whereas no significant effect on the gene expression of IL-1β and IL/8 was detected.

Conclusion
As the test item modulated two out of four endpoints selected as DC activation markers and thus did not fulfil the criteria for a positive DC activation response, the study authors concluded that Disperse Red 17 was not a sensitiser.

Ref.: 23

SCCS comment
The test is not conducted according to any OECD guideline. The relevance of this dendritic cell activation assay for risk assessment is unknown.

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428 (2004)
Tissue:	human (female) dermatomed abdominal skin, 400 µm thickness
Group size:	2 x 9 skin membranes from 3 different donors
Diffusion cells:	9 mm flow-through automated diffusion cells, 0.64 cm^2
Skin integrity:	permeation coefficient for tritiated water (< 2.5 x 10^{-3} cm/h)
Test substance:	Disperse Red 17
Batch:	40T60N4520
Purity:	Dye content 31%
Test item:	formulation with 0.2% (w/w) test substance (non-oxidative conditions), formulation with 2.0% (w/w) test substance (oxidative conditions)
Doses:	non-oxidative: 16.0 mg (25 mg/cm^2), oxidative: 13.1 mg (20 mg/cm^2)
Receptor fluid:	phosphate buffered saline containing 0.01% sodium azide (w/v) and 0.25% Tween 80® (w/v)
Solubility receptor fluid:	10.68 µg/ml
Stability:	/
Method of Analysis:	HPLC
GLP:	in compliance
Study period:	11 – 31 October 2005

The *in vitro* percutaneous absorption of Disperse Red 17 was determined in human dermatomed skin mounted in flow-through diffusion cells. Disperse Red 17 was tested as a semi-permanent (direct) hair dye under 'in-use'-conditions in two formulations: under non-oxidative conditions with a target concentration of 0.2% (w/w) and under oxidative conditions with a target concentration of 2.0% (w/w).

The integrity of 27 skin samples, of which 18 were used in the final study, was evaluated by measuring the permeability coefficient for tritiated water.

The percutaneous absorption of Disperse Red 17 from both formulations was evaluated for each of 9 human skin preparations from 3 different donors. The contact time was 60 minutes. The post exposure time was 23 hours. The concentration of the test substance was determined by HPLC in the receptor fluid. The residues remaining in/on the skin membranes and in the stratum corneum were obtained with tape striping 10 times per skin membrane, 24h after application.

Results
The mean recovery of Disperse Red 17 was 98.5% (non-oxidative) and 98.6% (oxidative formulation). Most of Disperse Red 17 was recovered in the skin wash after 60 minutes of exposure. Virtually no penetration of Disperse Red 17 into the receptor fluid after 24 hours was observed.

Table 1: recovery of Disperse Red 17 in human skin (in µg/cm² of dose, non-oxidative)

	Amount recovered (µg/cm²)										
Cell number	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**mean**	**SD**
Donor	**1**	**1**	**1**	**2**	**2**	**2**	**3**	**3**	**3**		
Skin wash	35.3	37.3	41.8	47.4	58.7	37.0	49.0	54.3	39.6	**44.5**	**8.3**
Donor compartment wash	0.84	0.40	0.64	0.16	0.75	0.16	0.41	0.16	0.37	**0.43**	**0.26**
Dislodgeable dose	36.2	37.7	42.4	47.6	59.4	37.1	49.4	54.5	40.0	**44.9**	**8.3**
Tape strips	0.61	0.26	0.45	0.16	0.41	0.16	0.28	0.16	0.16	**0.29**	**0.16**
Unabsorbed dose	36.8	37.9	42.9	47.7	59.8	37.3	49.7	54.6	40.1	**45.2**	**8.3**
Skin	0.28	0.17	0.31	0.18	0.32	0.14	0.36	0.27	0.29	**0.26**	**0.08**
Receptor compartment wash	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	**0.03**	**0.00**
Receptor fluid	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	**0.12**	**0.00**
Absorbed dose	**0.43**	**0.32**	**0.46**	**0.33**	**0.46**	**0.28**	**0.51**	**0.42**	**0.44**	**0.41**	**0.08**
Total recovery	37.2	38.3	43.3	48.0	60.3	37.6	50.2	55.0	40.6	**45.6**	**8.3**

Table 2: recovery of Disperse Red 17 in human skin (in % of dose, non-oxidative)

	Amount recovered (%)										
Cell number	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**mean**	**SD**
Donor	**1**	**1**	**1**	**2**	**2**	**2**	**3**	**3**	**3**		
Skin wash	91.8	96.2	92.6	98.8	100.0	98.4	96.8	93.4	95.8	**96.0**	**2.9**
Donor compartment wash	2.19	1.03	1.41	0.33	1.28	0.42	0.81	0.27	0.90	**0.96**	**0.61**
Dislodgeable dose	94.0	97.3	94.0	99.1	101.3	98.8	97.6	93.7	96.7	**96.9**	**2.6**
Tape strips	1.58	0.67	0.99	0.33	0.69	0.42	0.55	0.27	0.38	**0.65**	**0.41**
Unabsorbed dose	95.6	97.9	95.0	99.4	102.0	99.2	98.1	94.0	97.1	**97.6**	**2.5**
Skin	0.73	0.45	0.69	0.37	0.54	0.36	0.71	0.47	0.71	**0.56**	**0.15**
Receptor compartment wash	0.08	0.08	0.07	0.07	0.05	0.08	0.06	0.05	0.08	**0.07**	**0.01**
Receptor fluid	0.30	0.30	0.26	0.24	0.20	0.31	0.23	0.20	0.28	**0.26**	**0.04**
Absorbed dose	**1.12**	**0.83**	**1.02**	**0.68**	**0.79**	**0.75**	**1.01**	**0.72**	**1.06**	**0.89**	**0.16**
Total recovery	96.7	98.8	96.0	100.1	102.7	100.0	99.2	94.7	98.1	**98.5**	**2.4**

Table 3: recovery of Disperse Red 17 in human skin (in µg/cm² of dose, oxidative)

	Amount recovered (µg/cm²)										
Cell number	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**mean**	**SD**
Donor	**1**	**1**	**1**	**2**	**2**	**2**	**3**	**3**	**3**		
Skin wash **Donor compartment wash**	405.4 0.16	455.6 0.16	478.9 0.29	423.1 0.16	473.7 0.16	455.9 0.16	442.3 0.16	488.7 0.16	463.3 0.16	**454.1** **0.17**	**26.8** **0.04**
Dislodgeable dose	405.6	455.8	479.2	423.2	473.9	456.1	442.5	488.9	463.4	**454.3**	**26.8**
Tape strips	0.25	0.25	0.47	0.27	0.35	0.27	0.37	0.16	0.43	**0.31**	**0.10**
Unabsorbed dose	405.8	456.0	479.6	423.5	474.2	456.4	442.8	489.0	463.9	**454.6**	**26.9**
Skin **Receptor compartment wash** **Receptor fluid**	0.18 0.03 0.12	0.31 0.03 0.12	0.24 0.03 0.12	0.26 0.03 0.12	0.43 0.03 0.12	0.25 0.03 0.12	0.55 0.03 0.12	0.54 0.03 0.12	0.43 0.03 0.12	**0.35** **0.03** **0.12**	**0.14** **0.00** **0.00**
Absorbed dose	**0.33**	**0.46**	**0.39**	**0.40**	**0.58**	**0.40**	**0.70**	**0.68**	**0.58**	**0.50**	**0.14**
Total recovery	406.1	456.5	480.0	423.9	474.8	456.8	443.5	489.7	464.4	**4551**	**26.9**

Table 4: recovery of Disperse Red 17 in human skin (in % of dose, oxidative)

	Amount recovered (%)										
Cell number	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**mean**	**SD**
Donor	**1**	**1**	**1**	**2**	**2**	**2**	**3**	**3**	**3**		
Skin wash **Donor compartment wash**	87.9 0.03	98.8 0.03	103.8 0.06	91.7 0.03	102.7 0.03	98.8 0.03	95.9 0.03	105.9 0.03	100.4 0.03	**98.4** **0.04**	**5.8** **0.01**
Dislodgeable dose	87.9	98.8	103.9	91.7	102.7	98.9	95.9	106.0	100.5	**98.5**	**5.8**
Tape strips	0.05	0.06	0.10	0.06	0.08	0.06	0.08	0.03	0.09	**0.07**	**0.02**
Unabsorbed dose	88.0	98.8	104.0	91.8	102.8	98.9	96.0	106.0	100.5	**98.5**	**5.8**
Skin **Receptor compartment wash** **Receptor fluid**	0.04 0.01 0.03	0.07 0.01 0.03	0.05 0.01 0.03	0.06 0.01 0.03	0.09 0.01 0.03	0.06 0.01 0.03	0.12 0.01 0.03	0.12 0.01 0.03	0.09 0.01 0.03	**0.08** **0.01** **0.03**	**0.03** **0.00** **0.00**
Absorbed dose	**0.07**	**0.10**	**0.08**	**0.09**	**0.13**	**0.09**	**0.15**	**0.15**	**0.13**	**0.11**	**0.03**
Total recovery	88.0	98.9	104.0	91.9	102.9	99.0	96.1	106.1	100.7	**98.6**	**5.8**

Conclusion
Under the experimental conditions, the study authors concluded that the mean total absorption (= amount present in the receptor fluid, receptor compartment wash and the skin, excluding tape strips) was 0.41 µg/cm² (0.89% of the applied dose) under non-oxidative conditions and 0.50 µg/cm² (0.11% of the applied dose) under oxidative conditions

Ref.: 24

SCCS comment
Too few chambers were used. The mean value + 2 standard deviations will be used for the calculation of the Margin of Safety: 0.57 µg/cm² (0.41 + 2 x 0.08) under non-oxidative conditions; 0.78 µg/cm² (0.50 + 2 x 0.14) under oxidative conditions.

3.3.5. Repeated dose toxicity

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

No data submitted

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

Taken from SCCNFP/0677/06

High dose study

Guideline:	OECD 408 (1981)
Species/strain:	Sprague Dawley rat, Crl:CD (SD)
Group Size:	10 males + 10 females
Test material:	Disperse Red 17 dispersed in purified water
Batch:	928017/02 (dye content: 41.2%)
Dose:	0, 100, 200 and 400 mg/kg bw/day
Exposure period:	13 weeks
GLP:	in compliance
Study period:	November 1995 – February 1996

Groups of 10 male and 10 female rats were dosed with the test substance by gavage at 100, 200 and 400 mg/kg bw/day, 7 days a week for 13 weeks. The dosing solutions were analysed during weeks 1, 12 and 13 for stability and verification of homogeneity and concentration. During the study, the animals were observed daily for clinical signs and mortality, and weekly for body weight and food consumption. During week 13, urine was collected overnight for urinalysis and blood was sampled from the lateral tail vein for haematology and blood biochemistry. At the end of the treatment period a full autopsy was conducted with recording of weights and macroscopic and microscopic examination of major organs. Ophthalmoscopy was conducted before the start of the study and at the end of the treatment period on control and high dose animals.

Results

The mean concentration of Disperse Red 17 in all formulations was found to be within 10% nominal concentration.

Two female animals treated with 100 mg/kg bw/day were killed *in extremis* on days 57 and 88 respectively. The reported clinical signs in these two animals were not seen in the group treated with 400mg/kg bw/day and thus the findings were not treatment related. One high dose female rat was found dead on day 9 and the only clinical signs prior to death were hair loss and pink coloration of the skin. The animal had been cannibalised and it was not possible to ascertain the cause of death but as no further deaths were reported it was considered unlikely to be due to toxicity of the test substance.

Staining of the fur in all treatment groups was considered to be attributable to the property of the compound and of no toxicological significance. Hair loss was reported in all treatment groups throughout the study, particularly those treated with 400mg/kg bw/day. The body weight gain of males given 400mg/kg bw/day was reduced slightly but not significantly (bodyweight 91% of control at termination). Bodyweights of other groups and food consumption of all groups were not affected. There were no treatment-related ocular findings or abnormalities. There were significant decreases in red blood cell counts, haemoglobin concentration and packed cell volume in all treated groups of both sexes, showing a dose-related trend. Increases were apparent in white cell counts and clearly dose-related in reticulocytes of all treated animals.

Alanine aminotransferase and aspartate aminotransferase were increased in both sexes dosed at 400mg/kg bw/day. Cholesterol levels were significantly higher in all treated female groups, but only the high dose group included individual values above the historical control range. Bilirubin levels were significantly increased at 400 and 200 mg/kg bw/day in males and at all doses in females. Calcium and inorganic phosphorus also showed dose-related increases in both sexes with high dose group mean values above the historical control range. Interpretation of urinalysis was made difficult by the strong coloration of the compound.

Absolute and relative spleen weights were statistically increased in a dose-related manner in all dose groups of both sexes (absolute weight: 148, 176 and 223% in males; 125, 149 and

185% in females; relative weight: 139, 168 and 233% in males; 127, 144 and 191% in females. There were also statistically significant increases in absolute and relative liver weights (up to 129% in males and 137% in females) of mid and dose groups of both sexes; absolute liver weights were also increased in the low dose males, as well as absolute and relative thyroid weights (up to 137%), with less clear dose-response relationships). Kidney weights were also increased in high dose males and ovary weights were increased in mid and high dose females. Histopathology revealed dose-related incidence and severity of haemosiderin deposits in the spleens of all female dose groups and mid and high male groups, corresponding to the changes in spleen weight and haematological parameters. Centrilobular hepatocyte hypertrophy was apparent in the livers of mid and high dose animals, with a greater incidence in the high dose and consistent with the liver weights and increases in ALT and AST.

Ref.: 25

SCCS comment

The significance of the increased incidence of hair loss in all dose groups is unclear. Since compound related effects were noted in all dose groups, a NOAEL could not be established from this study.

Low dose study

Guideline:	OECD 408 (1981)
Species/strain:	Sprague Dawley rat, Crl:CD (SD) BR
Group Size:	10 males + 10 females
Test material:	Disperse Red 17 formulated in purified water
Batch:	928017/02 (dye content: 41.2%)
Dose:	0, 10 and 30 mg/kg bw/day
Exposure period:	13 weeks
GLP:	in compliance
Study period:	14 May 1997 – 14 august 1997

Groups of 10 male and 10 female rats were dosed with the test substance by gavage at 10 and 30 mg/kg bw/day, 7 days a week for 13 weeks. The dosing solutions were analysed during weeks 1, 4, 8 and 13 for stability and verification of homogeneity and concentration.
During the study, the animals were observed daily for clinical signs and mortality, and weekly for body weight and food consumption. During week 13, urine was collected overnight for urinalysis and blood was sampled from the lateral tail vein for haematology and blood biochemistry. At the end of the treatment period a full autopsy was conducted with recording of weights and macroscopic and microscopic examination of major organs. Ophthalmoscopy was conducted before the start of the study and at the end of the treatment period on control and high dose animals.

Results
The mean concentration of Disperse Red 17 in all formulations was found to be within 8% nominal concentration.
Two male animals treated with 30mg/kg bw/day were found dead on days 14 and 43 respectively. The deaths were reported to be due to misdosing or regurgitation of the test compound causing respiratory failure. There were no treatment- related deaths or clinical signs of toxicity. Staining of the fur in all treatment groups was considered to be attributable to the property of the compound and of no toxicological significance. Hair loss was reported in control and treatment groups throughout the study. Body weight gain and food consumption were comparable in all groups. There were no treatment related ocular findings or abnormalities.
Minor changes in haematological and biochemical parameters were within or close to the normal range and not considered to be consistent with treatment-related effects. There were no differences in urinary parameters between control and treated groups of either sex. Increases in spleen weights were apparent in both sexes dosed with 30mg/kg bw/day (by

12-16%, but only statistically significant for the relative weight in males). As the differences were minimal and correlated with no relevant histopathological finding and/or abnormalities in red cell parameters, the authors concluded that the change was of no toxicological significance.
Nothing was mentioned on pigmentation in females, even though a macroscopic and microscopic examination of the spleen was carried out.
Thyroid weights were increased in females at 30 mg/kg bw/day (absolute weight: 125%, relative weight: 132%) and decreased in males at both dose levels. As these differences in thyroid weights were not associated with relevant histopathological findings, they were considered not to be related to treatment. Other minor differences were noted in organ weights but were not considered to be treatment-related. All microscopic findings were considered to be within the normal range for the strain and age of rat and were similar in control and treated animals and therefore of no toxicological importance.

Conclusion
The authors concluded that the dose of 30 mg/kg bw/day was a No Observed Adverse Effect Level.

Ref.: 26

SCCS comment
Because of the changes in spleen weights observed at 30 mg/kg bw/day, which were consistent with the effects at higher dose levels, a NOAEL of 10 mg/kg bw/day (or 4 mg/kg bw/day pure dye) was concluded by the SCCP.

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 Reverse Mutation Test

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA 1535 and TA 1537,
Replicates:	triplicates in one experiment
Test substance:	Disperse Red 17 (WR 18044)
Solvent:	Deionised water
Batch:	40T60N4520 (dye content: 31.0% by NMR)
Purity:	84.5% area at 254 nm (HPLC) and 97.1% area at 510 nm (HPLC)
Concentrations:	3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix
Treatment:	direct plate incorporation method with 48 incubation, without and with metabolic activation
GLP:	In compliance
Study period:	14 February – 17 February 2005

Disperse Red 17 was investigated for the induction of gene mutations in strains of *Salmonella typhimurium* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as an exogenous metabolic activation system. Test concentrations were based on the results of a pre-experiment for toxicity and mutation induction with all strains used 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 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 all strains used, the pre-experiment is reported as experiment I. The experiment was performed with the direct plate incorporation method. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment no toxic effects were observed at any concentration. Therefore 5000 µg/plate was chosen as top concentration.
A substantial and concentration-dependent increase in the number of revertants was observed in strain TA98 and TA100 with and without metabolic activation. A biologically relevant increase in the number of revertants was not observed in TA1535, TA1537 and TA102.

Conclusion
Under the experimental conditions used, Disperse Red 17 was mutagenic in this gene mutation tests in bacteria in strains TA98 and TA100.

Ref.: 27

SCCS comment
Since a clear positive result in strains TA98 and TA100 was observed, it was not considered necessary to perform a repeat experiment under modified conditions.

***In vitro* Gene Mutation Assay (*hprt*-locus)**

Guideline:	OECD 476 (1997)	
Species/strain:	Chinese Hamster V79 Cells	
Replicates:	Duplicate cultures in two independent experiments	
Test substance:	Disperse Red 17 (WR 18044)	
Solvent:	DMSO	
Batch:	40T60N4520	
Purity:	84.5% area at 254 nm (HPLC) and 97.1% area at 510 nm (HPLC)	
Concentrations:	Experiment I:	120.9, 241.7, 483.5, 725.3 and 1088 µg/ml without S9-mix 241.7, 483.5, 725.3, 1088 and 1632 µg/ml with S9-mix
	Experiment II:	17, 34, 68, 136, 153 and 204 µg/ml without S9-mix
Treatment	Experiment I:	4 h treatment without and with S9-mix; expression period 7 days and selection period of 8 days
	Experiment II:	24 h treatment without S9-mix: expression period 7 days and selection period of 8 days
GLP:	In compliance	
Study period:	3 May – 30 June 2005	

Disperse Red 17 was tested for gene mutations at the *hprt* locus in Chinese hamster V79 cells both in the absence and presence of S9 metabolic activation. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as an exogenous metabolic activation system. A pre-test for toxicity was performed to determine the concentration range for the mutagenicity experiments measuring the colony-forming ability using 8 concentrations between 12.8 and 1632 µg/ml.
In the main tests, cells were treated for 4h without and with S9-mix or for 20h without S9-mix followed by an expression period of 7 days to fix the DNA damage into stable *hprt* mutations. Negative and positive controls were included.

Results
The data on cloning efficiency after the selection period did not indicate strong cytotoxicity. Without metabolic activation, the reduction in the cloning efficiency was more than 50%, whereas with metabolic activation no toxic effects occurred up to the highest concentration.

In experiment I, precipitation was observed at 483.5 µg/ml and above without metabolic activation and at 725.3 µg/ml and above with metabolic activation. In experiment II, precipitation was observed at 136 µg/ml and above.
No biological relevant increases of the mutant frequency were observed in both experiments with and without metabolic activation. Some single cultures exceeded the mutant frequency of the corresponding control. However, the increases were not dose dependent, did not occur in the duplicate cultures and they fell within the historical control range of the negative and solvent controls. Therefore, these isolated effects were considered not biologically relevant.

Conclusion
Under the test conditions used, Disperse Red 17 did not induce gene mutations at the *hprt* locus in V79 cells and is not considered mutagenic in this assay.

Ref.: 28

In vitro **micronucleus test**

Guideline:	Draft OECD 487	
Species/strain:	Human lymphocytes from two healthy non-smoking male donors.	
Replicates:	Duplicate cultures in 2 independent experiments	
Test substance:	Disperse Red 17 (WR 18044)	
Batch no:	40T60N4520 (dye content: 31.0% by NMR)	
Purity:	84.5% area at 254 nm (HPLC) and 97.1% area at 510 nm (HPLC)	
Solvent:	Sterile water	
Concentrations:	Experiment 1:	20, 30 and 40 µg/ml without S9-mix 80, 120 and 190 µg/ml with S9-mix
	Experiment 2:	20, 40 and 100 µg/ml without S9-mix 100, 150 and 250 µg/ml with S9-mix
Treatment:	Experiment 1:	24 h PHA, 20 h treatment and 28 h recovery without S9-mix. 24 h PHA, 3 h treatment and 45 h recovery with S9-mix.
	Experiment 2:	48 h PHA, 20 h treatment and 28 h recovery without S9-mix. 48 h PHA, 3 h treatment and 45 h recovery with S9-mix.
GLP:	In compliance	
Study period:	17 December 2004 – 7 March 2005	

Disperse Red 17 has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in cultured human peripheral blood lymphocytes. The dye content of the batch was 33.9% by NMR. Liver S9 fraction from Aroclor 1254-induced rats was used as an exogenous metabolic activation system. Both in the cytotoxicity range-finder and the micronucleus test, treatment of lymphocytes commenced approximately 24 or 48 h after mitogen stimulation by phytohaemagglutinin. Test concentrations were based on the results of a cytotoxicity range-finder; cultures of human peripheral blood lymphocytes were treated with a range of 10 increasing concentrations of Disperse Red 17 up to 600 µg/ml.
Cells were treated for 3 h both in the presence of S9-mix or 20 h in the absence of S9-mix; cells were harvested 48 h after the beginning of treatment. The final 28 h of incubation was in the presence of cytochalasin B (final concentration 6 µg/ml).
Toxicity was determined by measuring the reduction in replication index (RI). The top concentration for analysis was to be the one with approximately 50-60% reduction in RI. The lower concentrations were chosen as such so that a range from maximum to little or none cytotoxicity is covered. Micronucleus preparations were stained with Giemsa and examined microscopically for RI and micronuclei. Negative and positive controls were included.

Results
In experiment 1, without and with metabolic activation, the reduction in the replication index was 65% and 52%, respectively. In experiment 2, without and with metabolic activation, the reduction in the replication index was 63 and 57% respectively.
In both experiments a biologically relevant increase in the number of binuclear cells with micronuclei was not observed as compared to the vehicle control. In experiment 1 without S9-mix, the lowest (20 µg/ml) and the highest concentration (40 µg/ml) were statistically significantly different from the control. However, at 40 µg/ml only one replicate marginally exceeded the 95% reference range of the historical control data set; at 20 µg/ml none of the replicates exceeded the historical control data set. In experiment 2 with metabolic activation the intermediate dose (150 µg/ml) was statistically significant from the control. Again, only one replicate marginally exceeded the 95% reference range of the historical control data set. Consequently, these isolated statistically significant increases are not considered to be of biological importance.

Conclusion
Under the experimental conditions used, Disperse Red 17 was not genotoxic (clastogenic and/or aneugenic) in human lymphocytes *in vitro*.

Ref.: 29

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte micronucleus test

Guideline:	OECD 474 (1997)
Species/strain:	male NMRI mice
Group size:	5 male mice/group
Test substance:	Disperse Red 17 (WR 18044)
Batch no:	40T60N4520 (dye content: 31.0%)
Purity:	84.5% area at 254 nm (by HPLC) and 97.1% area at 510 nm (by HPLC)
Dose level:	0, 437.5, 875 and 1750 mg/kg bw
Route:	oral administration
Vehicle:	PEG 400
Sacrifice times:	24 h and 48 h (the highest dose only) after start of the treatment
GLP:	In compliance
Study period:	26 April – 17 June 2005

Disperse Red 17 has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based in a pre-experiment on acute toxicity measured at various intervals of 1, 2-4, 6, 24, 30 and 48 h after treatment with the 100, 500, 1500, 1750 and 2000 mg/kg body weight. In the main experiment male mice were exposed orally to 0, 437.5, 875 and 1750 mg/kg bw. Bone marrow cells were collected 24 h or 48 h (high dose only) after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE). The mice of the high dose group were examined for acute toxic symptoms at intervals of around 1, 2-4, 6 and 24 h after treatment. Bone marrow preparations were stained with May-Grünwald/Giemsa and examined microscopically for the PCE/TE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
In a pre-experiment on acute toxicity with exposures of 1000, 1500 and 2000 mg/kg bw Disperse Red 17 induced reduction of spontaneous activity, ruffled fur and at the highest dose abdominal position, apathy and eyelid closure between 24 and 48 h, whereas 1 male mouse died at 2000 mg/kg bw. Exposures with 100 and 500 mg/kg bw did not result in clinical signs. All treated mice had reddish to dark red urine.

In the main experiment, the mice treated with 875 and 1750 mg/kg body weight showed reduction of spontaneous activity, ruffled fur after 24 h and one mouse died after 24 h in the highest treatment group. Mice treated with 437.5 mg/kg body weight did not show toxic reactions. All treated mice again had discoloured urine after 6 h.
After treatment with the highest dose the ratio PCE/TE decreased compared to the control, indicating that Disperse Red 17 had reached the target organ. Moreover, the coloured urine also indicated to systemic availability of Disperse Red 17.
After 24h treatment, a biologically relevant increase in the number of cells with micronuclei compared to the control group was not observed. After 48h treatment, the increase in the number of cells with micronuclei was statistically significantly increased compared to the control group. However, the micronuclei frequency observed at the group level and at individual animal level fell within the range of historical control data. Therefore, the increase is not considered of biological importance.

Conclusion
Under the test conditions used Disperse Red 17 did not induce micronuclei in the bone marrow cells of mice.

Ref.: 30

In Vivo Unscheduled DNA Synthesis (UDS) Test

Guideline:	OECD 486 (1997)
Species/strain:	rat, Wistar HsdCpb: WU (SPF)
Group size:	4 male rats per test group
Test substance:	Sedona Red, WR18044
Batch no:	40T60N4520 (dye content: 31.0%)
Purity:	84.5% area at 254 nm (by HPLC) and 97.1% area at 510 nm (by HPLC)
Vehicle:	deionised water
Dose level:	0, 1000 and 2000 mg/kg bw
Route:	oral
Sacrifice times:	4 h and 16 h after dosing
GLP:	In compliance
Study period:	3 February – 16 March 2009

Sedona Red was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test concentrations were based on a pre-experiment for toxicity, measuring acute toxic symptoms at 1, 2-4, 6 and 24 h after oral administration of 422.5, 845 and 2000 mg/kg bw. In the main experiment rats were treated orally with 0, 1000 and 2000 mg/kg bw and were examined for toxic symptoms at 1, 2 and 4 h (4 h treatment) or at 1, 2-4 and 16 h (16 h treatment) after treatment.
Hepatocytes for UDS analysis were collected by perfusion with 0.05% w/v collagenase approximately 4 and 16 h after administration of Sedona Red. The quality of the hepatocytes after perfusion was determined by the trypan blue dye exclusion method. At least three cultures were established for each animal. At least 90 minutes after plating, the cells were incubated for 4 h with 5 µCi/ml ^{3}H-thymidine (specific activity 20 Ci/mmol) followed by overnight incubation with unlabelled thymidine. Evaluation of autoradiography was done after 14 days.
The nuclear and cytoplasmic grain counts, the net grains counts (nuclear minus cytoplasmic grains) as well as the mean and percentage of cells in repair (cells with a net grain count >5) were reported. Increased net grain counts should be based on enhanced nuclear grain counts rather than on decreased cytoplasmic grain counts. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment for toxicity at 1000 and 2000 mg/kg bw all rats survived. All 845 and 2000 mg/kg bw and most rats treated with 422.5 mg/kg bw showed reduction of

spontaneous activity and ruffled fur. For all treated mice red, coloured urine was reported from 6 h after treatment indicating systemic distribution and thus bioavailability of Sedona Red. For the main experiment 2000 mg/kg bw was chosen as top dose.
In the main test, the rats demonstrated the same toxic reactions, reduction of spontaneous activity and ruffled fur, and had red coloured urine as well.
The viability of the hepatocytes determined by means of the trypan blue dye exclusion assay was not substantially affected by the treatment with Sedona Red at any of the treatment periods or dose groups.
A biological relevant increase in UDS induction as compared to the untreated control was not found in hepatocytes of any treated animal both for the 4 h and the 16 h treatment time. No substantial shift to higher values was obtained in the percentage of cells in repair.

Conclusions
Under the experimental conditions used, Sedona Red did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in this *in vivo* UDS test.

Ref.: 1 (Subm. IV)

3.3.7. Carcinogenicity

Comment on reference 31:
From Submission III: "This bioassay is only available as a summary report and has several limitations with regard to reporting and experimental design. The study is not in line with relevant guidelines. Disperse Red 17 could not be clearly identified under the test items listed in the available parts of the report. Furthermore, neither quality data nor precise concentrations of Disperse Red 17 used in the study were given."

No information concerning the possible carcinogenic potential of Disperse Red 17 in humans can be obtained from this study.

3.3.8. Reproductive toxicity

3.3.8.1. One generation reproduction toxicity

Taken from SCCP/1161/08

Guideline: OECD 415
Species/strain: Sprague Dawley HSD:SD (SPF)
Group size: 28 per sex and dose
Test substance: Disperse red 17
Batch: 40T60N4520 (dye content: 31%)
Purity: 97.1 %
Dose: 0, 10, 30 and 200 mg/kg bw/day
Route: oral, gavage
Exposure: 10 weeks (males) and 2 weeks (females) prior to mating
GLP: in compliance
Study period: 5 October 2004 – 15 February 2005

Groups of 28 male and 28 female Sprague Dawley rats received Disperse Red 17 orally once daily at dose levels of 0, 10, 30 or 200 mg/kg body weight for a period of 10 weeks (males) and 2 weeks (females) prior to mating. Dosing of males was continued during the whole mating period until sacrifice (approx. week 11 - 13 of the study), and treatment of mated females was continued during gestation and parturition until day 21 of lactation. Animals were observed daily for mortality/morbidity and clinical signs were checked once daily. Body weights and food consumption were recorded throughout the study. During necropsy, the

animals were examined for macroscopically visible abnormalities with special emphasis on reproductive organs. Sexual organs, pituitary glands, brain, liver, spleen, kidneys and gross findings were preserved and processed for microscopic examination. The dams were allowed to litter and rear their progeny to the stage of weaning. Growth, development and behaviour of the offspring were assessed during lactation until scheduled necropsy. All pups were examined for external abnormalities.

Results
There were no unscheduled deaths which could be related to the administration of the test item. One female of dose group 3 killed on day 59 exhibited a massive follicular atrophy of the spleen and extensive enteritis. Males at 30 mg/kg bw/day (mid dose) exhibited a reddish staining of the fur from day 46 onwards. All males and females at 200 mg/kg bw/day exhibited red stained fur and urine from day 14 and 11, respectively, onwards. Body weight gain at 200 mg/kg bw/day was initially decreased (1st week only) for males and moderately decreased for females (-12 to -24%) throughout the pre-mating, gestation and lactation periods as compared to the control. Food consumption was not affected in any group. Dams at 200 mg/kg bw/day exhibited slightly lower numbers of implantations and slightly lower numbers of live pups/litter. Birth indices were not affected. Necropsy confirmed that the test item reached the adipose tissue and/or the body surface from 10 mg/kg bw/day upwards. Extension and severity of this discolouration was dose dependent. At 10 mg/kg bw/day, only the adipose tissue of most males and females showed orange discolouration. At the high dose, some animals exhibited large spleens which were dark brown.
The mammary glands cryoblocks were slightly orange discoloured in the high dose females. A very slight to slight erythroid hyperplasia was noted in animals dosed with 200 mg/kg bw/day with higher incidence than in animals of the control and intermediate dose groups. Additionally, a pigmentation of the splenic red pulp (either haemosiderin and/or stored test item) could be observed in nearly all animals at 200 mg/kg bw/day and in the females at 30 mg/kg bw/day.
In contrast to the slightly discoloured cryoblocks in high dose females, the 16 µm cryocuts of the mammary glands of the high dose group did not show any signs of discolouration or pigment incorporation/deposit in the cells. It is concluded that the test item did not penetrate into the glandular cells of the mammary gland. There were no effects on live offspring during lactation, including viability index, weaning index, and survival rate in any treated group as compared to the controls. Mean pup body weight at birth appeared to be slightly decreased at 200 mg/kg bw/day as compared to the control, in particular in males. Slightly lower body weight was also noted on lactation days 14 and 21. No behavioural or other physical abnormalities were observed in any dose group. Unlike their high dose parents, the suckling pups were not discoloured at necropsy, nor were there any macroscopic observations.

Conclusion
The NOAEL for developmental toxicity was set at 30 mg/kg bw/day. Because of the pigmentation of the splenic red pulp in the females at 30 mg/kg bw/day, the NOAEL for maternal toxicity was set at 10 mg/kg bw/day (or 3.1 mg/kg bw/day pure dye).

Ref.: 32

3.3.8.2. Teratogenicity

Taken from SCCNFP/0677/06

Guideline:	OECD 414 (1981)
Species/strain:	Sprague-Dawley rat, Crl : CD (SD) BR
Group size:	24 females (mated)
Test material:	Disperse Red 17 dispersed in water
Batch:	928017/02 (dye content: 41.2%)

Dose Levels: 0, 125, 250 and 500 mg/kg bw/day
Treatment Period: Days 6-15 of pregnancy, inclusive
GLP: in compliance
Study period: 8 January – 1 February 1996

Groups of 24 female rats were dosed with the test substance by gavage on days 6 to 15 after mating. The dose volume was 10ml/kg bw/day. The control group received the vehicle alone. The dams were observed daily for clinical signs and mortality, and for body weight (days 0, 6-15 and 20) and food consumption (days 0, 6, 9, 12, 15 and 20). They were sacrificed on day 20 of pregnancy, and examined for number of corpora lutea, number and distribution of live and dead foetuses, of early or late resorptions and of implantation sites, macroscopic external observations, and for skeletal and visceral abnormalities (half for each end point). The concentrations, homogeneity and stability of the dosing formulations were checked analytically.

Results
The dosing formulations for the 250 mg/kg bw/day group were measured to be 14-15% below nominal concentration during the second week of dosing. All other formulations were within 5% of nominal concentration.
Treatment related clinical signs were limited to red/pink staining of the fur, tail, extremities and excreta. No deaths or abortions occurred at any dose level. Food consumption and body weight gain were reduced in all treated groups, in a dose-related manner. The mean body weight of high dose animals was 92% of control at the end of the dosing period. At autopsy, staining of the fur, skin, body fat and mammary tissue were observed. No other treatment-related abnormalities were seen.
The mean numbers of corpora lutea, implantation sites, post-implantation loss, live foetuses and foetal body weights were similar for control and treated groups. A small number of foetal malformations were observed which were within the normal range, and treated groups did not differ significantly from control.

Conclusion
The test substance elicited maternal toxicity at the dose levels tested but was not embryotoxic or teratogenic. The NOAEL for maternal toxicity was less than 125 mg/kg bw/day.
The observation of accumulation of the test substance in mammary tissue raises concern with respect to potential effects on the offspring during lactation.

Ref.: 33

3.3.9. Toxicokinetics (ADME)

No data submitted

3.3.10. Photo-induced toxicity

No data submitted

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

/

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

/

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

(Disperse Red 17)
(Oxidative / permanent)

Maximum absorption through the skin	**A (µg/cm²) µg/cm²**	**=**	**0.78**
Skin Area surface	**SAS (cm²)**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.452 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60 mg/kg**	**=**	**0.0075**
No observed adverse effect level (one generation study, maternal toxicity, rat)	**NOAEL**	**=**	**3.1(mg/kg bw/day)***
Bioavailability 50%**	**adjusted NOAEL**	**=**	**1.55 mg/kg bw/day)**

Margin of Safety	**adjusted NOAEL / SED**	**=**	**205**

* corrected for dye content (31% dye)
** standard procedure according to the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation.

(Disperse Red 17)
(Non-oxidative / semi-permanent)

Maximum absorption through the skin	**A (µg/cm²) µg/cm²**	**=**	**0.57**
Skin Area surface	**SAS (cm²)**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.3306 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60 mg/kg**	**=**	**0.0055**
No observed adverse effect level (one generation study, maternal toxicity, rat)	**NOAEL**	**=**	**3.1 (mg/kg bw/day)**
Bioavailability 50%	**adjusted NOAEL**	**=**	**1.55 mg/kg bw/day)**

Margin of Safety	**adjusted NOAEL / SED**	**=**	**281**

3.3.14. Discussion

Physico-chemical properties
Disperse Red 17 is used as a non-reactive hair colouring agent (direct dye) in oxidative hair dye formulations at a maximum on-head concentration of 2 % inclusive of dispersant; and it is used as a non-reactive hair colouring agent (direct dye) in semi-permanent hair dye formulations at a maximum on-head concentration of 0.2 %, inclusive of dispersant.

2-[3-Methyl-4-(4-nitro-phenylazo)-phenylamine]ethanol) impurity (up to 2%) is a secondary amine and N-(2-hydroxyethoxyethyl),N-(2-hydroxyethyl)-3-methyl-4-((4-nitrophenyl)azo)-anilin impurity (up to 1.5%) is a tertiary amine. These impurities are prone to nitrosation and may generate nitrosamines. Nitrosamine content in Disperse 17 is not known. The dye should not be used in combination with nitrosating agents. The nitrosamine content should be < 50ppb.

The stability of Disperse Red 17 in the marketed products is not reported.

General toxicity
Disperse Red 17 demonstrated a low acute toxicity potential with LD50 values above 2000 mg/kg bw in rats.
On subchronic exposure, changes in spleen weights were observed at 30 mg/kg bw/day, which were consistent with the histopathology findings, i.e. dose-related incidence and severity of haemosiderin deposits in the spleen. A NOAEL of 10 mg/kg bw/day (or 4 mg/kg bw pure dye) is concluded.
The NOAEL for developmental toxicity was set at 30 mg/kg bw/day. Because of the pigmentation of the splenic red pulp in the females at 30 mg/kg bw/day, the NOAEL for maternal toxicity was set at 10 mg/kg bw/day (or 3.1 mg/kg bw/day pure dye). This NOAEL will be used for MoS calculation.

Irritation / sensitisation
No signs of irritation were noted on the skin. Red/orange staining was reported at all-time points.
A 3% test concentration caused only very slight short lasting conjunctival irritation of the rabbit eye. No alteration of the cornea was observed macroscopically. The pure test substance was considered to be slightly irritating to the rabbit eye.
Magnusson & Kligman study: excessive staining due to the test substance made assessment "difficult" in 6/10 animals and therefore the study should be considered as equivocal.
Peripheral blood monocyte derived dendritic cells study: as the test item modulated only two out of four endpoints selected as DC activation markers, and thus did not fulfil the criteria for a positive DC activation response, the study authors concluded that Disperse Red 17 was not a sensitiser. This test is, however, not validated.

Dermal absorption
In the *in vitro* dermal absorption study, too few chambers were used. The mean value + 2 standard deviations will be used for the calculation of the Margin of Safety: 0.57 µg/cm² (0.41 + 2 x 0.08) under non-oxidative conditions; 0.78 µg/cm² (0.50 + 2 x 0.14) under oxidative conditions.

Mutagenicity / genotoxicity
Overall, Disperse Red 17 has been tested for gene mutations and structural and numerical chromosomal aberrations. Disperse Red 17 induced gene mutations in bacteria but did not induce gene mutations in Chinese hamster V79 cells at the *hprt* locus. Disperse Red 17 did not induce structural or numerical chromosome aberrations in an *in vitro* micronucleus assay on cultured human peripheral blood lymphocytes.
In an *in vivo* micronucleus test, an increase in bone marrow cells with micronuclei was not seen indicating that Disperse Red 17 is not clastogenic nor aneugenic in this test.
The positive *in vitro* results found in the gene mutation test in bacteria were not confirmed in an *in vivo* unscheduled DNA synthesis test in rats and, consequently, Disperse Red 17 can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Carcinogenicity

One skin painting study is available. No information concerning the possible carcinogenic potential of Disperse Red 17 in humans can be obtained from this study.

4. CONCLUSION

The SCCS considers that Disperse Red 17 as an ingredient at 0.2% in non-oxidative hair dye formulations and at 2% in oxidative hair dye formulations is safe for the use by consumers.
The nitrosamine content in Disperse Red 17 should be < 50ppb, and it should not be used in the presence of nitrosating agents.
A sensitising potential of Disperse Red 17 cannot be excluded.
This safety assessment of Disperse Red 17 is based on the specification for use as a hair dye ingredient as described in Section 3.1.4.

5. MINORITY OPINION

Not applicable

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