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

CONCERNING

ACID RED 18

COLIPA n° C175

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 Acid Red 18 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

Acid Red 18 is listed as CI 16255 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.

Acid Red 18 was approved in 1983 by both JECFA and the SCF for use as a food colorant (E124). The ADI was set at 4 mg/kg bw.

2.1.1. Primary name

Acid Red 18 (INCI)

2.1.2. Chemical names

Chemical name	:	Trisodium 7-hydroxy-8-[(E)-(4-sulfonato-1-naphthyl)-diazenyl)]-1,3-naphthalenedisulfonate
CAS name	:	7-Hydroxy-8-[(4-sulfo-1-naphthalenyl)azo]-1,3-naphthalenedisulfonic acid, trisodium salt
Synonyms	:	6-Hydroxy-5-[(4-sulfonaphth-1-yl)azo]-2,4-naphthalenedisulfonic acid, trisodium salt

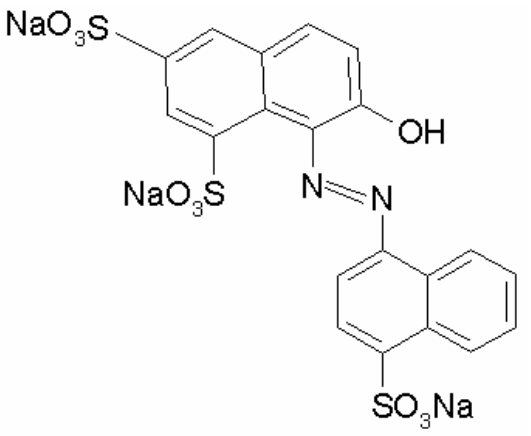
2.1.3. Trade names and abbreviations

Trade name	:	Covacap Rouge W 3102(LCW), Sicovit Cocineal Red 80 E124
COLIPA n°	:	C175
Other names	:	Cochineal Red A, Food Red 7, E124, Ponceau 4R, Food Red 102 (Japan)

2.1.4. CAS / EINECS /COLOR INDEX number

CAS	:	2611-82-7
EINECS	:	220-036-2
Colour Index	:	CI 16255

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{20}H_{11}N_2Na_3O_{10}S_3$
Mol weight : 604.5

2.1.7. Purity, composition and substance codes

Batch No. : Lot J00207
FDA certificate
Total colour content : > 82 % ($TiCl_3$ method); 90.4% (spectrophotometric at 510 nm)

Purity

NMR quantitative	:	> 82% (w/w)
HPLC qualitative	:	> 99% (w/w), loss on drying : < 10% (w/w)
Water content	:	< 10% (w/w)
Sulfated ash	:	< 45% (w/w)
Sum chloride + sulfate (calculated as Na-salts)	:	5.8 %
Water insoluble matter	:	0.01%

Impurities

4-Amino-1-naphthalensulfonic acid	:	< 0.1% (w/w)
7-Hydroxy-1,3-naphthalene disulfonic acid	:	< 0.05% (w/w)
Lead	:	< 20 ppm
Mercury	:	< 1 ppm
Arsenic	:	< 2 ppm
Iron	:	< 100 ppm
Water insoluble master	:	0.01%

Solvent residues : < 100 ppm (methanol, ethanol, isopropanol, n-propanol, acetone, ethyl acetate, methyl ethyl ketone and monochlorobenzene, all inclusive)

2.1.8. Physical properties

Appearance : Dark Red powder

Melting point	:	349.8°C (calculated by QSAR)*
Boiling point	:	880°C (calculated by QSAR)*
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	3.29 E-28 (calculated by QSAR)*
Log P_{ow}	:	1.63 (calculated by QSAR)*

* See General Comments below

2.1.9. Solubility

Water	:	> 20 % (w/w) (pH 9.8), Al-lake insoluble in water, but soluble in HCl
DMSO	:	8.4% (w/w)
Ethanol/water 4/6:		9%
Acetone/water 1/1:		> 10%

2.1.10 Stability

Stability of in a 10% (w/v) solution in water stored in dark at room temperature	:	100%
Stability of Acid Red 18 in a hair dyeing formulation stored for 10 months	:	100%

General comments on analytical and physicochemical characterisation

* 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).

2.2. Function and uses

Acid Red 18 is proposed for use as a direct dye in semi-permanent hair dye formulations at a maximum concentration of 0.5%.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

The dossier presented does not contain any study reports but is based on published literature. The acute toxicology and repeat dose toxicity paper was published in 1967. These studies were performed before the OECD guidelines were promulgated. The publication seems to be of a high standard but there are lacunae in the data.

2.3.1. Acute oral toxicity

In rats fed Acid Red 18, the LD_{50} is >8000 mg/kg bw. Coloured faeces are produced indicating the probable main route for excretion. After a single intraperitoneal injection, LD_{50} in rats was 0.6 and 2.6 mg/kg bw male/female respectively. In mice, the LD_{50} was 1.6 and 1.9 mg/kg bw male/female respectively. Evidence of tubular necrosis was seen. Tubular regeneration was seen in animals that survived the 7-day observation period.

Ref.: 4

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

No data

2.3.5 Repeated dose dermal toxicity

In rats, given up to 2% Acid Red 18 (approximately 1000 mg/kg bw/day) in diet, the main treatment-related effects were increased serum transaminase in both sexes and decreased haematological indices (depressed red cell counts, decreased haemoglobin concentration and haematocrit value) mainly in females. Based on the increased transaminase levels (suggesting potential liver injury) and blood parameters in the high dose group, the NOEL was considered to be 500 mg/kg bw/day (1 % Acid Red 18 in the diet).

Ref.: 4

At higher doses (> 1250 mg/kg bw/day) administered in other rat studies, effects were noted in the liver, heart, adrenal gland, caecum, brain arteries, testes and bodyweight development.

Ref.: 2

Mild and transient anaemia (reduced haemoglobin, haematocrit and red blood cell number) was seen in male pigs given 900 mg/kg bw/day in the diet for 90 days. A NOEL of 300 mg/kg bw/day was derived from this study.

Ref.: 2, 3

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

No data

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

Wistar rats were fed *Acid Red 18* in diet equivalent to 0, 50, 500 and 1250 mg/kg bw/day for 9 weeks prior to mating. The offspring were given equivalent doses to the parents, adjusted weekly for bodyweight from Day 21. Exposure continued until survival of either sex in any group reached about 20 % (114 and 118 weeks male/female).

All treated animals had a pink-coloured fur at all stages. In the two highest dose groups, red-coloured urine and faeces were produced. At week 78, all high-dose males showed diarrhoea.

Mortality rates between controls and treated groups were comparable at all times during the study. High-dose animals had lower bodyweight compared with controls from week 9 onwards. Food intake was higher in all treated groups, but not dose-related. Water intake showed a dose-related increase in two highest dose groups.

There were some haematological and biochemical changes that were not treatment-related. Urine concentration showed some variations in males and females, and in females of the high-dose group a higher protein-concentration in urine was found. The caecum and the testes showed treatment related weight differences. In the two higher doses, the full caecum and the caecal wall had significantly higher weight in both sexes. This was not considered an adverse effect but as a non-specific adaptation to an increase in the amount of osmotically active material in the intestinal content, linked with the increase in water intake and the observed softening of the faeces. The testes weights (absolute and relative) were elevated in the 500 and 1250 mg/kg bw/day male groups. This was not statistically significant compared with the concurrent control group. The relative brain weight was statistically significantly increased in high-dose males. The kidney weight was elevated at 50 and 1250 mg/kg bw/day (being statistically different to control) in males. Taken together with the increase in proteinuria in high dose females, this finding indicates that there might be some effect on the kidney at 1250 mg/kg bw/day *of Acid Red 18.* No other dose-related effects were noted with regard to organ weights.

Other than increased renal pelvic calculi at 1250 mg/kg bw/day in both sexes and 500 mg/kg/ bw in males, the authors of the paper considered there were no clear treatment related effects and concluded a NOEL of 500 mg/kg bw/day from this study. They considered other pathological findings as small and restricted to one sex.

In 1983, the SCF and the JECFA used this study to derive an ADI of 4 mg/kg bw/day for *Acid Red 18.* The final rationale for this decision is not known. The committees may have based this on the figures for food consumption and bodyweight given in the original report or used a slightly higher Safety Factor of 125 instead of 100.

The SCCNFP is concerned that some data reported by the authors of the paper is not considered relevant. The rationale for these decisions is unclear since there is scant data. There was a statistically significant increase in thyroid weight in females at 500 mg/kg bw/day and incidences of 'increase in degeneration of the granular layer neurones in the cerebellum' in mid and high-dose females.

Ref. : 5

A summary of a long-term toxicity/carcinogenicity study (82 weeks) in mice was cited. Acid Red 18 was in the feed at concentrations of 0.01, 0.05, 0.25 and 1.25 % (equivalent to about 14, 70, 350 or 1750 mg/kg bw/day). 'There were effects on the kidney and liver in both sexes, as well as short-lived anaemia in males, at dietary levels of 1750 mg/kg bw/day given for 82 wk. The mild transient anaemia was also apparent at 350 mg/kg bw/day in males and females, as was the effect on the kidney in females. No adverse effects were noted in animals dosed at 70 mg/kg bw/day.' The only treatment-related effect on tumour incidence was an increase in tumours of the ovary, which were found in 1, 1, 2 and 4 females of the four dose groups, respectively. Although these were not seen in any of the 60 female controls, the investigators noted that this tumour type had been seen at a similar incidence (3 out of 42) in a previous untreated control group in the same laboratories. These findings were therefore considered to be unrelated to treatment, and it was concluded that Acid Red 18 was not carcinogenic in mice.
This study was available for the SCF and JECFA evaluation, but was not used for the ADI evaluation.

Ref.: 2, 3

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

No data

2.4.2. Irritation (mucous membranes)

***In vitro* Irritation Potential – HET-CAM**

Guideline	:	/
Species/strain	:	fertilised fresh chicken eggs
Group size	:	6
Test substance	:	IT 413, Red 102
Batch number	:	lot J00207
Purity	:	not given
Dose	:	1% in water
GLP	:	reported in compliance, but not inspected

The HET-CAM assay was carried out according to the procedure developed for the COLIPA validation study on alternatives to the Draize Rabbit Eye Irritation Test (Brantom PG et al, Toxicology in Vitro 1997; 11: 141- 179).
300 µl of a 1% dilution of the test substance was exposed to the CAM of each prepared egg. The substance remained in contact with the CAM for 30 seconds and then rinsed off with physiological saline. Appropriate controls were used.
Results

The 1% dilution of the test substance caused no irritation to the CAM of fertilised chicken eggs. Based on these results a 1% aqueous dilution of the test substance was classified as a slight irritant according to the COLIPA classification system for assessing eye irritation, where "slight" is the best category obtainable.

Ref.: 6

Cytotoxicity in the neutral red uptake assay (NRU) on human keratinocytes

Guideline	:	/
Cell line	:	HaCaT human keratinocytes
Test substance	:	IT 413, Red 102
Batch number	:	lot A003401
Purity	:	not given
Dose	:	1% in water
GLP	:	reported in compliance, but not inspected

The NRU assay was carried out according to the procedure developed for the COLIPA validation study on alternatives to the Draize Rabbit Eye Irritation Test (Brantom PG et al, Toxicology in Vitro 1997; 11: 141- 179) with the modification that human keratinocytes of the HaCaT cell line were used, and the treatment performed in serum-free culture medium. Two independent NRU assays with identical doses were performed. Doses from 681 – 10000 µg/ml were applied together with appropriate controls. No NRU-50 value could be determined from the cytotoxicity curves as the viability was still 61% in the first assay and 70% in the second, so the NRU-50 is reported > 10000 µg/ml, and hence the test item classified as non-irritant.

Ref.: 7

2.5. Sensitisation

Local Lymph Node Assay (LLNA)

Guideline	:	Skin sensitization, Local Lymph Node Assay (OECD 429)
Species/strain	:	CBA/J mice
Group size	:	55 females, divided into 11 groups of 5 animals.
Test substance	:	CI 16255, Red 102
Batch number	:	J00207
Purity	:	84.2 weight % (NMR) and 99.7 area% (HPLC)
Dose	:	0.5–4% in DMSO and in acetone/aqua (1:1) i.e. AA mixed with olive oil (4:1)
GLP	:	In compliance

On days 0, 1 and 2 the animals received 25 µl of one of the test preparations or vehicle on the dorsal surface of each ear. On day 5 all mice received intravenous injection of tritium labelled thymidine in phosphate buffered saline, and 5 hours later they were killed humanely and the draining auricular lymph nodes were removed. Single cell suspensions were prepared for each animal, appropriately treated and measured by liquid scintillation counting.

Results
The stimulation indices were less than 3 at all tested concentrations, hence an EC3 value could not be calculated, and the test substance was classified as non-sensitizing in the vehicles tested.

Ref.: 8

2.6. Teratogenicity/Reproduction toxicity

The dossier presented does not contain any study reports but is based on published literature. The papers included in the dossier were published in 1981 and 1987. The studies were not performed to OECD guidelines and GLP was not indicated.

Acid Red 18 was fed to groups of 12-16 pregnant (vaginal plug positive) STD-ddY mice (10 weeks old, 26-31 g) at doses of 0, 0.04, 0.2 and 1.0 % in the diet from day 0 to day 18 of gestation (Group A) or during gestation, the 21 day lactation period and for further 35 days (group B). General appearance and wellbeing as well as bodyweight and food consumption were recorded daily.

At gestation day 18, half of the animals were killed under ether anaesthesia and a caesarean section was performed. The presence of resorption sites and foetuses (live or dead) was examined. The number of implantation sites and corpora lutea was also determined. Each live foetus was weighed, sexed and examined for gross external malformations. After fixation and staining a skeletal examination of the foetuses was performed.
The remaining dams were allowed to deliver and the postnatal development of newborn animals was examined for 56 days after birth. For each litter, number of pups, stillbirths and live births and the presence of gross anomalies were noted, the bodyweight of the neonates was determined weekly and the development (e.g. separation of ear auricula, eruption of low incisors, descent of testes, etc.) examined. After weaning, the Fl generation was treated further up to the 56 day after birth and food consumption was measured weekly. The general appearance, behaviour and survival of the offspring were checked daily.

No treatment-related effects in dams were noted with regard to bodyweight, food consumption, clinical observations and post-mortem findings. The uterus weight with foetuses, the number of corpora lutea, and implantations were similar to control in all treated groups.

There were no treatment related effects with regard to litter size, foetal mortality, foetal bodyweight or external malformations. Similarly, no treatment-related skeletal effects were noted.

The postnatal development was not affected by *Acid Red 18*. No effects were noted on bodyweight development, except in the high dose group where there were significantly higher values for survival rate and lactation index. No adverse effect was noted with regard to the general and sexual development of the offspring. A NOEL of > 1 % in the diet, approximately 1200 mg/kg bw/day was derived from this study.

Ref.: 9

In other studies, particularly a 3-generation feeding study in rats, there were no indications of toxicity to the developing foetuses up to the highest dose, 1250 mg/kg bw.

Animals in all dose groups showed a pink coloration of the fur and soft faeces in the two highest dose groups. No treatment-related effects were noted with regard to the observed mortalities, food intake and bodyweight development. Neither fertility nor pup development was affected in a dose-related manner by the treatment with *Acid Red 18*. In the teratology parts, no differences were found between control and treated groups in any generation.

Several lesions were recorded at necropsy, with higher frequency noted in treated groups for mottled livers, speckled thymus, renal stones and enlarged caecum (especially in the high-dose

group). None of these effects were consistently noted for all generations. The weight of the caecum (full or empty) showed a dose related increase for both the absolute and the relative weight in almost all generations and is most likely linked to the observed enlarged caecum. This effect is noted in older animals, but in young animals it was similar to controls. There were no further dose-related changes in organ weights or were only seen in one generation. No treatment related effects were seen histologically.

Based on the findings in this 3-generation study with Acid Red 18, A NOAEL of 1250 mg/kg bw/day was derived for dams, the unborn, pups, neonates and young adult rats.

Ref.: 10

2.7. Toxicokinetics (including Percutaneous Absorption)

Percutaneous absorption *in vitro*

Guideline	:	OECD 428 (2000)
Test system	:	split thickness pig skin (300-400 µm), 5-6 samples / experiment
Contact time	:	30 minutes under occlusion (donor chamber covered with parafilm)
Test substance	:	Exp. I : *CI 16255, Red 102 WR 23087*, dissolved in acetone/water 1:1 (20 mg/ml)
		Exp. II : *CI 16255, Red 102 WR 23087*, in a typical hair dye formulation (ref. 8150992A), composition partially stated
Control	:	caffeine, tested every 3 months, results available
Purity	:	97.9 %
Batch no	:	J 00207
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	:	GLP statement present, though unsigned

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 16255/ml solution was applied.

Experiment II : CI 16255 was applied in a typical hair dye formulation (ref. 8150992B), 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 1.03 µg/cm² in the first and 0.96 µg/cm² in the second experiment.

The individual amounts found in the upper dermis extract were:
Experiment I : 0.13 - 0.10 - 0.48 - 0.05 - 0.02 µg/cm².
Experiment II : 0.03 - 0.04 - 0.04 - 0.04 - 0.05 - 0.02 µg/cm².

The individual amounts found in the epidermis extract were:
Experiment I : 0.75 - 0.23 - 12.3 - 1.93 - 0.30 - 0.49 µg/cm².
Experiment II : 0.57 - 2.53 - 2.18 - 0.38 - 1.20 - 0.81 µg/cm².

The mean recovery of the test item was 101.6 % in the first and 91.7 % 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 the lowest detection limit in the receptor fluid was 0.021 µg/ml. When measurements reached this value, they were "calculated" using an unclear methodology.
- There is a large variability in the skin extract measurements, which makes it impossible to perform a correct assessment of the percutaneous absorption of CI 16255.

For all the above-mentioned reasons, the percutaneous absorption study cannot be accepted.
Ref.: 12

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Mutation Reverse Testing Using Bacteria

Acid Red 18 (86.9 %) was been tested on *S. typhimurium*, TA 1535, TA1537, TA1538, TA98, TA 100 in the absence and in the presence of Aroclor induced rat or hamster liver homogenate at the doses between 333 and 10000 µg/plate. The results indicate that the test item is non mutagenic.

Ref.: 13

***In* vitro Mammalian Cell Gene Mutation Test**

Acid Red 18 (86.9 %),has been tested on Mouse Lymphoma L 5178 Y (Thymidine Kinase Locus) in the absence and in the presence of Aroclor induced rat liver homogenate at doses of 4857; 6143; 7429; 8714; 10000 µg/ml (-S9) and 500;750;1000;1250;1500 µg/ml (+S9),for 4 hours. The results indicate that the test item is non mutagenic.

Ref.: 13

2.8.2 Mutagenicity/Genotoxicity *in vivo*

***In* vivo Mammalian Erythrocyte Micronucleus Test**

Guideline	:	OECD 474 (1997)
Species/strain	:	NMRI mice (5animals/sex/group)
Test item	:	RED 102 WR 23087
Batch no.	:	J00207
Purity	:	84.2 weight % (NMR spectrum)
Dose level	:	500, 1000, 2000 mg/kg (24 h); 2000 mg/kg (48 h)
Administration	:	oral
Positive contr.	:	CPA, orally, 40 mg/kg
GLP	:	in compliance

Results
Toxicity: In a preliminary experiment, a dose of 2000 mg/kg induced several types of toxic effects during a period of 48 h of observation.

Mutagenicity: the positive control (CPA) induced a high percentage of MN in PCEs; the test item treated animals presented the same range of MN per 2000 PCEs as the untreated animals. There was no sign of toxicity in the bone marrow cells in the test item treated animals; there was no indication of the presence of the test item in the target cells.

Conclusion
The study is not adequate for the evaluation due to the absence of a demonstration that the *in vivo* target cells have been exposed to the test item.

Ref.: 15

Acid Red 18, purity of 91 %, was given once by i.p. to mice and analyzed after 24 h; the doses were: 300, 600, 1200 and 2400 mg/kg. No MN induction was observed.

The paper reports that the test item has been found to induce chromosome aberrations on mammalian cells treated *in vitro*.

Ref.: 16

2.9. Carcinogenicity

Oral administration, rats

Male and female Wistar rats of the F0 generation received Acid Red 18 (purity 81%) in the diet providing 0 (control), 50, 500 or 1250 mg Acid Red 18 per kg body weight/day for 60 days. The control groups consisted of 114 males and females and the treated groups of 66 males and females. The animals were mated and allowed to rear their litters. At weaning, pups were selected for the long-term study to give treated groups 54 animals of each sex and a control group 96 males and females, with offspring always receiving the same treatments as their parents. Treatments continued until approximately 20% of animals survived, resulting in duration of 114 weeks for males and 118 weeks for females. Bodyweight, food and water intake and clinical conditions were monitored regularly throughout the study. At the end of the study each animal was autopsied, selected organs were weighed and a full range of tissues were prepared. High dose animals showed a lower body weight gain without any reduction in food intake. Water intake was higher than in the controls in the medium and high dose groups, and this was related to caecal enlargement and softening of faeces. No adverse changes were seen in the investigation of blood or urine apart from a higher incidence of females with higher level of proteins in the urine at the high dose. No other findings of significance were seen and survival and tumours incidence were similar in all groups.

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		=	0.5 %
Systemic exposure dose (SED)		=	0.29 mg/kg/day
No observed effect level (mg/kg)	NOAEL	=	1000 mg/kg
Margin of Safety	**NOAEL / SED**	**=**	**3429**

2.12. Conclusions

Acid Red 18 has low toxicity. In rats fed Acid Red 18, the LD_{50} is >8000 mg/kg bw. A NOEL was considered to be 500 mg/kg bw/day (1 % Acid Red 18 in the diet) in rats. Acid Red 18 was not considered maternotoxic or foetotoxic.

No data on skin irritation. 1% aqueous solution of Acid Red 18, assessed for eye irritation potential with HET-CAM and Neutral-Red Uptake *in vitro* assays, was slightly irritating in a HET-CAM and non irritant in the NRU assays. These assays have limitations for use with coloured substances. Neither test has been validated.
Acid Red 18 was classified as a non-sensitiser.

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 test item has been tested on mice for the induction of MN in the bone marrow cells. The study is inadequate as it does not provide demonstration that the compound has reached the target cells *in vivo*.

In a series of published papers, the test item has been found negative for the induction of gene mutations in bacterial and in mammalian cells and positive for the induction of chromosome aberrations on mammalian cells. It is possible that the metabolic activation system employed in these experiments is not the most adequate for the chemical class of the test item (azo-dye). The data are insufficient for a conclusive evaluation of the potential mutagenicity/genotoxicity

A long-term carcinogenicity study with rats did not indicate any cancer hazard.

2.13. References

1. Otterstätter, G. (1995): Die Färbung von Lebensmitteln, Arzneimitteln, Kosmetika. Behr's Verlag.
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3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the use of Acid Red 18 as a hair colouring agent ('direct' dye) in semi-permanent hair dye formulations at a maximum concentration of 0.5% 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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