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

CONCERNING

CURRY RED

COLIPA n° C174

adopted by the SCCNFP on 23 April 2004
by means of the written procedure

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 Curry Red safe for use in cosmetic products as a hair dye ingredient?

* 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

2.1. General

Curry Red is listed as CI 16035 in Annex IV, part 1 – list of colouring agents allowed for use in cosmetic products – to Directive 76/768/EEC on cosmetic products; field of application 1: colouring agents allowed in all cosmetic products.

Curry Red is used and approved as a food colorant since decades. Numerous toxicity studies including long-term toxicity, carcinogenicity and reproductive toxicity are available for this colorant.
The dye is approved in the EU for the use in food (E 129) and for general use in cosmetics. In the US, Curry Red is approved for usage in food, drugs and cosmetics (with the exception of eye area use). Both the SCF in 1987 and the JECFA in 1981 derived an ADI of 7 mg/kg bw/day for Curry Red based on the findings obtained in a rat long-term toxicity/carcinogenicity study (Reference 3).

2.1.1. Primary name

Curry Red (INCI)

2.1.2. Chemical names

Disodium 6-hydroxy-5-[(E)-(2-methoxy-5-methyl-sulfonatophenyl)diazenyl]-2-naphthalene sulfonate (IUPAC)
Disodium 6-hydroxy-5-[(2-methoxy-4-sulphonato-m-tolyl)azo]naphthalene-2-sulphonate
2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-sulfonyl)azo], disodium salt (CA index name, 9CI)
6-Hydroxy-5-((2-methoxy-5-methyl-4-sulfophenyl)azo)-2-naphthalenesulfonic acid, disodium salt

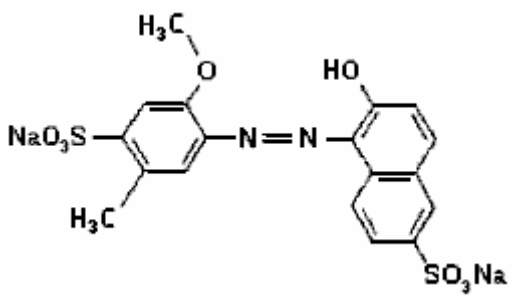
2.1.3. Trade names and abbreviations

COLIPA n°	:	C 174
Trade name	:	FD&C Red 40 W093 (LCW)
Other Names	:	Allura Red AC
		Food Red 17
		E 129
		FD&C Red No. 40

2.1.4. CAS / EINCES / COLOR INDEX number

CAS	:	25956-17-6
EINECS	:	247-368-0
Colour Index	:	CI 16035

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{18}H_{14}N_2Na_2O_8S_2$
Mol weight : 496.43

2.1.7. Purity, composition and substance codes

Substance code : A013078
Batches used : 0103061012; FDA certified Lot AK5827

The analytical data given by the summary report and FDA certificate for the same batch are summarized in the following table.

	Summary report	*FDA specifications*
Purity	> 85%	91.6 %
Total color (by weight)	–	88 %
Relative chromatographic purity (HPLC - UV/VIS peak area method)	>95% at 254 nm	97,9% at 254 nm 95.2% at 510 nm
Loss on drying (vacuum dessication)	< 10 %	4.2 %
Water content (Karl-Fischer)	< 10 %	5.7 %
Water insoluble matter (alkaline solution)	–	0.07 %
Sulfated Ash content	< 40 %	32 %
Sum of volatile matter and chlorides and sulfates (calc. as sodium salts)	–	< 14 %
Lead	< 10 ppm	< 20 ppm
Mercury	< 1 ppm	< 1 ppm
Arsenic	< 3 ppm	< 3 ppm
Iron	< 100 ppm	
6-Hydroxy-2-naphthalenesulfonic acid, disodium salt	< 100 ppm	< 0.3 %
4-Amino-5-methoxy-2-methylbenzene-sulfonic acid	< 100 ppm	< 0.2 %
6,6'-Oxy-bis(2-naphthalenesulfonic acid)	< 100 ppm	< 1 %
6-Hydroxy-8-(2-methoxy-methyl-4-sulfophenoxy)-5-(2-methoxy-methyl-4-sulfophenoxylazo)-naphthalene-2-sulfonic acid	–	< 1%
Lower sulfonated subsidiary colors	–	< 1 %

Solvent residues
Solvents such as methanol, ethanol, isopropanol, n-propanol, acetone, ethyl acetate, cyclohexane, methyl ethyl ketone and monochlorobenzene were detected.

2.1.8. Physical properties

Appearance	:	Dark red powder
Melting point	:	349.8°C (calculated by QSAR)*
Boiling point	:	872°C (calculated by QSAR)*
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{OW}	:	– 0.55 (calculated by QSAR)*

* See Comments below

2.1.9. Solubility

Soluble	in water	:	> 20 weight % (pH 9.7)
	in acetone / water 1:1	:	1.3 weight %
	in DMSO	:	4.4 weight %
	in water / ethanol 6:4	:	3 weight %

2.1.10 Stability

In water (3% w/v): adequate stability during 7 days at room temperature.

General comments on analytical and physico-chemical characterisation

* The purity data given in the summary report are different from those of the FDA certificate for the same batch, including different impurities.
* No pKa are reported for the ionisable group.
* The physical properties has been calculated without indicating the method used. Furthermore, calculated values can not be accepted as estimates of the true physical constants without justification, indicating that the reported values are realistic (possible decomposition of the test substance at elevated temperatures).
* The pH corresponding to the calculated log P_{ow} is not stated. Since log P_{ow} is known to strongly depend from the pH, the reported value is useless unless information is given about its relation to physiological conditions and to the pH conditions of the percutaneous absorption studies.
* No information is provided for the stability of the dye in a common market formulation.

2.2. Function and uses

Curry Red is proposed to be used as a hair colouring agent (”direct” dye) in semi-permanent hair dye formulas at a maximum concentration of 0.4% in the finished cosmetic product.

TOXICOLOGICAL CHARACTERISATION

Although a reasonable part of the studies presented in the submission is only available in summaries a scientifically valid evaluation with regard to toxicological endpoints is possible (see also references 1, 2, 3).

2.3. Toxicity

2.3.1. Acute oral toxicity

Rat
LD_{50} > 10000 mg/kg bw

Dog
LD_{50} > 5000 mg/kg bw

Ref.: 2

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

Mouse
In a 6-week drinking water study groups of six male per group received doses of 0, 0.05 and 0.25 mg/ml *Curry Red* respectively in the drinking water. Bodyweight, drinking water consumption and investigations of brain, kidney, liver and spleen revealed no substance-related effects. A NOEL of 0.25 mg/ml in drinking water (equal to about 50 mg/kg bw/day, assuming a bodyweight of 25 g and a drinking water consumption of about 5 ml/day) can be derived from this study.

Ref.: 2

Rat
Groups of 6 male rats were fed 0 or 5.0 % *Curry Red* in a standard and a specially purified diet for 7 or 14 days. No effects on weight development were noted for the standard diet, whereas a slight reduction of bodyweight was noted for the purified diet. Based on this study administering 5 % *Curry Red* in the diet (equals about 3750 mg/kg bw/day, assuming an average bodyweight of 200 g and a food consumption of 15 g/day) for 14 days a NOAEL of about 3750 mg/kg bw/day can be derived.

Ref.: 2

Dog
In dogs (one female, one male), the oral administration of *Curry Red* for 6 weeks at doses of 125, 250 and 500 mg/kg bw/day did not effect the body and organ weights. Clinical, macroscopic and microscopic investigations did not reveal substance related effects; a NOEL of 500 mg/kg bw/day for the dog can be deduced.

In a 2-year oral toxicity study, diets containing 0.37, 1.39 and 5.19 % of *Curry Red,* respectively, were fed to groups of four dogs (two female/two male) each. Eight animals (four per sex) were used for the concurrent control group. An interim sacrifice took place after 1 year (one animal per sex and group). Behaviour and clinical chemistry as well as macroscopic and microscopic investigations were performed. Besides some not further specified tissue effects after 1 year which were not seen after the final 2-year treatment no substance related effects were noted. From this 2-year feeding study a NOEL of 5.19 % *Curry Red* in the diet can be derived which is equal to about 1500 mg/kg bw/day (assuming a feed consumption of 250 g/day and an average bodyweight of 8-9 kg).

Pig

Pigs (number of animals not indicated) were treated with *Curry Red* at an oral dose of 1000 mg/kg bw/day for 21 days followed by a dose of 1500 mg/kg bw/day until necropsy at day 76 (11 w). No effects were noted with regard to organ weights of liver, kidney and spleen. Haematology, clinical chemistry and histology did not reveal any substance related effects. For the described exposure scenario a NOAEL in pigs of 1500 mg/kg bw/day can be assumed.

2.3.5 Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

See 2.3.4

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

See also point 2.9. (combination studies chronic toxicity/carcinogenicity)

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Primary skin irritation in rabbits

Guideline	:	/
Species/strain	:	New Zealand albino rabbit
Group size	:	3 animals
Test substance	:	D&C red # 40; 10 % dilution in propylene glycol
Batch No.	:	/
Dosages	:	10 % solution in propylene glycol
GLP	:	/

Curry Red (synonym used in the report: D&C RED 40) was investigated as a 10 % dilution in propylene glycol for its irritation potential in three New Zealand albino rabbits. The diluted test item was spread repeatedly (once daily, 5 days a week for two weeks) to the inner surface of one ear of each animal. The untreated ear served as control.

Results
Under the described test conditions Curry Red produced a slight skin irritation. The noted grade of irritation after the repeated open application was 2 (maximum score 5).

Conclusion
The reported test in rabbits is not in line with current guidelines for the evaluation of the skin irritating potential *in vivo* (e.g. OECD 404).

Ref.: 6

Some additional data with regard to skin compatibility in rabbits are summarised in a review. Curry Red applied to skin of rabbits (4 animals per groups, intact and scarified skin treated) at single doses of 0.316, 1, 4, 3.16 and 10 g/kg was evaluated to be not irritating to the rabbit skin.
In another test the irritation potential of Curry Red was investigated after repeated application to rabbit skin. 0, 0.1 and 1 % solutions in water or a hydrophilic cream preparation were applied to the skin. The treatment scheme for intact skin was 5 times a week for 13 weeks (7 animals per group) and for scarified skin 5 times a week for 3 weeks with 2 animals per group. Under these stringent test conditions, slight to moderate irritation was noted, but clinical investigations did not reveal any substance related effects. The concentration and the test conditions at which the effects were noted is not given in the summary.
(Cited in Reference: 2 from Hazleton 1967/68 but no other information available)

Primary skin irritation in humans

In a patch test in which the neat test item and 25 % dilution in water was applied to the skin of 200 volunteers (males and females) neither skin irritating nor skin sensitising properties were noted.
(Cited in Reference: 2 from Osbourne 1972 but no other details available))

2.4.2. Irritation (mucous membranes)

Eye irritation potential in rabbits

Based on a summary report in the literature, 0.1 ml of a 1 % dilution in water applied to eyes of 6 rabbits revealed no eye-irritating properties.
(Cited in Reference: 2 from Avon 1982 but no other details available)

Assessment of the eye irritation potential in the Hen's egg test on the chorioallantoic membrane (HET-CAM)

Guideline	:	/
Group size	:	6 eggs per groups
Test substance	:	FD +C Red Nr. 40 (WR 20674)
Batch No.	:	0103061012 (Ellis & Everard)
Purity	:	/
Dosages	:	1 % aqueous dilution
GLP	:	in compliance

HET-CAM assays were performed with a 1 % aqueous dilution of Curry Red. The diluted test item was applied onto the CAM of fertilised chicken eggs at day 9 of incubation and irritation parameters evaluated.
The endpoint assessment as recommended for non-transparent test items was used. For this assessment the test item was rinsed off 30 sec after application onto the CAM and evaluation of the parameters mentioned above was performed. Reference substances (Texapon ASV, NaOH, acetic acid in NaCl) were investigated in parallel to calibrate the test and demonstrate the sensitivity and validity of the assay.

Results
The 1% aqueous solution did not cause any damaging effect on the CAM as the obtained evaluation resulted in score 0 for both independent experiments

Ref.: 7

Assessment of the eye irritation potential by cytotoxicity measurement in the neutral red uptake assay (NRU) on human keratinocytes (HaCat)

Guideline	:	/
Number repetitions	:	2 independent experiments
Test substance	:	FD +C Red Nr. 40 (WR 20674)
Batch No.	:	0103061012 (Ellis & Everard)
Purity	:	/
Test concentrations	:	3.162 to 10000 µg/ml medium
GLP	:	/

An *in vitro* screening assay using the measurement of cytotoxicity in human keratinocytes (NRU-assay) was performed. Monolayers of human keratinocytes (HaCat) were exposed in 96-well microtiter plates to various concentrations of *Curry Red* for 24 hours and cell viability was measured by neutral red uptake. The concentration causing a 50 % reduction in neutral red

uptake compared to the concurrent control (NRU-50) is determined. This figure, as a measure for cytotoxicity of a test item in this cell culture, is compared to findings with known eye irritants under identical test conditions and allows a prediction of the eye irritation potential of a test item. Sodium lauryl sulfate (SLS, 1 - 50 µg/ml) is tested in parallel as a positive control.

Results
No NRU-50 value could be determined as the viability was still 71 % in the first and 69 % in the second assay after treatment with the highest test concentration of 10000 µg/ml medium. Thus, the NRU-50 was > 10000 µg/ml. The NRU-50 values obtained with the positive control SLS (12.5 and 15.6 µg/ml) were in the range of the historical control data of the laboratory and demonstrate the validity and sensitivity of the assay.
According to the classification system of the performing laboratory discriminating between non-irritant, not-classified and severe, Curry Red is classified as non-irritant, as the cut-off for this classification is ≥ 750 µg/ml under the described test conditions.

Ref.: 8

2.5. Sensitisation

Local Lymph Node Assay (LLNA)

Guideline	:	OECD 429 (Draft 2000)
Species/strain	:	Mice CBA/J
Group size	:	5 females per group
Test substance	:	CI 16035, FD&C Red 40
Batch No.	:	0103061012 (Ellis & Everard)
Purity	:	/
Concentrations	:	a) 0.5, 1, 2, 4 % (w/v) in DMSO b) aqua/acetone (1:1) mixed with olive oil (4:1)
GLP	:	in compliance

The skin sensitising potential of Curry Red was investigated in CBA/J mice by measuring the cell proliferation in the draining lymph nodes after topical application onto the ears.
25 µl of 0 (vehicle only), 0.5, 1, 2 and 4 % (exceeding the maximal solubility in both vehicles used) of Curry Red in either DMSO or a mixture of aqua/acetone (1:1) with olive oil (4:1) were applied on three consecutive days to the surface of the ear of 5 female CBA/J mice per group. After application, the ears were dried for about 5 minutes by means of a hair dryer. A positive control (p-phenylenediamine at 1 % in DMSO) was investigated in parallel under identical tests conditions.

Animals were checked twice daily for morbidity/mortality. Observation for clinical signs was done daily before and at least once after dosing. Bodyweight was determined on day 1 and day 5. On day 5 the mice received an intravenous injection of 250 µl phosphate buffered saline containing 20.8 µCi of [H^3] methyl thymidine. About five hours later the mice were sacrificed by CO_2-inhalation and the draining auricular lymph node was removed and weighed. After preparing a single cell suspension for each animal, cells were precipitated by TCA and the radioactivity due to incorporation of [H^3] methyl thymidine in the pellets was determined by liquid scintillation counting as disintegration per minute (dpm).
The mean dpm per treated group was determined and the stimulation index (test item compared to the concurrent vehicle control) was calculated.

Results
With the test item in DMSO mean stimulation indices of 0.6, 0.7, 0.9 and 0.9 were obtained for the 4 test concentrations of 0.5, 1, 2 and 4 %, respectively.
In the second vehicle (aqua/acetone/olive oil) the indices were 1.2, 1.0, 0.9 and 0.9 for the 4 test concentrations.
As no relevant increase in the mean stimulation indices was observed (all figures were well below the trigger value of three), no indication was found that Curry Red might be a skin sensitiser under the given test conditions.
The positive control (PPD, 1 % in DMSO) caused an increase in the stimulation index by a factor of 7.8.

Conclusion
In the local lymph node assay, Curry Red did not reveal any potential to be a skin sensitiser at tested 4 % . Based on these findings Curry Red is evaluated not to be a skin-sensitiser.

Ref.: 9

Human data

In a provocation test (10 – 14 repeated patch applications for 48 h contact) with 200 volunteers, the neat test item and a 25 % dilution in water revealed neither skin irritating nor skin sensitising properties after 72 h skin contact. In a Draize-Shelanski repeated insult patch test (induction: 10 applications, challenge 14 days later) no indication for a skin irritating or a skin sensitising potential was found.
(Cited in Reference: 2 from Osbourne 1972 and BT 1973 but no other details available)

2.6. Teratogenicity

Developmental prenatal toxicity study in rats

Guideline	:	/
Species/strain	:	Osborne Mendel rats
Group size	:	42 - 43 presumably pregnant rats based on vaginal smear
Test substance	:	FD&C Red 40
Batch No.	:	AA-4181 (FDA certified batch)
Purity	:	/
Concentrations	:	0, 30, 75, 150, 300, 600 and 1000 mg/kg bw/day
Treatment period	:	day 0 - 19 of gestation
GLP	:	/

Curry Red diluted in distilled water was administered daily to groups of 42 to 43 presumably pregnant Osborn Mendel rats (12-21 week old, 220-270 g) at doses of 0, 30, 75, 150, 300, 600 and 1000 mg/kg bw/day via gavage from day 0 to day 19 of gestation. General appearance and well being as well as bodyweight were recorded daily. Food consumption was monitored weekly.
At day 20 of treatment, animals were killed by CO_2-asphyxiation and caesarean sections were performed and the corpora lutea counted. The position and stage of all implantations were determined. Each live foetus was weighed, the sex registered and examined for gross external malformations. About one-half of the foetuses were investigated for skeletal anomalies the remaining ones for soft tissues variations.

Results
No treatment-related effects in dams were noted with regard to bodyweight or food consumption. The clinical observations also revealed no indications for systemic toxicity of the tested doses. The numbers of corpora lutea and implants were similar to control in all treated groups. Some variations (increase) with regard to the number of viable male foetuses were noted at 30 and 1000 mg/kg bw. As these findings were not dose-related, they are considered to be accidental and not due to the treatment with *Curry Red.* Some variations, reaching statistical significance at 600 mg/kg bw were also noted with regard to the percentage of animals with two or more resorptions. Again no dose-relation was noted. Hence, this variation is also considered accidental. The mean foetal weight and crown-rump lengths as well as the occurrence of runts were similar in all groups including controls.
No treatment related effects were noted at gross necropsy or with regard to skeletal and visceral investigations. Variations/effects sometimes reaching statistical significance (e.g. sternebral variations, increase in the incidence of 14^{th} rib bud) were noted at single doses only and did not reveal a dose response relationship. All of these variations were therefore considered to be accidental, moreover as they were within the historical controls range.
In summary, the prenatal developmental toxicity study in Osborn Mendel rats investigating doses up to the limit dose of 1000 mg/kg bw *Curry Red*, did not reveal any indication for the test item to be embryo- or foetotoxic or teratogenic. Neither in the dams nor in the foetuses indications were noted for treatment-related adverse effects. Thus, for both the maternal and developmental toxicity a NOEL of ≥ 1000 mg/kg bw/day can be deduced from this study.

Ref.: 10

In the literature, there are additional prenatal developmental studies in rats and rabbits cited. Based on this information *Curry Red* is not a teratogen in rabbits (cited in reference 5) and no embryo-, foetotoxic or teratogenic effects were noted in rats treated during pregnancy with up to 200 mg/kg bw/day; highest dose tested in this test (cited in reference 2).
Furthermore, the findings of a 2-generation study in rats are summarised in the mentioned review (reference 2).

Rats (number of animals not indicated) were fed a diet containing 0, 0.37, 1.39 or 5.19 % of *Curry Red* over two generations. Several parameters were evaluated including number of living pups per animal, weight of pups, number of implantations and resorptions, number, weight and size of the foetuses as well as a macroscopic investigation.

No substance related effects were noted with regard to embryo-toxicity and/or teratogenicity. This is in line with findings obtained in the teratogenicity studies in rats reported above.

No other effects were noted with the exception of a reduction of the fertility index in the F2 generation. The information at which dose this effect was observed and to which extent a reduction was observed is not given in the summary (Reference 2).

This study was performed in the late 1960s and is of limited value only, as no detail information is available. The study is insufficient to evaluate potential fertility effects of a test item based on today's requirements. As far as it can be estimated from other studies performed at that time, the number of animals investigated was most likely rather low and effects on the fertility index in the F1 or F2 generation were often due to the study design (rather old males and females, no historical data about variations in control groups for the specific study design). Consequently the mentioned effects in the F2 generation, most likely observed at a very high dose (5.19 % in feed

is equal to more than 3000 mg/kg bw/ day) cannot be evaluated and are not considered of concern for the overall risk assessment. This conclusion is supported by the scientific evaluation and ADI deduction of *Curry Red* by the SCF and JECFA in the 1980th as in none of the evaluations the fertility effects mentioned above were of concern.

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Kinetics, *in vivo*

The adsorption, distribution, excretion and metabolism of *Curry Red* has been investigated in rats and dogs. The main findings are summarised in a review.
(Cited in Reference 2 from Hazleton 1975 but no other details available).

^{35}S-labelled Curry Red was administered orally to rats pre-treated for 5 days with the same amount of unlabelled compound. Within 72 h 76-92 % and 5.7-19.8 % of the administered dose were excreted via faeces and urine, respectively. The tissue residues were low with a maximum of 0.4 % of the administered dose. In urine the main metabolite was cresidine sulfonic acid. Two further metabolites were not identified. In faeces, the parent compound, cresidine sulfonic acid and 2 unknown metabolites were found

A similar picture was obtained in dogs using the above described test design.
Within 72 hours 92 and 95 % of the administered dose was excreted via faeces. The corresponding figures for urine were 2.7 and 4.4 %. Main metabolite in urine was again cresidine sulfonic acid. In faeces the parent compound, cresidine sulfonic acid and 3 unknown metabolites were found.

From the limited information given in the summaries the following conclusion regarding the toxicokinetic of Curry Red in two different species can be drawn:

* No significant differences were noted with regard to absorption, excretion and metabolism in rats and dogs
* The absorption after oral administration is limited (4-19.8 %)
* If absorbed, Curry Red is rapidly excreted (96-99.5 %) within 72 h
* Tissue levels in general are very low (0.4 % of the applied dose)
* Main excretion takes place via faeces
* Main metabolite in urine and faeces is due to the cleavage at the azo-linkage resulting in cresidine sulfonic acid.
* The metabolites noted in the faeces are most likely due to enzyme activities of intestinal micro-organisms in the lower gastrointestinal tract (cleavage at the azo-linkage).

Percutaneous Absorption, *in vitro*

Guideline	:	OECD 428 (2000)
Test system	:	split thickness pig skin (400-450 µm), 5-6 samples/experiment
Contact time	:	30 minutes under occlusion (donor chamber covered with parafilm)
Test substance	:	Exp. I : *CI 16035, FD&C Red nr. 40 WR 20674*, dissolved in acetone/water 1:1 (20 mg/ml)
		Exp. II : *CI 16035, FD&C Red nr. 40 WR 20674*, in a typical hair dye formulation (ref. 8150992A), composition partially stated

Control	:	caffeine, tested every 3 months, results available
Purity	:	99.3 %
Batch no	:	0103061012
Application	:	Exp. I : 100 µl/cm² (1 mg pure dye)
		Exp. II : 200 mg/cm² (1 mg pure dye)
Receptor fluid	:	Saline solution, pH 3.0
GLP	:	in compliance

Porcine ear obtained from the slaughter house immediately after slaughter and before steam cleaning were used for this experiment. The outer ear region was washed, carefully shaved and the skin was removed by dissection. Thickness of the dissected skin was approximately 400-450 µm. The surface of the skin that was in contact with the test substance during permeation-assay was 1.0 cm². Two experiments were performed:

Experiment I : 2 mg/cm² of the 20 mg CI 16035/ml solution was applied.
Experiment II : CI 16035 was applied in a typical hair dye formulation (ref. 8150992A), containing :

8.50%	Cetearyl Alcohol
5.40%	Sodium Laureth Sulphate
3.75%	Cocamidopropyl Betaine
0.50%	Acid Color
0.80%	Phenoxyethanol
0.75%	Ceteareth-12
0.70%	Aminomethylpropanol
0.30%	Methylparaben
0.20%	Propylparaben

The skin was mounted in glass flow-through diffusion chamber with diameter of 1.135 cm.
Each donor chamber was filled with 1 ml of the test item dissolved in saline, pH 3.0, and covered with parafilm. Since the pH of the representative hair dye formulation is 3.0, this pH was used in both experiments. Saline, pH 3.0 was pumped through the chambers with a flow rate of 1-2 ml/hour and chamber. Buffer solution of the acceptor chamber was collected in plastic vials that were replaced according to the sampling times and stored at – 20 ^{o}C. The whole test system was set up in an incubator adjusted to 32 ^{o}C. After 30 min of incubation, test item was removed from skin with 10% aqueous shampoo solution.

Following the washing procedure the donor chamber were filled with 1 ml of saline pH 3.0. The collecting vials were changed after 0, 0.5, 1, 2, 4, 6, 8 and 24 hours.
At the end of the experiment, the epidermis and upper dermis were prepared from the full thickness skin using scalpels and forceps.

Results
No measurable permeation through the skin occurred at any time point within the time frame of both experiments. The lowest detection limit under the conditions reported is 0.021 µg/ml. Together with the upper dermal extracts the average amounts of test item considered having passed the skin are 1.19 µg/cm² in the first and 1.28 µg/cm² in the second experiment. The individual amounts found in the upper dermis extract were:

Experiment I : 0.38 - 0.53 - 0.38 - 0.18 -0.25 µg/cm².
Experiment II : 0.14 - 0.34 - 0.15 - 1.26 - 0.12 - 0.11 µg/cm².

The individual amounts found in the epidermis extract were:

Experiment I : 149 - 4.08 - 1.56 - 123 - 1.23 - 2.67 µg/cm².
Experiment II : 0.613 - 2.44 - 0.68 - 0.42 - 1.14 - 0.84 µg/cm².

The mean recovery of the test item was 95.7 % in the first and 104.3 % in the second experiment.

Conclusion
Many shortcomings can be formulated with regard to this study
- No separate measurements have been performed on stratum corneum, epidermis and dermis.
- No data on the solubility of the test substance in the receptor fluid are given.
- When calculating the total amount of percutaneously absorbed substance, the values measured in the epidermis are not taken into account, while these (except the amount in the SC) should be added.
- The lab states that no measurable permeation through the skin occurred at all time points, the lowest detection limit in the receptor fluid being 0.021 µg/ml. Nevertheless, the individual measurements were "calculated" using an unclear methodology, resulting in cumulative receptor fluid values ranging from 0.8731 to 0.9405 µg/cm².
- There is a large variability in the skin extract measurements, which makes is impossible to make a correct assessment of the percutaneous absorption of CI 16035.
- The test has been performed with an 0.5% Acid Red 52 formulation, while the requested maximum authorized concentration is 0.4%.

For all the above-mentioned reasons, the percutaneous absorption study cannot be accepted.

Ref.: 11

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Reverse Mutation Testing Using Bacteria

Guideline	:	/
Strains	:	*Salmonella typhimurium* TA1535, TA1537, TA98, and TA100
Doses	:	7.5-750 mg/plate; Ether extracts of the dye
Metabolic Act.	:	Aroclor 1254-induced and uninduced rat liver homogenate; uninduced Syrian golden hamster liver homogenate
Replicate	:	/
Batch No.	:	samples approved for food use. Four productions samples
Purity	:	/
GLP	:	/

Results
Published paper
C174 was found positive (in all samples) in the presence of a reduction of metabolic activation system. No complete evaluation can be made in relation with the data reported in this paper.
The study is inadequate for a complete evaluation.

Ref.: 12

***In Vitro* Mammalian cell gene mutation test**

Guideline	:	OECD/476 (1997)
Species/Strain	:	Mouse lymphoma L5178Y cell line (Forward mutations at TK locus)
Doses	:	156.3-312.5-625-1250-2500-5000 µg/ml with and without S9
Replicate	:	2 experiments
Metabolic Act.	:	uninduced Syrian golden hamster liver homogenate
Positive control	:	MMS (without metabolic activation) DMNA (with metabolic activation):not suggested by OECD
Substance	:	FD+C RED NR. WR 20674
Batch No	:	0103061012
Purity	:	HPLC: 99.3% (HPLC)
Stability	:	good for 7 days
GLP	:	in compliance

Results
Test item concentrations between 39.1 and 5000 µg/ml were used to evaluate toxicity in the presence (4h treatment) and in the absence (4h and 24h treatment) of metabolic activation.
Only a moderate toxic effect was observed at the highest concentration with metabolic activation (40.7% cell growth compared with control). The osmolality of the solutions was also tested.
In this cell line, forward gene mutations are scored at the thymidine kinase locus.

Mutagenicity Data
Positive controls. MMS (without metabolic activation induced 293-333/10^6 small size mutant colonies (control 45-82/10^6) and 54-21/10^6 large size mutant colonies (control 19-21/10^6) in the two cultures of experiment I. The corresponding values for experiment II were 415-681/10^6 (control 108-121/10^6) and 16-72/10^6 (control 17-47/10^6) for the two cultures.
DMNA (with metabolic activation induced 131-137/10^6 small size mutant colonies (control 80-97/10^6) and 80-67/10^6 large size mutant colonies (control 30-62/10^6) in the two cultures of experiment I.
The corresponding values for experiment II were 42-392/10^6 small size mutant colonies (control 54-75/10^6) and 236-58/10^6 large size mutant colonies (control 91-17/10^6).
According to the Authors, the part of the experiment I with metabolic activation was not considered valid since the positive controls of both parallel cultures failed to respond properly.
DMNA is not suggested for this mutational assay by the OECD Guidelines.
There is no indication of increase in mutation frequency induced by the substance in both experiments in the absence of metabolic activation and in the second experiment in the presence of metabolic activation.
In experiment II the frequencies of the spontaneous mutant colonies exceeded the historical frequency.

It may be concluded that the test substance does not induce any gene-mutation and, possibly, chromosomal aberrations in this cell line; however, this test is being repeated.

Ref.: 13

2.8.2 Mutagenicity/Genotoxicity *in vivo*

in vivo Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD/470 (1997)
Species/Strain	:	NMRI Mice (male and female)
Group size	:	5 males /5 females / group dosed
Test substance	:	FD+C RED No. 40 WR 20674
Batch No	:	103061012
Purity	:	HPLC: 99.3%
Stability	:	good for 7 days
Dose level	:	A toxicity test was performed on 4 animals (2 males and 2 females) by oral treatment with a dose of 2000 mg/kg and observed for 48h. Final study: 500, 100, and 2000 mg/kg
Positive control	:	CPA: 40 mg/kg
Negative control	:	Deionized water
Administration	:	Oral (no description of modality)
Sacrifice time	:	24 and 48h
GLP	:	in compliance

Results
Toxicity studies: A toxicity study was performed with 4 animals (2 males and 2 females), administered orally with a dose of 2000 mg/kg and observed for 48 hours. A reduction of spontaneous activity was observed only in one case.
Main assay: Different toxic reactions were observed in many animals (reduction of spontaneous activity, abdominal position, eyelid closure, apathy). Data on the number of Micronucleated cells for 2000 erythrocytes have been reported, as well as the number of immature PCE (polychromatic erythrocytes) but not the number of mature normochromatic erythrocytes (NCE): therefore, it was not possible to calculate the ratio (PCE/CPE+NCE) a value which gives information about the presence of the test item in the bone marrow cells.

Evaluation
The study is not adequate; it cannot be used to evaluate the potential of the test item to induce structural/numerical chromosome aberrations in somatic cells *in vivo*.

2.9. Carcinogenicity

Oral administration, rat

Male and female Charles River CD rats of the F0 generation received Curry Red (purity 88%) in concentration of 0 (control), 0.37, 1.39 or 5.19%. The groups consisted of 30 males and 30 females. The rats received Curry Red from 1 week before mating throughout the 3-week breeding period and during the gestation and lactation period. The F1 rats were exposed to the same diary dietary concentration as the F0 rats. Each groups consisted of 50 males and 50 females. The exposure continued until survival of either sex in any group reach approximately 20% at which point all rats at that sex were killed. Deaths, morbidity and gross signs of toxicity were recorded daily. Individual body weights, food consumption and clinical signs of gross observation were recorded every 4 week after 26 weeks. F1 male and female rats received Curry Red for 118 and 121 weeks, respectively. Food consumption among the high dose males and females was elevated, but not significantly so, when compared with controls. The authors

conclude that lifetime exposure of rats to Curry Red as a dietary admixture at concentrations up to 5.19% did not demonstrate carcinogenic effects.

Ref.: 4

Oral administration, mice

Curry Red was fed to Charles River HaM/ICR (CD-1) (study A) and CD-1 outbred (study B) mice as a dietary admixture in two separate lifetime toxicity/carcinogenicity studies. Each study included an *in utero* exposure phase during which the colouring was fed at dietary concentrations of 0 (control), 0.37, 1.39 or 5.19% throughout the mating, gestation and lactation periods. The F0 mice consisted of 50 males and 50 females in study A and 70 males and 70 females in study B. After random selection the lifetime exposure phase was initiated using the same dietary concentrations with 50 male and 50 female per group in study A and 100 males and 100 females per group in study B. Exposure was for 104 weeks in study A and 109 weeks in study B. There were no compound related effects on survival. There were no consistent statistical significant compound-related effects on mean food consumption in either study A or study B. The appearance of lymphocytic lymphoma occurred in study A earlier among treated groups than among controls. Lymphomas were observed in 1 low-dose male, 1 mid-dose female and 2 males and females from the high-dose group between week 31 and week 37. Lymphomas were not observed among controls until week 85 and 70 for males and females, respectively. Increased incidence of lymphocytic lymphoma or acceleration of the appearance of the lymphoma were not observed in study B. The authors state that study B was conducted to determined whether Curry Red had an effect on the appearance of lymphocytic lymphoma (a different strain of mice was used). A critical analysis of the data failed to establish a relationship between the incidence of lymphoma and Curry Red. The authors state that no compound-related adverse effects were observed.

Ref.: 5

Human studies

No data.

2.10. Special investigations

No data

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

The percutaneous absorption study results cannot be used to calculate the MoS. Therefore, the worst case of 100% will be used in the calculation. The Notes of Guidance (SCCNFP/0690/03) indicate a weekly use of 35 ml for a semi-permanent hair dye, with a retention factor of 0.1.

Absolute worst case calculation (assuming a daily use of 35 ml and a relative density of approximately 1.0 for the hair dye formulation) :

Maximum absorption through the skin	A (%)	=	100 %
Typical body weight of human		=	60 kg
Daily exposure to hair dye formulation		=	35 g/day
Retention Factor		=	0.1
Concentration of dye in the formulation		=	0.4 %
Systemic exposure dose (SED)		=	0.233 mg/kg/day
No observed adverse effect level (mg/kg)	NOAEL	=	700 mg/kg

Margin of Safety	**NOAEL / SED**	**=**	**3000**

2.12. Conclusions

Toxicity

Numerous oral toxicity studies administering *Curry Red* via gavage, drinking water or feeding to different species and for different treatment lengths are reported in the literature. The data package also covers long-term toxicity studies in two different rodent species and in dogs.

Although the studies do not fulfil the current OECD, EU or EPA guidelines, a scientifically sound evaluation of the systemic toxic potential of *Curry Red* after repeated application can be drawn, taking together all available data obtained with different species under different exposure scenarios.

In summary, toxic effects were only noted, if at all, at high doses (> 1000 mg/kg bw/day) independent of the exposure duration and species. No specific target organ for the systemic toxicity of *Curry Red* was found in rodents or any other species. In none of the combined long term toxicity/carcinogenicity studies in two rodent species any indication was found, for *Curry Red* being carcinogenic. Based on this finding, a NOEL of 1.39 % *Curry Red* in the diet, was deduced from this study by the study authors.

Based on the available information, the study is considered to be scientifically valid and suitable for a NOEL deduction for a repeated exposure scenario. The SCF and the JECFA also used this study to derive an ADI of 7 mg/kg bw/day for this compound. The derived NOEL would be based on the mid dose male group receiving 1.39 % *Curry Red* in the diet and being equivalent to 701 mg/kg bw/day.

Thus the corresponding NOAEL of 700 mg/kg bw/day can be used as the reference figure for the final risk assessment according to the SCCNFP-guidelines. However, it should be noted that compared to the expected frequency via application in hair colorants, the exposure duration in this study clearly represents a worst case scenario.

Skin irritation
Experimental findings with regard to skin compatibility in rabbits and in humans are available as short summaries lacking key information (e.g. concentration tested, concentrations causing effects after repeated applications, etc.) or were obtained under non-standard test conditions. However, taken together, the available information suggests, that Curry Red is not likely to cause skin irritation, especially at the intended maximum concentration in hair dye formulations of 0.4%.

Eye irritation
Curry Red was considered non-irritant when assessed for eye irritation potential with HET-CAM and Neutral-Red Uptake *in vitro* assays. These assays have limitations for use with coloured substances. Neither test has been validated.

Sensitisation
In the local lymph node assay, Curry Red did not reveal any potential to be a skin sensitiser at tested 4 %. Based on these findings Curry Red is evaluated not to be a skin-sensitiser.
In a provocation test (10 – 14 repeated patch applications for 48 h contact) with 200 volunteers, the neat test item and a 25 % dilution in water revealed neither skin irritating nor skin sensitising properties after 72 h skin contact. In a Draize-Shelanski repeated insult patch test (induction: 10 applications, challenge 14 days later) no indication for a skin irritating or a skin sensitising potential was found.

Percutaneous absorption
The percutaneous absorption study cannot be accepted. Therefore, the worst case of 100% was used in the calculation of the Margin of Safety.

Mutagenicity/Genotoxicity
Curry Red (C174) was tested for gene mutation in mammalian cells and for structural/numerical chromosome aberrations in bone marrow cells of mice.
Data from literature indicate that this substance could be considered mutagenic on *Salmonella* but the study cannot be fully evaluated because of the lack of basic information on the results and protocol.
C174 does not induce gene mutations on the thymidine kinase locus of mouse lymphoma cells; however, because of some protocol inadequacy the Authors decided to repeat the study. The *in vivo* study on the induction of MN on mice cannot be evaluated because of inadequacy of the test report (lack of the number of normochromatic mature erythrocytes) which did not allow to evaluate the presence of the substance in the target cells.

Carcinogenicity
Long-term carcinogenicity studies with rats and mice do not indicate that Curry Red represents a cancer hazard.

2.13. References

1. Otterstätter, G. (1995): Die Färbung von Lebensmitteln, Arzneimitteln, Kosmetika. Behr's Verlag.
2. DFG (1991): Kosmetische Färbemittel, VCH Weinheim, page 237-244
3. Poulsen, E. (1991): Safety evaluation of substances consumed at technical ingredients (food additives). Food Additives and Contaminants **8 (2)**, 125-134.

4. Borzelleca, J.F. et al. (1989): Lifetime toxicity/carcinogenicity study of FD & C Red No. 40 (Allura Red) in Sprague Dawley rats. Food and Chemical Toxicology **27 (11)**, 701-705.
5. Borzelleca, J.F. et al. (1991): Lifetime toxicity/carcinogenicity studies of FD & C Red No. 40 (Allura Red) in mice. Food and Chemical Toxicology **29 (5)**, 313-319.
6. Fulton Jr, J. E. (1989): Comedogenicity and irritancy of commonly used ingredients in skin care products. J. Soc. Cosmet. Chem. **40**, 321-333.
7. Bracher, M and Faller, C (2002): Assessment of the eye irritation potential of ALLURA Red AC (WR 201674) in the hen's egg test on the chorioallantoic membrane (HET-CAM). Study number HC 139, Cosmital SA, Switzerland.
8. Bracher, M and Faller, C (2002): Assessment of the eye irritation potential of CI-Nr. 16035 by cytotoxicity measurement in the neutral red uptake assay (NRU) on human keratinocytes (HaCaT). Study number NU 118, Cosmital SA, Switzerland.
9. Christ, M. (2002): CI 16035; FD&C RED 40 - Local lymph node assay. Study report number 762/008, MDS Pharma Services, France.
10. Collins, T.F.X., et al. (1989): Study of the teratogenic potential of FD & C Red No. 40 when given by gavage to rats. Food and Chemical Toxicology **27 (11)**, 707-713.
11. Wollny, E. and Röder A.(2002): Skin permeability in vitro absorption through porcine ear skin with CI 16035, FD+C RED No. 40 WR 20674. Study No. 724902, RCC-Cytotest Cell Research GmbH, Germany.
12. Prival, M.J. et al. (1988): Evaluation of azo food dyes for mutagenicity and inhibition of mutagenicity by methods using *Salmonella typhimurium*. Mutation Research **206**, 247-259.
13. Wollny, H-E. (2002): Cell Mutation Assay at the Thymidine Kinase Locus ($TK^{+/-}$) in Mouse Lymphoma L5178Y cells with FD+C Nr. 40 WR 20674. Study No. 726701, RCC-Cytotest Cell Research GmbH, Germany.
14. Honavar, N. (2002): Micronucleus Assay in Bone Marrow Cells of the Mouse with FD+C Nr. 40 WR 20674. Study No. 726705, RCC-Cytotest Cell Research GmbH, Germany.
15. Wella AG A2001/345; Jan. 28, 2002. LAN-Analysenbericht
16. Certificate of Analysis by Ellis & Everard

3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the use of Curry Red as a hair colouring agent ('direct' dye) in semi-permanent hair dye formulas at a maximum concentration of 0.4% in the finished cosmetic product does not pose a risk to the health of the consumer.

4. Other considerations

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5. Minority opinions

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