Scientific Committee on Consumer Products

SCCP

OPINION ON

Disperse Red 17

COLIPA n° B5

The SCCP adopted this opinion at its 18th plenary of 16 December 2008

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

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

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

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

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Opinion to be cited as: SCCP (Scientific Committee on Consumer Products), Opinion on Disperse Red 17, 16 December 2008

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1. BACKGROUND

Submission I for Disperse Red 17, with the chemical name 1-(4'-Nitrophenylazo)-2-methyl-4-bis-(β-hydroxyethyl)aminobenzene, was submitted in December 1998 by COLIPA[1].

Submission II was submitted in November 2001.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at the 24th plenary meeting of 24-25 June 2003 opinion SCCP/0677/03 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 data on physico-chemical properties and chemical characterisation of the test material.*
* *a developmental toxicity study including the weaning period.*
* *data on genotoxicity/mutagenicity following the relevant SCCNFP opinions and in accordance with the Notes of Guidance*".

According to the current submission III, submitted by COLIPA in July 2005, Disperse Red 17 it is used as a direct dye in oxidative and non-oxidative hair dye formulations.

Submission III presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider Disperse Red 17 safe for consumers, when used as an ingredient in non-oxidative hair dye formulations with a concentration on the scalp of maximum 0.2% taking into account the scientific data provided?*

2. *Does the SCCP 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*

[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

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-Hydroxy-ethyl)-[3-methyl-4-(nitro-phenylazo)-phenyl]-amino]-ethanol

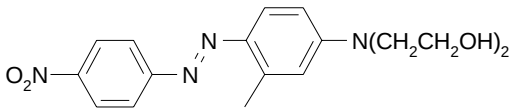
3.1.1.3. Trade names and abbreviations

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

3.1.1.4. CAS / EINECS number

CAS: 3179-89-3 (Disperse Red 17, INCI dictionary)
EINECS: 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

Description of sample	928017	40T60N4520
References of Analyses	A 2005/350 Submission I of B005 (October 1998)	A 2005/350 G 2004/009
NMR content / %, w/w	40.3	31.0
HPLC purity / area % 210 nm 254 nm 510 nm	 70.4 80.5 98.9	 74.6 84.5 97.1
HPLC content	39.1	32.3
Loss on drying / %, w/w	5.9	6.0
Water content / %, w/w	6.6	9.2
Sulphated ash / %, w/w	11.7	14.9
Sodium ligninsulfonate / %, w/w	54	57
p-Nitroaniline * / ppm	50	13
m-Tolyldiethanolamine / ppm	< 200 **	9
2-[3-Methyl-4-(4-nitro-phenylazo)-phenylamine]ethanol) / %, w/w	0.8	0.053
Heavy metals	< 10 ppm with the exception of Fe with 220 ppm	< 10 ppm

* classified by German MAK as carcinogenic category 3A
** below detection limit; indicated value is the detection limit of the used method

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 Po/w: (3.575, for Sedona Red purity 31.5%) EU Method A8

3.1.8. Additional physical and chemical specifications

pH-value:	3.5 (saturated aqueous solution commercial dye, 20°C)		*(Ref. 4)*
Melting point:	150-152 °C	(EU – A.1)	*(Ref. 6)*
Boiling point:	decomposition at 175°C	(EU – A.2)	*(Ref. 8)*
Relative density:	D_4^{20} = 1.0923 (20°C)	(EU - A.3)	*(Ref. 9)*
Vapour pressure:	2.8 exp – 6 hPa (20°C)	(EU - A.4)	*(Ref. 10)*
Viscosity:	/		
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%).

General Comments to physico-chemical characterisation

- Complete physico-chemical properties of Disperse Red 17, as required by SCCNFP opinion n° SCCNFP/0677/03 are not submitted. The physico-chemical properties reported are of Disperse Red 17 with different amounts of the dispersant sodium ligninsulfonate.
- The dye content (40.3% and 31.0%) described in the two batches of Disperse Red 17 + dispersant is significantly different from each other. However, no information is provided to ascertain maximum dye content in the marketed products.
- 0.8% 2-[3-Methyl-4-(4-nitro-phenylazo)-phenylamine]ethanol) impurity is a secondary amine.
- In the HPLC analysis of the dye with dispersant, 70-80% peak area at 210 nm and 254 nm of the dye indicates that some other peaks (impurities) should be present in the chromatogram. However, they are not reported.
- The stability of Disperse Red 17 in oxidative hair dye formulations has not been demonstrated.
- 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 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

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 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. The primary irritation index was reported to be 0.0.

Ref.: 17

3.3.2.2. Mucous membrane irritation

Newly submitted study

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 neantine (Diethylphthalate)
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 neantine (DEP) was instilled into the one eye of 6 rabbits. The untreated eye served as controls. 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

Taken from SCCNFP/0677/06

Guideline:	OECD 405 (1992)
Species/strain:	New Zealand albino rabbits
Group size:	3 females
Test material:	Disperse Red 17
Batch:	928017/02 (dye content: 41.2%)
Dose:	0.1g
Max Irrit Score:	3.3
GLP:	in compliance
Study period:	13 – 17 December 1994

0.1g of the neat substance was applied once to the right eye of each animal without rinsing. The left eye served as control. Ocular reactions were recorded at 1, 24, 48 and 72 hours after instillation. Evaluation of ocular irritation was calculated according to the modified Kay and Calandra scale.

Results
Some red/black staining apparent in the treated eye of all animals, which hindered some evaluations. Slight chemosis and hyperaemia were reported in the conjunctiva of two rabbits one hour after instillation. In the third rabbit heavy staining prevented evaluation but some discharge was evident. At 24 hours all rabbits were showing slight hyperaemia and there was no evidence of reaction 48 and 72 hours after instillation. No corneal reactions were reported. The Maximum Score of Ocular Irritation was calculated to be 3.3, 1 hour after instillation.
According to the defined criteria the pure test substance was classified as slightly irritant to the rabbit eye.

Ref.: 19

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 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 and the following day, 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

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

Comment
The test is not 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 cm²
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/cm²) oxidative: 13.1 mg (20 mg/cm²)
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 each on 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**

	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	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	405.4	455.6	478.9	423.1	473.7	455.9	442.3	488.7	463.3	**454.1**	**26.8**
Donor compartment wash	0.16	0.16	0.29	0.16	0.16	0.16	0.16	0.16	0.16	**0.17**	**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	0.18	0.31	0.24	0.26	0.43	0.25	0.55	0.54	0.43	**0.35**	**0.14**
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	**0.33**	**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	**455.1**	**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	87.9	98.8	103.8	91.7	102.7	98.8	95.9	105.9	100.4	**98.4**	**5.8**
Donor compartment wash	0.03	0.03	0.06	0.03	0.03	0.03	0.03	0.03	0.03	**0.04**	**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	0.04	0.07	0.05	0.06	0.09	0.06	0.12	0.12	0.09	**0.08**	**0.03**
Receptor compartment wash	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	**0.01**	**0.00**
Receptor fluid	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	**0.03**	**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**

	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**		
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

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.
It is not clear from the dossier whether the active dye or Disperse Red 17 was measured.

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, and absolute liver weights was also increases in the low dose males, and 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

Comment
The significance of the increased incidence of hairloss in the top dose group 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 weight 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

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 NOEL 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
Species/strain:	TA 1535, TA 1537, TA 98, TA 100 and TA 102
Replicates:	triplicates in one experiment
Test substance:	Disperse Red 17 (WR 18044) (COLIPA B5)
Solvent:	Deionised water
Batch:	40T60N4520 (dye content: 31.0%)
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
Treatment:	With and without Phenobarbital/β-Naphthoflavone induced S9-mix using the standard plate incorporation assay
GLP:	In compliance
Study period:	14 – 17 February 2005

COLIPA B5 was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test) with and without metabolic activation in one experiment. Eight concentrations were used between 3 and 5000 μg/plate. No toxic effects were observed at any concentration.

Results
A substantial and dose dependent related increase in revertants was observed with treatment in strain TA 98 with and without metabolic activation and in strain TA 100 without metabolic activation. In TA 100 with metabolic activation a weak but clear dose dependent increase was observed. In strains TA 1537, TA 1537 and TA 102 there were no signs of any increases in the number of revertants.

Conclusion
Under the test conditions used COLIPA B5 did induce base pair and frameshift mutations both with and without metabolic activation and is considered to be mutagenic in the *Salmonella typhimurium* assay.

Ref.: 27

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

Guideline:	OECD 476
Species/strain:	Chinese Hamster V79 Cells
Replicates:	Duplicate cultures in two independent experiments
Test substance:	Disperse Red 17 (WR 18044) (COLIPA B5)
Solvent:	DMSO
Batch:	A 019600501
Purity:	≥ 98%
Concentrations:	Experiment 1 without S9-mix: 120.9, 241.7, 483.5, 725.3 μg/ml Experiment 1 with S9-mix: 241.7, 483.5, 725.3, 1088 and 1632 μg/ml Experiment 2 without S9-mix: 17, 34, 68, 136 and 153 μg/ml
Treatment	Experiment 1 with and without Phenobarbital/β-Naphthoflavone induced S9-mix: 4 h treatment Experiment 2 without S9-mix: 24 h treatment
GLP:	In compliance
Study period:	3 May – 30 June 2005

COLIPA B5 was tested for gene mutations at the *hprt* locus in Chinese Hamster V79 Cells.
A pre-test for toxicity was performed to determine the concentration range for the mutagenicity experiments. The highest concentration used in the pre-test was chosen with regard to the solubility of the test item. Eight concentrations were used between 12.8 and 1632 μg/ml.

Results
In experiment 1, toxic effects in both cultures were observed at 241.7 μg/ml and above without metabolic activation, indicated by a reduction in the cloning efficiency to below 50%. With metabolic activation no toxic effects occurred up to the highest concentration. 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 2, toxic effects were observed at 34 μg/ml and above. Precipitation was observed at 136 μg/ml and above.

No reproducible increases of the mutant frequency were observed in both experiments with and without metabolic activation. Some single cultures exceeded three times the mutant frequency of the corresponding control. However, the increases were not dose dependent and did not occur in the duplicate cultures. The mutant frequency values of these increases were also within the historical control range.

Conclusion
Under the test conditions used COLIPA B5 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 the pooled blood of two male donors.
Replicates:	Duplicate cultures
Test substance:	Disperse Red 17 (WR 18044) (COLIPA B5)
Solvent:	Sterile water
Batch:	40T60N4520 (dye content: 31.0%)
Purity:	84.5% area at 254 nm (HPLC) and 97.1% area at 510 nm (HPLC)
Concentrations:	Experiment one without S9-mix: 20, 30 and 40 μg/ml Experiment one with S9-mix: 80, 120 and 190 μg/ml Experiment two without S9-mix: 20, 40 and 100 μg/ml Experiment two with S9-mix: 100, 150 and 250 μg/ml
Treatment:	Experiment one (24 h PHA): 20 h treatment and 28 h recovery without S9-mix. Experiment one (24 h PHA): 3 h treatment and 45 h recovery with Aroclor 1254 induced S9-mix Experiment two (48 h PHA): 20 h treatment and 28 h recovery without S9-mix. Experiment two (48 h PHA): 3 h treatment and 45 h recovery with Aroclor 1254 induced S9-mix.
GLP:	In compliance
Study period:	17 December 2004 – 7 March 2005

COLIPA B5 was tested in the *in vitro* micronuclei test to identify structural and/or numerical chromosome aberrations in cultured human peripheral blood lymphocytes. A preliminary cytotoxicity range-finding study was performed in ten concentrations between 6.047 and 600 μg/ml.

Results
In experiment 1, with and without metabolic activation, a reduction in the replication index was 52 and 65%, respectively. 52% is less than the target 60% cytotoxicity, a steep curve

of cytotoxicity was observed, so that the next concentration (200 μg/ml) induced 69% cytotoxicity. Because a big variation was observed between the two replicates at 200 μg/ml, it was decided that 190 μg/ml would be suitable for testing. In experiment 2, with and without metabolic activation, a reduction in the replication index was 57 and 63%, respectively.

There were no indications of dose dependent increases in frequencies of micronucleated binucleate cells compared to the vehicle control. In experiment 1 without S9-mix, the highest concentration (40 μg/ml) was statistically significant from the control. However, only one replicate marginally exceeded the 95% reference range of the historical control data set. In experiment 2 with metabolic activation the intermediate dose (150 μg/ml) was statistically significant from the control. However, only one replicate marginally exceeded the 95% reference range of the historical control data set. These isolated incidents are not considered to be of biological importance.

Conclusion
Under the conditions used COPLIPA B5 did not induce structural chromosome aberrations in cultured human peripheral blood lymphocytes.

Ref.: 29

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte micronucleus test

Guideline:	OECD 474
Species/strain:	NMRI mice
Group size:	5 males per dose
Test substance:	Disperse Red 17 (WR 18044) (COLIPA B5)
Lot 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:	24 h preparation interval: 437.5, 875 and 1750 mg/kg body weight 48 h preparation interval: 1750 mg/kg body weight
Route:	Oral administration
Vehicle:	PEG 400
Sacrifice times:	24 h and 48 h (only the highest dose) after application of the test substance
GLP:	In compliance
Study period:	26 April – 17 June 2005

COLIPA B5 was tested to assess the chromosomal damage in the bone marrow cells of mice.
A pre-experiment for toxicity was performed. Two mice per sex were treated orally with the test item and examined for acute toxic symptoms at intervals of 1 h, 2-4 h, 6 h, 24 h, 30 h and 48 h after administration of the following doses: 100, 500, 1500, 1750 and 2000 mg/kg body weight. Males were more sensitive towards the test item than females. The doses in the main experiment were based on the observations from this pre-experiment. At least 2000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei.

Results
The mice treated with 1750 mg/kg body weight showed toxic reactions after 24 and 48 h and one mouse died after 24 h. The mice treated with 875 mg/kg body weight showed toxic reactions after 24 h, whereas mice treated with 437.5 mg/kg body weight did not show toxic reactions but had discoloured urine after 6 h.

After treatment with the highest dose the number of PCEs decreased compared to the control, indicating that the test item had reached the target organ. After 24h treatment, the frequency of micronuclei was comparable to the control group. After 48h treatment, the

mean value of 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 COLIPA B5 did not induce micronuclei in the micronucleus test in the bone marrow cells of mice.

Ref.: 30

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

New study, submission III - 2005

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 to 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,

and for macroscopic observations. The foetuses were examined for bodyweight, sex and 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

No data submitted

3.3.10. Photo-induced toxicity

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
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; 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 dispersant.
The dye content described in the two batches of Disperse Red 17 + dispersant is significantly different from each other (40.3% and 31.0%).
Complete physico-chemical properties of Disperse Red 17, as required by SCCNFP opinion n° SCCNFP/0677/03 were not submitted. The reported physico-chemical properties are for Disperse Red 17 with different amounts of the dispersant sodium ligninsulfonate.
2-[3-Methyl-4-(4-nitro-phenylazo)-phenylamine]ethanol), an impurity in Disperse RED 17 at 0.8%, is a secondary amine.
In the HPLC analysis of the dye with dispersant, the 70-80% peak area at 210 nm and 254 nm indicates that some other peaks (impurities) should be present in the chromatogram. However, they were not reported.
The stability of Disperse Red 17 in the marketed products is not reported.
The stability of Disperse Red 17 in oxidative hair dye formulations has not been demonstrated.

General toxicity
Disperse red 17 demonstrated a low acute toxicity potential with LD_{50} 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 NOEL 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).

Irritation
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 irritant to the rabbit eye.

Sensitisation
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 chromosome or numerical aberrations in an *in vitro* micronucleus assay on cultured human peripheral blood lymphocytes or micronuclei in the bone marrow cells of mice.
Because of the clear positive result seen in the bacterial reverse mutation test, an additional *in vivo* test should be performed to exclude a gene mutation potential of Disperse Red 17.

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 SCCP is of the opinion that the safety of Disperse Red 17 can not be 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.

5. MINORITY OPINION

Not applicable

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