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

CONCERNING

DISPERSE RED 17

COLIPA n° B5

adopted by the SCCNFP during the 24th plenary meeting
of 24-25 June 2003

1. Terms of Reference

1.1 Context of the question

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

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is Disperse Red 17 safe for use in cosmetic products?

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

1.3 Statement on the toxicological evaluation

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

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

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

2. Toxicological Evaluation and Characterisation

The dossier of Disperse Red 17 has been presented in two separate submissions. The first submission included all required studies for the safety evaluation. Additional (new) data on percutaneous absorption were presented in the second submission.

According to the first submission, Disperse Red 17 is a mixture of 41.2% dye 1- (4'-Nitrophenylazo) -2-methyl-4-bis-(beta-hydroxyethyl) aminobenzene (CAS no. 3179-89-3) together with dispersing agents (sodium lignosulfonate). Neither the identity of the dispersants nor their content in the mixture were reported.

The second submission describes Disperse Red 17 as a mixture of 40 ± 2.5% dye 2,2'-[[3-methyl-4-[(4-nitrophenyl) azo] phenyl] imino] bisethanol and the dispersants sodium lignosulfonate (CAS no 8061-51-6) (% not reported) and sodium lignosulfonate sulfomethylated (CAS no 68512-34-5) (% not reported). This material is used for the percutaneous absorption study of the dye active principle taking into account its concentration in the mixture.
The SCCNFP considers that Disperse Red 17 is in fact a preparation containing the dye itself (40 ± 2.5%) plus two dispersing agents with unknown content.

Since the toxicological evaluation of this product is mainly related to the dye as the active principle, a clarification is needed about the actual concentration of the dye in the Test Material used for each toxicological study, in order to present a consistent Opinion on the request presented to the SCCNFP.

2.1. General

2.1.1. Primary name

Disperse Red 17 (INCI name)

2.1.2. Chemical names

Chemical name	:	1-(4'-Nitrophenylazo)-2-methyl-4-bis-(beta-hydroxyethyl)aminobenzene
Synonyms	:	Ethanol,2,2'-[[3-methyl-4-[(4-nitrophenyl)azo]phenyl]imino]bis
	:	2,2'-[[3-methyl-4-[(4-nitrophenyl)azo]phenyl]imino]bisethanol
	:	2-[(2-Hydroxy-ethyl)-[3-methyl-4-(nitro-phenylazo)-phenyl]-amino]-ethanol

2.1.3. Trade names and abbreviations

Trade name	:	Intraperse Red YNB Conc.(Crompton & Knowles)
Colour Index	:	CI 11210

2.1.4. CAS/EINECS no.

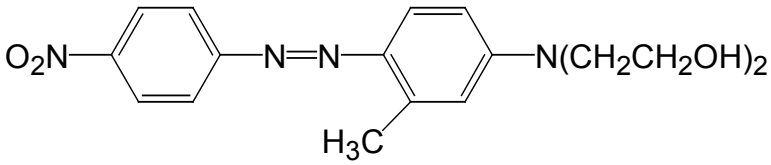
2,2'-[[3-methyl-4-[(4-nitrophenyl)azo]phenyl]imino]bisethanol (active dye)

CAS No.	:	3179-89-3
EINECS No.	:	221-665-5

Sodium Lignosulfonate (dispersant)
CAS No. : 8061-51-6
EINECS No. : /

Sodium Lignosulfonate Sulfomethylated (dispersant)
CAS No. : 68512-34-5
EINECS No. : /

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{17}H_{20}N_40_4$ (active dye)
Mol weight : 344 (active dye)

2.1.7. Purity, composition and substance codes

COLIPA B5 is a mixture of :

2,2'-[[3-methyl-4-[(4-nitrophenyl)azo]phenyl]imino]bisethanol : 40 ± 2.5 %
Sodium Lignosulfonate (dispersant) : % not reported
Sodium Lignosulfonate Sulfomethylated (dispersant) : % not reported
Water : < 6 % (deduced)

Purity
Titre as determined by HPLC (Batch 928017) : 41.2%
Water Content (Batch 928017) : 5.9%w/w
Ash Content (Batch 928017) : 11.7%w/w
Heavy Metals (Batch 928017) : <10ppm

Potential impurities and reaction intermediates (Batch 928017)
p-Nitroaniline : < 50ppm (detected)
m-Tolyldiethanolamine : < 200ppm (not detected)
2-[3-methyl-4-(4-nitro-phenylazo)-phenylamine]-ethanol (*synonym: Disperse dye, CI 11180)* : 0.8%
4-(nitro-phenyl)-o-tolyl-diazene : No authentic standard available

Solvent Residues (Batch 928017)
Methanol : <100ppm (detected)
Others (Loss on drying) : 5.9 %

2.1.8. Physical properties

Appearance	:	A black powder almost odourless
Melting point	:	/
Boiling point	:	/
Density (apparent)	:	400-600 kg/m^3
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	3.2 [calculated]
Storage	:	Protect from light and moisture

2.1.9. Solubility

Water	:	/
Ethanol	:	/
Water/ethanol (50:50)	:	soluble (no quantitative data)
Receptor fluid*	:	10.68 µg/ml at 32 °C

* 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

2.1.10. Stability

A loss of 8.6% dye in the test formulation used for percutaneous absorption was noted during the storage period of 1 month at 45°C followed by one month at room temperature. The degradation products of the dye have not been reported

General comments on analytical and physico-chemical characterisation

* Disperse Red 17 is not a single compound, but a mixture of a dye with other chemicals. Consequently the chemical name presented is not correct. All tests are performed using the dye preparation containing approximately 40% 2,2'-[[3-methyl-4-[(4-nitrophenyl)azo]phenyl]imino]bisethanol.

* The reported properties seem to refer to the mixture of active ingredient and dispersants. The individual properties of the active substance and dispersants remains unknown.

* The composition of the mixture is qualitative; the contents of dispersants are not reported, and an approximate water content < 6 % can be deduced from the loss on drying analysis (= 5.9%).

* Solubility information is inadequate; the reported "% insolubilities" are difficult to interpret; missing information on solubility of the individual components of the mixture are necessary to explain the insolubility in two solvents and solubility in their mixture.

* Information on impurities is inadequate, and do not include impurities of the dispersants; the impurity 2-[3-methyl-4-(4-nitro-phenylazo)-phenylamine]-ethanol (0.8%) is another disperse dye (CI 11180), which is a secondary amine.

* Purity of the chemical assessed reliably (HPLC) and reported for only one batch : it would be advisable to have an statement of the range of impurities that be may be present, based on the analysis of more than one batch.

* p-Nitroaniline (potential impurity in Disperse Red 17) is listed as a category 3 carcinogen in the German list of MAK and BAT values.

* The degradation products of the dye over 2 months storage have not been addressed..

2.2. Function and uses

Disperse Red 17 will be incorporated in semi-permanent hair dye formulations at a maximum concentration of 0.2% (of which only 40 % represents the active principle). It is common practice for 35 ml of the formulation to be applied for a period of 30minutes before washing. Application may be repeated at weekly intervals

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

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 no	:	928017/02
Dose	:	2000 mg/kg bw
Observ. Period	:	14 days
GLP	:	in compliance

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 100-2000 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 Limit-study one death was reported at 1000mg/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 in 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. : 1

2.3.2. Sub-chronic oral toxicity

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 no	:	928017/02
Dose	:	0, 100, 200 and 400 mg/kg bw/day
Exposure period	:	13 weeks
GLP	:	in compliance

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 100mg/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. A NOAEL could not be established from this study. The significance of the increased incidence of hairloss in the top dose group is unclear.

Ref. : 5.1

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 no	:	928017/02 (dye content : 41.2%)
Dose	:	0, 10 and 30 mg/kg bw/day
Exposure period	:	13 weeks
GLP	:	in compliance

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.
Thyroid weight were increased in females at 30mg/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. The authors concluded that the dose of 30mg/kg bw/day was a No Observed Adverse Effect Level.
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 is concluded by the SCCNFP.

Ref. : 5.2

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline	:	OECD 404 (1992), 92/69/EEC. Annex V
Species/strain	:	New Zealand albino rabbits
Group size	:	3 females
Test material	:	Disperse Red 17
Batch No	:	928017/02
Dose	:	0.5g
GLP	:	in compliance

The substance (0.5 g moistened with water) was applied to a 6.25cm^2 area of intact skin of 3 male 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. : 3

2.4.2. Irritation (mucous membranes)

Guideline	:	OECD 405 (1992); 92/69/EEC Annex V
Species/strain	:	New Zealand albino rabbits
Group size	:	3 females
Test material	:	Disperse Red 17
Batch No	:	928017/02
Dose	:	0.1g
Max Irrit Score	:	3.3
GLP	:	in compliance

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. : 2

2.5. Sensitisation

Magnusson and Kligman study

Guideline	:	OECD 406 (1992); 92/69/EEC Annex V	
Species/strain	:	Dunkin-Hartley guinea pigs	
Group size	:	10 test + 5 control, females	
Test material	:	Disperse Red 17 dispersed in water	
Batch No	:	928017/02	
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 (Day8)
		challenge :	2.5% test substance for 24 hours, occluded
GLP	:	in compliance	

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.
Excessive staining due to the test substance made assessment “difficult” in 6/10 animals and therefore the study should be considered as equivocal.

Ref. : 4

2.6. Teratogenicity

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 No	:	928017/02
Dose Levels	:	0, 125, 250 and 500 mg/kg bw/day
Treatment Period	:	Days 6-15 of pregnancy, inclusive
GLP	:	in compliance

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.
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 in mammary tissue raises concern with respect to potential effects on the offspring during lactation.

Ref. : 11

2.7. Toxicokinetics (incl. Percutaneous Absorption)

2.7.1. Percutaneous Absorption *in vitro*

Study 1

Guideline	:	/
Tissue	:	Human female breast skin
Method	:	Franz diffusion cell
Test material	:	Disperse Red 17, 0.2 % in formulation
Batch No	:	928017/02 (dye content : 41.2 %)
Dose Levels	:	circa 40 mg formulation in the presence/absence of 10 mg of bleached hair
Replicate cells	:	8 with and without hair
GLP	:	not in compliance

The skin penetration of Disperse Red 17 was evaluated in a static Franz diffusion cell system, using human epidermis prepared by heat-separation from previously frozen breast skin. The test substance was prepared at a concentration of 0.2% in a formulation. Approximately 40 mg of the mixture was applied to 2 cm^2 of epidermal membrane with and without addition of 10 mg finely chopped bleached hair for 30 minutes and then excess washed off with 2% sodium lauryl sulphate solution and dried. Four and 24 hours later the levels of substance were measured in the receptor fluid (physiological saline) using HPLC. Integrity of the epidermal membrane was checked by microscopy before the study, and by means of addition of Chinese ink at the end of the study. Any cells showing penetration of the ink were eliminated from the analysis.

Results
The quantity of test substance penetrating through the epidermal layer after 4 hours was 0.56% of applied dose in the presence of hair and 0.72% in the absence of hair. After 24 hours penetration had increased to 1.04% of the applied dose in the presence of hair and 1.27% of applied dose in the absence of hair.

This study did not include determination of recovery of the test substance. Physiological saline was used as the receptor fluid, which may not be adequate for a relatively lipophilic substance.

The study is considered inadequate.

Ref. : 12

Study 2

Guideline	:	/
Tissue	:	Human female abdominal skin, dermatomed (2cm²)
Method	:	Franz diffusion cells
Test material	:	Disperse Red 17, 0.2 % in a dispersant formulation
Batch No	:	443964 (dye content : 38.9 %)
Dose Levels	:	19.52 mg/cm² of formulation. 14.95 µg/cm² of Disperse Red 17.
Receptor fluid	:	PBS buffer w/o Ca^{2+}, Mg^{2+} Instamed® 9.55g/l containing 0.25% of Tween 80. Disperse Red 17 as active agent was soluble at a 10.68 µg/ml concentration, volume: 3 ml.
Replicate cells	:	7 (interpreted)
Analytical method	:	HPLC methodology validated (UV – Visible detection) Quantitation limit in the receptor fluid 0.005 - 0.1 µg/ml
Stability	:	A loss of material was observed (-8.6 %) after 1 month at 45 °C followed by 1 month at room temperature.
GLP	:	in compliance

The skin penetration of Disperse Red 17 was evaluated in a static Franz diffusion cell system using dermatomed human abdominal skin (582 ± 128 µm). The integrity of the skin was checked by TEWL. The solubility of Disperse Red 17 in the receptor fluid was evaluated taken into account the maximal amount of substance which could be found in the receptor fluid (2.26 mg of Disperse Red 17 in 200 ml).

The dye formulation (19.52 mg/cm² with a content of Disperse Red 17 of 14.95 µg/cm²) was applied on the skin surface for 30 min. Then, the skin surface excess was washed off with a 2% sodium dodecyl sulphate solution, rinsed with water and finally dried. Twenty four hours after application, the content of Disperse Red 17 was determined by HPLC in the following compartments: skin excess, SC, epidermis + dermis and receptor fluid.

Results
Under the present experimental conditions, a total recovery of Disperse Red 17 of 92.1% has been obtained. Most of the hair dye applied on the skin surface was removed with the washing procedure (92.0% of the applied dose). The content of the test substance detected in the SC was: 0.014 µg/cm². The amount of the Disperse Red 17 in the epidermis and dermis represented 0.011 µg/cm² and the diffusion of that substance in the receptor fluid was extremely low (0.003 µg/cm²).
The global absorbed amount of Disperse Red 17 (epidermis + dermis + RF) was 0.014 µg/cm².

Ref. : 13

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	/
Species/strain	:	*S. typhimurium,* TA98, TA100, TA1535, TA1537, TA1538 *E. coli,* WP2uvrA
Replicates	:	Triplicate plates, 2 independent tests
Test material	:	Disperse Red 17
Batch No	:	928017/02
Concentrations	:	1.6-1000 μg/plate with and without metabolic activation.
GLP	:	in compliance

Disperse Red 17 has been investigated for gene mutation in *S. typhimurium* and *E. coli,* using the plate incorporation method. Liver S9 (10%) fraction from β-naphthoflavone and sodium phenobarbitone-induced Fischer344 rats was used as the exogenous metabolic activation system. The concentration range 1.6-1000 μg/plate was selected on the basis of a preliminary toxicity indicating that concentrations above 1000 μg/plate were cytotoxic.

Results
The test substance induced significant and concentration-related increases in the number of revertants in TA98, TA100 and TA1538 with and without metabolic activation in two independent experiments. Significant increases in revertants were found with TA1537 in one experiment. The negative and positive control agents gave the expected results.
The test substance was mutagenic in the Ames incorporation assay with or without metabolic activation.

Ref. : 6

***In Vitro* Mammalian Cell Gene Mutation Test**

Guideline	:	/
Cells	:	L5178Y mouse lymphoma cells ($TK^{+/-}$)
Replicates	:	2 independent tests
Test material	:	Disperse Red 17 in DMSO solution
Batch No	:	928017/02
Exposure time	:	3 hours (± S9)
Concentrations	:	50 to 500 μg/ml with and without metabolic activation
GLP	:	in compliance

Disperse Red 17 has been investigated for gene mutation at the TK locus in L5178Y ($TK^{+/-}$) mouse lymphoma cells. Liver S9 (10%) fraction from β-naphthoflavone and sodium phenobarbitone-induced Fischer344 rats was used as the exogenous metabolic activation system. The concentration range 50-500μg/ml was selected on the basis of a preliminary toxicity indicating that concentrations above 500 μg/ml were cytotoxic.

Results
In the first experiment the test substance increased the mutation frequency at all concentrations in the presence of S9 and at 250 and 500μg/ml in the absence of S9. In the second experiment statistically significant increases in mutation frequency were seen at 300 and 400 μg/ml in the absence of S9 and 100μg/ml in the presence of S9. The negative and positive control agents gave the expected results.
The test substance caused an increase in mutation frequency in two independent experiments. The compound is considered mutagenic in this test.

Ref. : 7

***In Vitro* Mammalian Chromosome Aberration Test**

Guideline	:	/
Species/strain	:	Chinese Hamster Ovary Cells
Replicates	:	Duplicate cultures, 2 independent tests
Test material	:	Disperse Red 17 dispersed in an aqueous solution
Batch No	:	928017/02
Concentrations	:	5-50 μg/ml without metabolic activation (3 doses). 50-500 μg/ml with metabolic activation (3 doses).
GLP	:	in compliance

Disperse Red 17 has been investigated for induction of chromosomal aberrations in CHO cells. Liver S9 fraction from β-naphthoflavone and sodium phenobarbitone-induced Fischer344 rats was used as the exogenous metabolic activation system. The test concentrations were selected on the basis of a preliminary toxicity study indicating maximal concentrations that decreased the mitotic index by more than 50% of control.

Results
The results of the study showed a statistically significant increase in aberrations at 500μg/ml with metabolic activation. The positive control agents gave the expected result.
The test substance was clastogenic to CHO cells in the presence of metabolic activation, in one experiment. Exposure times were not indicated, therefore, the study is unsuitable for evaluation.

Ref. : 8

2.8.2. Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	/
Species/strain	:	Mouse, CD1 outbred
Group size	:	5 males + 5 females
Test material	:	Disperse Red 17 in 0.5% aqueous carboxymethylcellulose
Batch No	:	928017/02
Dose Levels	:	0, 125, 200 and 320 mg/kg bw by gavage
Sacrifice Times	:	24, 48 and 72 hours (Positive control: 24 hours).
GLP	:	in compliance

Disperse Red 17 has been investigated for induction of micronuclei in the bone marrow cells of mice. Dose levels were determined by a preliminary range finding study in which 3/4 mice died

at 500 mg/kg bw two hours after dosing. The substance was administered once by gavage to groups of animals sacrificed at 24, 48 and 72 hours for harvest of bone marrow cells.

Results
There was no evidence of the mean increased incidence of micronucleated polychromatic erythrocytes in any of the test groups when male and female results were pooled. There was no change in the ratio of polychromatic to normochromatic erythrocytes.
The study is unsuitable for evaluation, because there is no indication that the target cells were reached.

Ref. : 9

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*

Guideline	:	OECD draft guideline of 1991 (475)
Species/strain	:	Wistar rat, HanIbm:WIST (SPF)
Group size	:	3 males
Test material	:	Disperse Red 17 in Polyethylene glycol 300
Batch No	:	928017/02
Dose Levels	:	0, 200 and 2000mg/kg, by gavage
Sacrifice Times	:	16 hours: all dose groups; 2 hours: high dose group
GLP	:	in compliance

Disperse Red 17 has been investigated for induction of unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* following *in vivo* dosing. A preliminary toxicity study showed signs of toxicity but no deaths at 2000mg/kg bw and therefore this was used as the highest dose, in accordance with the OECD draft guideline. Negative and positive controls were in accordance with the OECD guideline. Animals were sacrificed after 16 hours and for an additional high dose group after 2 hours. Hepatocytes were isolated and at least 3 cultures were established per animal. The hepatocytes were subsequently treated with ^{3}H-thymidine *in vitro*. Incorporation of radiolabel was assessed using autoradiography.

Results
There were no differences in the viability of hepatocytes isolated from rats of different dose groups. The positive control agent for the 16 hours treatment gave the expected result. No positive control was used for 2 hours of exposure. The study is considered unsuitable because not conforming the OECD guideline.

Ref. : 10

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

NOT APPLICABLE

2.12. Conclusions

Disperse Red 17 contains 41.2% of the dye component and the remainder of the formulation consists of sodium lignosulphonate and sodium lignosulfonate sulfomethylated as dispersing agents and a number of impurities. No appropriate information on physico-chemical properties and purity are provided in order to get an acceptable characterization of the compounds included in that mixture. Also, the information submitted on solubility and on impurities is inadequate. The degradation products of the dye over 2 months storage have not been addressed..

Disperse Red 17 was minimally toxic in an acute rat oral toxicity test.
When administered during organogenesis, the substance adversely affected maternal food consumption and bodyweight gain at doses of 125, 250 and 500mg/kg bw/day. There was no evidence of foetotoxicity or teratogenicity. Two 13 week oral rat studies have been conducted. The first showed evidence of dose-related effects on the liver spleen and thyroid at doses of 100, 200 and 400 mg/kg bw/day. The second study established a NOAEL of 10 mg/kg bw/day.
The observation of accumulation in mammary tissue raises concern with respect to potential effects on the offspring during lactation.

It was slightly irritating when applied neat to the rabbit eye but not to rabbit skin. It has been tested for sensitising potential using a Magnusson and Kligman protocol. Discoloration due to the staining properties of the dye precluded accurate evaluation of the skin and therefore the study should be considered equivocal.

Percutaneous penetration has been investigated using human skin *in vitro*. A value of 0.014 µg/cm² is the amount of dermal absorption determined.

Disperse Red 17 has been tested for mutagenicity/genotoxicity in several assays *in vitro* or *in vivo*. In vitro test have produced positive results for the induction of gene mutation and chromosome aberration. None of the two in vivo tests are in accordance with the current OECD guidelines and are unsuitable for an accurate genotoxic evaluation.

2.13. References

1. Toxicol Labs Ltd, UK, Report No LRL/97/95 (June 1995)
2. Toxicol Labs Ltd, UK, Report No A/E/40913 (Jan 1995)
3. Toxicol Labs Ltd, UK, Report No A/S/40912 (Dec 1994)
4. Toxicol Labs Ltd, UK, Report No A/K/40914 (June 1995)
5.1. Quintiles England Limited, UK, Study No. LRL/102/96 (Jan 1998)
5.2. CIT, France. Study No15209 TCR (Jan 1998)
6. Toxicol Labs Ltd, UK, Report No M/Ames/38550 (Jan 1994)
7. Toxicol Labs Ltd, UK, Report No M/PML/40266 (Sept 1994)
8. Toxicol Labs Ltd, UK, Report No M/CCA/38551 (May 1994)
9. Toxicol Labs, UK. Study No. M/MMN/38552 (Oct 1994)
10. Cytotest Cell Research GmbH, Germany, Report No. 491600 (Feb 1995)

11. Quintiles Preclinical Services, UK, Report No LRL/103/96 (Jan 1997)
12. L'Oreal, France. Ref. 95/11/21, 95/12/11 and 96/02/26 (June 1996)
13. L'Oréal Recherche, France. In vitro percutaneous absorption of Disperse Red 17(COLIPA n° B5). Study n° 16160; 20/08/2001

3. Opinion of the SCCNFP

The SCCNFP is of the opinion 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.

4. Other considerations

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5. Minority opinions

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