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

CONCERNING

ACID YELLOW 23

COLIPA n° C29

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 Yellow 23 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 Yellow 23 is listed as CI 19140 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 Yellow 23 is permitted as a food colorant (E102) in the EEC. It is also listed in the priority-based Assessment of Food additives for use in the USA. JECFA and the SCF have set an ADI of 7.5 mg/kg.

2.1.1. Primary name

Acid Yellow 23 (INCI)

2.1.2. Chemical names

Trisodium 5-oxo-1-(4-sulfonatophenyl)-4-[(E)-(4-sulfonatophenyl)diazenyl]-4,5-dihydro-1H-pyrazole-3-carboxylate (IUPAC)
Trisodium 5-hydroxy-1-(4-sulphophenyl)-4-(4-sulphophenylazo)pyrazole-3-carboxylate
1H-Pyrazole-3-carboxylic acid, 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl) azo]-trisodium salt (C index name 9CI)
4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-((4-sulfophenyl)azo)-1H-pyrazole-3-carboxylic acid, trisodium salt

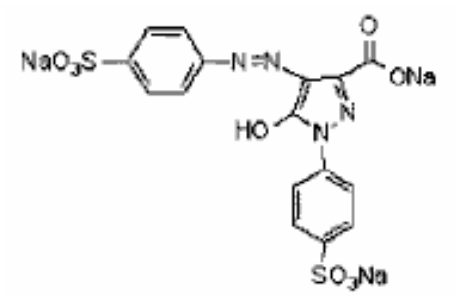
2.1.3. Trade names and abbreviations

COLIPA n^{o}	:	C 29
Trade names	:	Covacap Jaune W 1100 (LCW) Sicovit Tartrazine 85 E 102 (BASF)
Other names	:	Tartrazine Food Yellow 4 E 102 Amarillo Japan Yellow 4 FD&C Yellow No. 5

2.1.4. CAS no. / EINECS /COLOUR INDEX number

CAS	:	1934-21-0
EINECS	:	217-699-5
Colour Index	:	19140

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{16}H_{12}N_4O_9S_2$
Mol weight : 534.37

2.1.7. Purity, composition and substance codes

Substance code : A003109
Batches used : 3098GA; FDA certified Lot AJ9508
Purity : > 87 % (NMR quantitative)- see General comments below

Relative chromatographic purity
(HPLC - UV/VIS peak area method): > 98% at 254 nm

Loss on drying : < 10 %
Water content : < 10 %
Ash content : < 45 % (sulphated)
Loss on drying : < 10 %

Potential impurities

Sulfanilic acid sodium salt	:	< 0.2 weight %
Pyrazolone T ethyl ester	:	< 0.2 weight %
Pyrazolone T	:	< 0.2 weight %
Aniline	:	< 100 ppb
Benzidine	:	< 1 ppb
4-Aminobiphenyl	:	< 5 ppb
4-Aminoazobenzene	:	< 75 ppb
Azobenzene	:	< 40 ppb
Lead	:	< 10 ppm
Mercury	:	< 1 ppm
Arsenic	:	< 2 ppm
Iron	:	< 100 ppm

Solvent residues
No 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	:	Orange powder
Melting point	:	349.8°C (calculated by QSAR)*
Boiling point	:	870°C (calculated by QSAR)*
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{OW}	:	– 10.17 (calculated by QSAR)*
pKa	:	/

* see General Comments below

2.1.9. Solubility

Soluble in water (quantitative data not reported).

2.1.10 Stability

Stability data provided for a common market formulation are not acceptable.

General comments on analytical and physico-chemical characterisation

* The information provided on the compound is incomplete and confusing, not conforming to SCCNFP Notes of Guidance. There is confusion between absolute content of the dye and its chromatographic purity determined by the relative peak-area HPLC method. According to a FDA certificate for the same batch, the purity is 93% (instead of 87%).

* 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 pKa are reported for the 3 ionisable groups.

* The reported stability data on a common market formulation are based on a single determination and comparison of the result with a "theoretical" content"; this is not an acceptable stability test.

2.2. Function and uses

Acid Yellow 23 is proposed to be used as a hair colouring agent (”direct” dye) in semi-permanent hair dye formulas at a maximum concentration of 0.5% in the finished cosmetic product.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

The dossier presented does not contain any study reports but is based on published literature. The acute toxicology study was published in 1985. It was considered inadequate.

Ref.: 1

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

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

Acid Yellow 23 was administered to rats in drinking water. In the 5 % dose group, 6 males and all females died. Body weight gain was depressed by more than 10 %. At this dose, significant lower absolute organ weights of thymus, lungs, heart, liver, spleen, kidneys (no information whether both sexes were affected), and testes were measured. However, the relative organ weights of the brain, lungs, adrenals, kidneys, and testes were increased by 5 %, but the relative thymus weight in males was decreased. At the 2.5 % dose level, the absolute liver weights in both sexes and the relative liver weight in females were significantly lower. Histological examination showed severe atrophy and/or degeneration in haematopoietic organs (thymus, bone marrow, lymph nodes and spleen) in both sexes

of the 5 % group animals that died during the experiment. No information was given on histology of animals at the end of treatment.
No NOAEL in mg/kg/day could be derived due to the missing data on water consumption.

Ref.: 2

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No chronic study was provided. Several carcinogenicity studies were provided but there were many data gaps. It would seem from these studies that other than coloured fur and faeces there were no dose-related effects from Acid Yellow 23. A NOAEL was derived of 5% in food, equivalent to 2640 and 3348 mg/kg/day in males and females respectively.

Ref.: 9, 2, 3

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

No data concerning skin irritation of Acid Yellow 23 are available. No conclusions from limited reported patch tests are possible.

2.4.2. Irritation (mucous membranes)

Repeated eye irritation study in rabbits

Guideline	:	/
Species	:	New Zealand White rabbits
Group size	:	6 of each sex
Test substance	:	Acid Yellow 23; commercial sample; no information on purity
Batch No.	:	/
Purity	:	/
Dose levels	:	3 % (w/v) in aqueous solution
Route	:	ocular
Exposure	:	1 application daily for 21 days
GLP	:	not in compliance : animals were kept according to "Good animal husbandry" (NIH, 1985)

30 µl of test solution (3 % (w/v) Acid Yellow 23 in aqueous solution with 0.5 % (w/v) hydroxypropyl methylcellulose and 0.25 % (w/v) laureth-10 acetate) or vehicle were administered to the right eye of 6 female and 6 male New Zealand White rabbits, followed by closing the eye for approx. 1 sec to prevent substance loss. Ocular irritation was determined according to a modification of the Draize test. All animals were checked for viability twice daily.

Ocular irritation was scored 24 h after each treatment and prior to the next instillation. On days 1, 3, 7, 14, and 21 the eyes were also evaluated 1 h after treatment. 24 h after each treatment additionally eye stain and particle depositions were scored. Ophthalmic observations were conducted on days 3, 7 and 14 always prior to instillation. The left eyes served as untreated controls.

Results
No lethality or significant clinical signs, and no substance-related weight change were observed. Except slight conjunctival redness or discharge, that were seen sporadically in the eyes of the animals, all animals were free of significant signs of ocular irritation. No significant signs of eye staining or particle depositions were observed. At ophthalmoscopic examinations, no ocular abnormities were noticed.

Conclusion
Acid Yellow 23 it is not expected to cause irritant effects following repeated treatment of eyes in a concentration of 3 % in aqueous solution.

Ref.: 3

2.5. Sensitisation

Maximisation (Magnusson and Kligman) Test

Guideline	:	OECD Guideline No. 406		
Species	:	Albino guinea pigs		
Strain	:	Ibm: GOHI (synonym: Himalayan spotted)		
Group size	:	15 (10 test, 5 control female animals)		
Test substance	:	Acid Yellow 23; commercial sample; certified colour content 93 %		
Batch No.	:	3098GA ; lot AJ9508		
Purity	:	/		
Dose levels	:	intradermal induction	:	5 % solution
		epidermal induction	:	50 % solution
		challenge	:	25 % solution
GLP	:	in compliance		

A 5 % Acid Yellow 23 solution (in 1 % carboxymethylcellulose solution; prepared with bi-distilled water) was applied in an emulsion of Freund´s Complete Adjuvant (FCA) for the intradermal induction. 1 week later, following treatment with sodium lauryl sulfate (SLS) the epidermal induction was conducted for 48 h under occlusion with a 50 % Acid Yellow 23 solution. 2 wk later the animals were challenged by epidermal application of Acid Yellow 23 (25 % solution) under occlusive dressing. Cutaneous reactions were evaluated at 24 and 48 h after removal of the dressing. For challenge reading, all animals were depilated 3 h before examination to remove the discolouration.

Results
None of the control and test animals showed skin reactions after the challenge treatment with Acid Yellow 23. The 50 % test item stained the skin orange, therefore it was not possible to determine whether erythema were present or not. However, no oedema was observed.

Ref.: 4

Human patch test, study 1

Guideline	:	/
Species	:	human
Group size	:	32 patients (20 women, 12 men) with a positive patch test reaction to p-aminoazobenzene (0.25 %)
Age	:	women : mean age 39.9 years; men: mean age 46.6 years
Test substance	:	Acid Yellow 23; no information on purity
Batch No.	:	/
Dose levels	:	2 %
GLP	:	/

Eleven patients had previously also shown sensitisation to para-phenylenediamine (PPD). 10 (4 women, 6 men) out of the 32 cases were negative to all the test allergens of the European standard series. 30 patients with an allergic contact dermatitis but negative to p-aminobenzene and to PPD were also tested as control group with the same patch test series. Patch tests were performed using uniform patch tests (no further information given).

Results
Acid Yellow 23 did not elicit an allergic reaction in any of this group.

Conclusion
The data are only of limited relevance for risk assessment.

Ref.: 31

Human patch test, study 2

Guideline	:	/
Species	:	human
Group size	:	28 patients
Age	:	not stated
Test substance	:	Acid Yellow 23 aluminium lake; no information on purity
Batch No.	:	/
Dose levels	:	5 %
GLP	:	/

Patients had pigmented cosmetic dermatitis. The test substance was mixed at 5 % in a vehicle composed of 88 % polyethylene glycol 400 and 12 % polyethylene glycol 6000. The test were fixed with A1-test on Dermicel® tape. The artificial ultraviolet light source used for photopatch testing was equipped with 15 fluorescent lamps: five with shortwave UVL (280-355 nm, max. 305 nm) and ten with long wave (320 – 440 nm. Max. 352 nm). Irradiation with UVL was carried out at a distance of 35 cm for 10 min of long wave exposure and ½ MED of shortwave exposure; the energy of the former estimated by the intensity estimator was found to be 7.2 mW/cm^2 (at 365 nm under these conditions). The results were read at 48 and 72 h after application.

Results
Acid Yellow 23 did not elicit an allergic reaction in the patch test. One of 28 persons developed a light erythema in the photopatch test.

Conclusion
The study is not in accordance with current standards. However it must be regarded as a hint on possible phototoxic effects of Acid Yellow 23.

Ref.: 32

Oral *'intolerance'* in humans

Guideline	:	/
Species	:	human
Group size	:	25 patients (15 males, 10 females) with symptoms suggesting food allergy
Age	:	18 - 153 months
Test substance	:	Acid Yellow 23; commercial sample; no information on purity
Batch No.	:	/
Dose levels	:	1, 10 mg
Route	:	oral
GLP	:	/

48 h prior to administration of Acid Yellow 23 (in opaque capsules) the children were submitted to a dye-free diet. The first dose was given on empty stomach, and a second dose was given 1 h later when the physical examination proved negative. After another hour, the children were examined again. If results remained negative, the dye-free diet was maintained for further 48 h to cover retarded symptoms. Results were judged as positive if either a reproduction of the previous symptoms was reached, or an objective worsening of the pre-existing patterns had been observed.

Results
3 patients gave 'immediate', and 2 gave 'retarded' precipitation of symptoms after oral administration of Acid Yellow 23.

Conclusion
The study is not in accordance with current standards.

Ref.: 5

***'Intolerance'* in humans**

Studies concerning the testing of Acid Yellow 23 for adverse reactions in humans listed in the following tables have been reviewed. It is known that in certain individuals adverse reactions described as 'food allergy' (pruritus, oedema, urticaria, asthma or rhinitis) result from ingestion of the food colouring Acid Yellow 23. In dermal testing of Acid Yellow 23, usually conducted as patch test, mainly negative results were gained. In the corresponding review, for none of the cited studies detailed information on material, guidelines, or GLP is given. Often, no information on study design is pointed out, and dose scheme is not explained. In several cases the information on observed effects is limited to the statement "reaction" or "no reaction", without further explanations on the type of reactions.

Ref.: 6

Oral challenge

n	Dose	Reaction	Previous allergic reactions / additional remarks
44	Up to 20 mg	No reaction in double-blind study	perennial asthma
13	0.22 mg	3 reacted in a double-blind study	chronic urticaria; 10 with aspirin intolerance
1	0.22 mg	Reaction	chronic urticaria
53	1 or 10 mg	6 reacted	urticaria or angioedema
111	1 or 10 mg	14 reacted in a single-blind study	urticaria; 11 of the 14 positive also react to aspirin
48	0.1-5 mg	11 % reacted	urticaria or facial oedema; patients have been children
39	0.1-10 mg	21 reacted (19 with urticaria); the additional 33 controls were negative.	recurrent urticaria or angioedema; 5 with asthma; 17 with aspirin sensitivity; multiple sensitivity to other azo dyes in 2/3 of *Acid Yellow 23* positives
12	1 or 10 mg	3, actually without urticaria, reacted at high dose; 2 with active urticaria reacted to both doses.	previous responses to 10 mg *Acid Yellow 23* (weals); some with urticaria
317	10 mg	40 reacted	urticaria; 20 of the positives also responded to salicylates
23	2-10 mg	7 reacted when tested without or with minimal symptoms of urticaria	urticaria; aspirin-sensitive
96	17 mg	20 reacted when tested during asymptomatic periods	urticaria and / or angioedema
277	1-50 mg	11 showed reduction in forced expiratory volume within 4 h	asthma; 11 positives also react to aspirin
15	25 mg	2 showed reduction in forced expiratory volume; maximum reaction after 30-45 min	asthma; aspirin-sensitive
80	25 mg	3 developed rhinorrhoea and bronchoconstriction; double-blind study	angioedema, rhinitis and / or asthma; 40 (including all 3 positives) also react to aspirin
114	0.1, 1, 10 mg (increasing dose)	20 % exhibited reduced pulmonary function; few cases of urticaria, itching, headache, rhinitis or facial oedema	asthma; many of *Acid Yellow 23* sensitive were also aspirin-sensitive.
40	0.22 or 0.44 mg	6 reacted with bronchospasm or urticaria; the additional 40 controls were negative; double-blind study	asthma or rhinitis
28	1.5 or 15 mg	1 showed reduced lung function	asthma
504	0.1-10 mg	14 developed reduced lung function or other pronounced responses; single-blind study	asthma or rhinitis
56	25 mg	no reactions; double-blind study	asthma; 5 reacted to aspirin; patients have been children
22	15 mg	no reactions; double-blind study	asthma
32		20 % revealed respiratory symptoms, urticaria, eczema;	respiratory allergy; patients have been children
122	50 mg	32 reacted (heat wave, weakness, blurred vision, nasopharyngeal secretions, feeling of suffocation, palpitation, itching, urticaria, prolonged bleeding time, angioedema); single-blind study	97 with various "allergic" disorders
8	1-5 mg	7 exhibited symptoms of asthma or urticaria.	aspirin-sensitive and/or urticaria suffers; all reacted also to various benzoates
1	1 mg	erythematous macules on the legs within 2 h; no effect on asthma	asthma; recurrent purpuric eruption on the legs
7	1-10 mg	5 developed purpuric skin lesions; administration at intervals of 1 - 2 h during asymptomatic periods	recurring allergic vascular purpura; 3 positives also reacted to aspirin, 1 positive reacted to sodium benzoate

3	tablets	3 adult patients developed various reactions (generalized urticaria, itching, oedema of the tongue and lips) after ingesting corticosteroid tablets dyed with *Acid Yellow 23*	not reported

n : number of patients

Sublingual challenge

n	Dose	Reaction	Previous allergic reactions / additional remarks
2	1000 ppm	both reacted	1 reacted to aspirin and suffered from collagen disease; 1 with asthma
506	not clear	4 reacted; single-blind study	one or more allergic conditions (asthma or rhinitis); statements on *Acid Yellow 23* dilution contradictory
100	1600 ppm or less	12 reacted (behavioural agitations, asthma, headache, rapid heartbeat).	variety of complaints (not further specified); 5 positives also react to aspirin

n: number of patients

Patch test

n	Dose	Duration	Reaction	Previous allergic reactions / additional remarks
100	5 % solution	20 min	No immediate reactions	atopy: 36; urticaria: 23; non-atopic dermatitis: 20
56	2 % solution	1 h	No immediate reactions; no reactions after 48 h	chronic urticaria 4 patients experienced wealing of skin after ingestion of 1 or 10 mg of the colouring
1	2 % solution	48-72 h	Clear reaction	dermatitis
1	Piece of dyed shirt	48-72 h	No reaction	dermatitis
1	Orange juice	48-72 h	No reaction	dermatitis
1	2 % solution	Not given	No reaction	recurrent asthmatic symptoms; positive reaction in oral challenge

n: number of patients

Skin-prick test

n	Dose	Reaction	Previous allergic reactions / additional remarks
1	1000 ppm	Strong reaction within 10 min	previous anaphylactic reaction due to an enema with soap dyed with *Acid Yellow 23* and sunset yellow; seasonal asthma; positive skin-prick test to various allergens

n: number of patients

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 1990, 1992 and 1988. They were not performed to OECD guidelines and GLP was not indicated. They were considered relevant for risk assessment as despite data gaps, sufficient animal numbers and relevant endpoints were examined

Mated, sperm-positive female rats were dosed with 0, 60, 100, 200, 400, 600 or 1000 mg /kg/day by gavage with Acid Yellow 23, from gestation day (GD) 0 to 19.

No substance-related differences between treatment groups and control group were noted in behaviour, external maternal findings, and body weight. The mean daily food consumption was significantly higher in the 1000 mg/kg dose group, indicating an effect on food utilization. No dose-related changes were observed in pregnancy rate, implantation efficiency, maternal findings, foetal viability or foetal development. Examination of offspring for external and skeletal variations revealed no dose-related increase of incidence. The NOAEL for teratogenicity of Acid Yellow 23 was > 1000 mg/kg/day in this study. The study is regarded as valid and relevant for risk assessment, as a sufficient number of animals and relevant endpoints were examined.

Ref.: 7

Mated, sperm-positive female rats were dosed with 0.05, 0.1, 0.2, 0.4, or 0.7 % in drinking water (equivalent to 67 –1064 mg/kg/ day) with Acid Yellow 23, from GD 0 to 19.

No unusual behaviour or remarkable external maternal findings were noted. Mean daily food consumption and body weight gain were similar in all groups. A significant increase in fluid consumption was observed in the 0.2, 0.4, and 0.7 % dose groups. No dose-related changes were observed in pregnancy rate, implantation efficiency, foetal viability or foetal development. Reproductive findings at necropsy were unremarkable. Examination of offspring for external, skeletal and soft tissue variations revealed no dose-related increase of incidence.
The NOAEL for teratogenic toxicity of Acid Yellow 23 was > 0.7% corresponding to 1064 mg/kg/day based on the mean fluid consumption in this study. A sufficient number of animals was regarded and relevant endpoints were examined. The study is judged as valid and relevant for risk assessment.

Ref.: 8

A 2-generation chronic toxicity/carcinogenicity study was conducted with different concentrations of Acid Yellow 23. Male and female rats were fed a basal diet (control group) or basal diet blended with commercial Acid Yellow 23 (0.1, 1.0, 2.0, 5.0 %) for approx. 2 months prior to mating. No treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of alive and still-born pups were observed. Slight decreases in body weight (4-5 %), and slight increases in food consumption were noted at the 5.0 % dose group.
The NOAEL for reproductive and teratogenic toxicity of Acid Yellow 23 was 5 % in the diet.

Ref.: 9

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Due to the effects of Acid Yellow 23 following ingestion as a food colorant, it is assumed that intestinal absorption of Acid Yellow 23 occurs. However, no metabolism studies are available.

2.7.1. Percutaneous Absorption *in vitro*

Guideline : OECD Draft Guideline (1996)
Species : Pig

Study 1

Test substance	:	Acid Yellow 23; commercial sample; certified colour content 93 %
Batch No.	:	3098GA ; lot AJ 9508
Dose levels	:	5 mg/ml in saline

Study 2

Test substance	:	Acid Yellow 23; representative hair dye formulation (C1 L3731)
Batch No.	:	AJ 9508 (dyestuff)
Purity	:	/
Dose levels	:	1.0 g of the final hair dye product containing 0.5 % Acid Yellow 23
GLP	:	in compliance

The skin absorption of Acid Yellow 23 was investigated on the outer skin of porcine ears (freshly obtained from the local slaughter-house, approx. 400 µm thick) with amounts corresponding to realistic use conditions. Two studies were performed. In the first study, 5 mg of the pure dye dissolved in saline transferred to the donor chamber with 1 ml was applied to the skin. In the second study, about 5 mg of the dye was applied to the skin in a commercial hair dye formulation containing 0.5 % Acid Yellow 23.

A glass flow-through diffusion chamber (donor chamber volume about 1 ml, skin surface 1.01 cm^2) was used. The receptor solution (saline, pH 3.0) was pumped through the receptor chamber by a rate of 1- 2 ml/h.

Six chambers per experimental group were investigated. The donor chambers were covered by Parafilm® after adding the test substance to the chamber.

Before application of the test item, receptor fluid was added to the donor chamber to check the integrity of the skin by means of conductivity measurement and to obtain the blank samples for each chamber. Only intact skin samples were used for the study.

The integrity of the skin was monitored over the entire duration of the study measuring the conductivity across the skin at each sampling time. Intact skin usually ranges from 100-500 µS with aqueous solutions. The maximum conductivity with gross damage to the skin or without skin is 2-5 mS.

30 min after substance application, the test item was removed by washing the skin three-times with 1 ml washing solution (10 % diluted shampoo-formulation). The washing solutions were combined and the amount of dye was determined by HPLC (limit of quantification: 150 ng/ml). For the remaining time of the experiment (24 h total) the donor chamber was filled with 1 ml saline solution.

Fractions of the receptor fluid were collected at 0, 0.5, 1, 2, 4, 6, 8, and 24 hours, weighed and stored at –20°C until analysis. The donor solution was also collected after 24 h and analysed. At termination of the experiment, the skin was extracted (extraction solution: 10 mM tetra butylammonium bromide + 50 mM NH_4OAc containing CH_3CN/H_2O = 30/70 (v/v)) and the dye content was quantified by HPLC.

Caffeine is used in the performing lab every 3 months as a positive control substance to demonstrate the validity of the used system.

Results
The conductivity over time ranged within the historical controls. No abrupt change in conductivity, indicating a loss of barrier properties of the skin, occurred in any chamber up to the maximal duration of the experiments. The mean recovery of the test item for experiment I and II were 94.5 ± 6.88 % and 97.5 ± 4.24 %. The majority of the test item (91 % in the first experiment, 78 % in the second experiment) was determined in the donor chamber solution.

No measurable penetration through the skin was observed at any time point in the first and in the second study.

Taking into account the detection limits a worst case of penetrated Acid Yellow 23 gives an upper limit of 5.0 µg of the test item (0.11 % of the applied total amount) in the first experiment and 5.9 µg of the test item (0.12 % of the applied total amount) in the second experiment. Together with the skin extracts (total skin) the worst case considerations of penetrated test item result in 5.4 µg/cm^2 (0.11 % of the applied total amount) in the first and 13.2 µg/cm^2 (0.26 % of the applied total amount) in the second study.

Conclusion
Under the described test conditions, skin penetration of Acid Yellow 23 is low. A skin penetration rate of 5.4 µg Acid Yellow 23/cm² (about 0.11 % of the applied amount) and 13.2 µg Acid Yellow 23/cm² (0.26 % of the applied dose) within 24 hours has been calculated for the pure substance and a commercial formulation, respectively, including the amounts present in the stratum corneum and thus assuming worst case conditions.
A penetration rate of 13.2 µg/cm² (0.26 %) representing the highest value derived from the above described test will be used as a worst case scenario for the final risk assessment.

Ref.: 11

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Reverse Mutation Testing Using Bacteria

Guideline	:	OECD/471 (1997)
Species/Strain	:	*Salmonella typhimurium (* TA1535, TA1537, TA98, TA100); *E.coli* (wP2uvrA)
Test item	:	FD&C Yellow 5 (C.I. 19140)
Batch No.	:	3098GA
Lot No.	:	AJ9508
Purity	:	Certified total colour content: 93%
Replicate	:	2 experiments
Doses	:	33, 100, 333, 1000, 2500, 5000 µg/plate (± S9)
Metabolic Act.	:	uninduced Syrian Hamster liver homogenate (S9)
Positive controls	:	according to OECD Guideline
GLP	:	in compliance

Results
There was no increase in the number of revertants with the dose in all tested strains and all conditions.

Conclusion
The test agent is non mutagenic on bacterial cells.

Ref.: 12

Reverse Mutation Testing Using Bacteria

Published papers

Non mutagenic in *Salmonella typhimurium* (TA92, TA1535, TA1537, TA94, TA100 and TA98 ± S9 rat treated liver homogenate)

Ref.: 13, 19

Non mutagenic in *Salmonella typhimurium* (TA98, TA100 ± S9 rat treated liver homogenate)

Ref.: 14

Non mutagenic in *Salmonella typhimurium* (mutagenic at ether contact of the dye) (TA98, TA100, TA1535, TA1537, ± S9 rat/hamster liver homogenate)

Ref.: 15

Non mutagenic in *Salmonella typhimurium* (TA38, TA98, TA1535, TA1537, TA100, ± S9 from rat/hamster treated liver homogenate)

Ref.: 16

Non mutagenic in *Salmonella typhimurium* (TA1538, TA100, TA1535, TA1537, TA98, ± S9 Aroclor treated rat liver homogenate)

Ref.: 18

Mitotic recombination-*Saccharomyces cerevisiae*

Published paper

Non-convertogenic in *S. cerevisiae* (-S9)

Ref.: 20

***In vitro* Cell Transformation Assay**

Published paper

Negative for cell transformation on baby Syrian hamster kidney fibroblasts (BHK21, C13) cells.

Ref.: 18

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

Published paper

Not mutagenic in mouse lymphoma assay in the absence of metabolic activation.

Ref.: 16

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

Guideline	:	OECD/476 (1997)
Species/Strain	:	Mouse Lymphoma L51878Y cells (forward mutation at Thymidine Kinase ($TK^{+/-}$) locus
Test item	:	FD&C Yellow 5 (C.I. 19140)
Batch	:	3098GA
Lot No.	:	AJ 9508
Purity	:	Certified total colour content: 93%
Dose	:	156.3: 312.5; 625; 1250; 250; 5000 µg/ml (± S9) 4h
Replicate	:	only without S9 (24h). In both treatments 2 independent cultures were tested
Metabolic Act.	:	Phenobarbital / Naphthoflavone induced rat liver homogenate (S9)
Positive control	:	MMS (-S9); DMNA (+S9)
GLP	:	in compliance

Results
Toxicity: in a preliminary experiment, the concentration range of the test item was 39 t0 5000 µg/ml without S9 (4h/24h) and with S9 (4h). No toxicity was observed at all doses.

Mutagenicity

Small and large colonies were counted. The results (x 10^{-6}) with the positive controls were the following:

TREATMENT	4h (small colonies)		4h (large colonies)	
	Culture 1	Culture 2	Culture 1	Culture 2
MMS	425	476	160	146
control	31	44	30	19
DMNA	97	91	189	159
control	68	53	25	13
	24h (small colonies)		24h (large colonies)	
MMS	344	358	79	86
control	38	46	12	14

The results indicate that MMS induced small and large colonies (chromosome aberrations and gene mutation).
DMNA is a weak clastogenic agent and it is not recommended by OECD 476 Guideline.
This test is valid only for evaluating the ability of the test item to induce gene mutations and chromosome aberrations in the absence of S9 and gene mutations only in the presence of S9.

In the treated samples there was no significant increase in mutation frequencies under all condition doses.

Conclusion
The test item does not produce gene mutation and clastogenicity in the absence of S9 and gene mutation in the presence of S9 in this assay.

Ref.: 22

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

Published paper

Clastogenic in CHL cell line ± S9, and in M. muntjac's fibroblast cells.

Ref.: 19, 13, 23, 25

DNA Damage – Unscheduled DNA Synthesis - Mammalian Cells *in vitro*

Published paper
A 94% colour at a dose of $2x10^{-3}$ to $2x10^{-6}$ Molar was found negative in rat hepatocytes treated *in vitro*.

Ref.: 27

2.8.2 Mutagenicity/Genotoxicity *in vivo*

Unscheduled DNA Synthesis (UDS) test with Mammalian liver Cells *in vivo*

A 94% colour at a dose of 500 mg/kg bw was given to male Sprague Dawley rats by gavage and found negative for the induction of UDS *in vivo*.

Ref.: 27

2.9. Carcinogenicity

Oral administration, rats

Acid Yellow 23, dissolved in distilled water, was administered via drinking water (ad libitum) to 2 dose groups of Fischer 344 rats, each consisting of 48 - 49 males and 48 - 50 females, for 2 years. Acid Yellow 23 solutions of 1 and 2% were prepared weekly. All animals were observed daily, clinical signs, and deaths were recorded. Body weights were measured weekly for the first 13 weeks, and every 2 weeks afterwards. A complete examination (macro- and microscopy) was conducted of all animals. No dose-related mortality was observed. A pronounced decrease of growth was apparent at 2% Acid Yellow 23 (>30%, estimated from graphical presentations). No other signs of toxicity were reported. It was concluded that the colorant was not carcinogenic in F344 rats when 1 or 2% solutions were administered continuously in the drinking water for up to 2 years.

Ref.: 2

A chronic toxicity/carcinogenicity study, which included also reproductive effects, was conducted. Male and female CD rats received either a basal diet (control group) or basal diet

blended with commercial Acid Yellow 23 (commercial sample; 90 % pure colouring, 10% intermediates or volatile matter) (0.1, 1.0, 2.0, 5.0%). In the "in utero phase" of the study, 60 rats/sex/group (F_0) were exposed for approx. 2 months prior to mating. A maximum of 2 rats/sex/litter (F_1) were randomly selected for the "chronic phase" and exposed to the same dose levels as their parents (70 animals/sex/group). The maximum exposure was 113 and 114 weeks for males and females, respectively (The high dose animals [5%] received the colorant for 122 and 125 weeks for males and females, respectively). Mortality, morbidity, and gross signs of toxicity were recorded twice a day. Ophthalmoscopic examinations were conducted once with the F_0 generation and at initiation of the chronic phase (with F_1) and at month 3, 6, 12, 18, 24. 10 animals/sex/group were randomly selected for haematology, clinical chemistry, and urinalysis at month 3, 6, 12, 18 and 24 and at termination of the "chronic phase". At necropsy, organ weights (brain, gonads, kidney, liver, spleen, and thyroid) were measured and a complete histopathological examination was performed on all animals from the 0, 2.0, and 5.0% dose group and on 10 rats randomly selected from the other groups after an interim sacrifice at 12 months.

F_0: No compound related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of alive and still-born pups were observed following exposure to Acid Yellow 23 for approx. 2 months. Slight decreases in body weight (4 - 5%), and slight increases in food consumption were noted at the 5.0% dose group. **F_1**: A yellow tint to the fur was observed in all treated animals and the faeces of animals from the 1.0, 2.0, and 5.0% dose group were yellow. At the 5.0% dietary level a significant decreased group mean body weights of both sexes was measured, which was 12.2% in males and 16.9% in females ($p < 0.01$). No significant decrease in body weight has been noted in the 2.0% level, but in females of the 1.0% level (- 14.4%). The authors suggest that the reduced body weight might be due to the large amount (5.0%) of a non-nutritive compound in the diet. The haematology, clinical chemistry, urinalysis, and histological evaluation revealed some lesions, that seemed however not compound related. All observed lesions, including neoplasms, were of types commonly found in ageing rats and it was concluded that admixture of the substance at levels up to 5% did not demonstrate carcinogenic effects.

Ref.: 9

Mice

Male and female CD-1 mice, groups of 60 males and 60 females received either a basal diet (control group) or basal diet blended with commercial Acid Yellow 23 (commercial sample; 90 % pure colouring, 10 % intermediates or volatile matter) (0.5, 1.5, 5.0%) for 24 months or until the survival was lower than 10 animals per group. Mortality, morbidity and gross signs of toxicity were recorded twice a day. Individual body weights and food consumption were measured weekly (first 14 wk) or bi-weekly (wk 16 - 26) or monthly thereafter. Detailed physical examinations for signs of toxicity and palpation for masses were conducted weekly. Ten animals/sex/group were randomly selected for haematology at month 3, 6, 12, 18 and 24. Necropsy was conducted on all animals, and a complete histopathological examination was conducted on all animals from the 0 and 5.0% dose group.

Survival of mice was found to be similar for control and treatment groups. Animals of all treatment groups revealed amber or yellow-brown coloured urine within 1 wk and yellow hair and skin. The faeces of animals from the 1.5 and 5% dose groups were purple or yellow-brown. Slight, not significant decreases in mean body weights of both sexes were observed at 5.0%, and

of males at 1.5%. Male mice of the 5% dose group consumed slight, but significant more food than the control group. Neoplastic, inflammatory, and degenerative lesions, that were observed macroscopically and histologically, occurred in similar incidence among control and treated mice. It was concluded that admixture of the substance at levels up to 5% did not demonstrate carcinogenic effects.

Ref.: 29

Human studies

No data.

2.10. Special investigations

No data

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

(Acid Yellow 23)
(Semi-permanent)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**13.2 µg/cm^2**
Typical body weight of human		**=**	**60 kg**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**9.24 mg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.154 mg/kg**
No observed effect level (mg/kg) (rat, carcinogenicity, oral)	**NOEL**	**=**	**2640 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**17143**

2.12. Conclusions

Physical and chemical characterisation
The information provided on the compound is largely incomplete and confusing regarding purity and physical constants. The stability data are not acceptable.

The basic toxicity data for Acid Yellow 23 is poor. A NOAEL for Acid Yellow 23 was derived of 5% in food, equivalent to 2640 and 3348 mg/kg/day in male and female rats respectively. Acid Yellow 23 was not considered maternotoxic or foetotoxic up to 5% in food.

No data concerning skin irritation of Acid Yellow 23 are available. No conclusions from limited reported patch tests are possible.
Acid Yellow 23 it is not expected to cause irritant effects following repeated treatment of eyes in a concentration of 3 % in aqueous solution.
A guinea pig maximization test pointed to no skin sensitising potency of Acid Yellow 23 after dermal contact.

Based on the limited data from human studies, a slight skin sensitising capacity of Acid Yellow 23 cannot be ruled out. Acid Yellow 23 may cause intolerance after oral exposure in some individuals.

A penetration rate of 13.2 µg/cm² (0.26 %) represents the highest value derived from the submitted studies.

Several published papers reporting the mutagenic/genotoxic potential of the test item in different series of chemical testing methods under testing cannot be evaluated, due to the lack of information on the purity of the chemical tested, the lack of guidelines adopted, the scarce information on the positive controls, the absence of indication on the number of replicates. They are reported schematically in this opinion.
The studies performed with a known sample of the test item and following OECD Guideline are
(1) a bacterial reverse mutation test, and
(2) an *in vitro* mammalian cell gene mutation assay. It has been demonstrated that the test agent does not induce gene mutations in bacterial and in mammalian cells and, possibly, chromosome aberrations on mammalian cells in the absence of a metabolic activation system.
Two long-term carcinogenicity studies with rats and one with mice have been performed. No indications of carcinogenic potential have been obtained.

2.13. References

1. E W Schafer, W A Bowles. Acute oral toxicity and repellency of 933 chemicals to house and deer mice. Arch. Environ. Contam. Toxicol., 14 111-129 (1985).
2. A Maekawa, C Matsuoka, H Onodera, H Tanigawa, K Furuta, J Kanno, J J Jang, Y Hayashi. Lack of carcinogenicity of tartrazine (FD & C Yellow no. 5) in the F344 rat. Food Chem. Toxicol., 25 891-896 (1987).
3. S D Gettings, D L Blaszcak, M T Roddy, A S Curry, G N McEwen. Evaluation of the cumulative (repeated application) eye irritation and corneal staining potential of FD&C yellow no. 5, FD&C blue no. 1 and FD&C blue no. 1 aluminium lake. Food Chem. Toxicol., 30 1051-1055 (1992).
4. G Arcelin. FD&C Yellow 5 (C.I. 19140) Contact Hypersensitivity in Albino Guinea Pigs Maximization Test. RCC Project 740654. Unpublished Report. December 1999
5. M Ibero, J L Eseverri, C Barroso, J Botey. Dyes, preservatives and salicylates in the induction of food intolerance and / or hypersensitivity in children. Allergol. Immunopathol., 10 263-268 (1982).
6. L Hesser. Tartrazine on trial. Food Chem. Toxicol., 22 1019-1024 (1984).
7. T F X Collins, T N Black, L H Brown, P Bulhack. Study of the teratogenic potential of FD & C Yellow no. 5 when given by gavage to rats. Food Chem. Toxicol., 28 821-827 (1990).
8. T F X Collins, T N Black, M W O'Donnell, P Bulhack. Study of the teratogenic potential of FD&C Yellow no. 5 when given in drinking-water. Food Chem. Toxicol., 30 263-268 (1992).
9. J F Borzelleca, J B Hallagan. Chronic toxicity/carcinogenicity studies of FD&C Yellow no. 5 (tartrazine) in rats. Food Chem. Toxicol., 26 179-187 (1988a).
10. C M Burnett, H P K Agersborg, Jr., J F Borzelleca, E Eagle, A G Ebert, E C Pierce, J C Kirschman, R A Scala. Teratogenic studies with certified colors in rats and rabbits. Abstr. No. 118. Toxicology and Applied Pharmacology, 29 121 (1974).
11. N.N. Skin Permeability In Vitro Absorption through Porcine Ear Skin with FD&C Yellow 5 (C.I. 19140). RCC - CCR Project 641203. Unpublished Report. January 2001

12. N.N. Salmonella typhimurium and Escherichia coli Reverse Mutation Assay for Azo Dyes with FD&C Yellow 5 (C.I. 19140). RCC - CCR Project 641201. Unpublished Report. June 2000
13. M Ishidate, T Sofuni, K Yoshikawa, M Hayashi, T Nohmi, M Sawada, A Matsuoka. Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol., 22 623-636 (1984).
14. J P Brown, P S Dietrich. Mutagenicity of selected sulfonated azo dyes in the Salmonella/microsome assay: use of aerobic and anaerobic activation procedures. Mut. Res., 116 305-315 (1983).
15. M J Prival, V M Davis, M D Peiperl, S J Bell. Evaluation of azo dyes for mutagenicity and inhibition of mutagenicity by methods using Salmonella typhimurium. Mut. Res., 206 247-259 (1988).
16. T P Cameron, T J Hughes, P E Kirby, V A Fung, V C Dunkel. Mutagenic activity of 27 dyes and related chemicals in the Salmonella/microsome and mouse lymphoma $TK^{+/-}$ assays. Mut. Res., 189 223-261 (1987).
17. K-T Chung, G E Fulk, A W Andrews. Mutagenicity testing of some commonly used dyes. Appl. Environ. Microbiol., 42 641-648 (1981).
18. E Longstaff, D B McGregor, W J Harris, J A Robertson, A Poole. A comparison of the predictive values of the Salmonella/microsome mutation and BHK21 cell transformation assays in relation to dyestuffs and similar materials. Dyes Pigm., 5 65-82 (1984).
19. M Ishidate, T Sofuni, K Yoshikawa. Chromosomal aberration tests in vitro as a primary screening tool for environmental mutagens and/or carcinogens. GANN Monogr. Canc. Res., 27 95-108 (1981).
20. N Sankaranarayanan, M S S Murthy. Testing of some permitted food colours for the induction of gene conversion in diploid yeast. Mut. Res., 67 309-314 (1979).
21. N K Tripathy, K K Patnaik, M J Nabi. Genotoxicity of tartrazine studies in two somatic assays of Drosophila melanogaster. Mut. Res., 224 479-483 (1989).
22. N.N. Cell Mutation Assay at the Thymidine Kinase Locus (TK) in Mouse Lymphoma L5178Y Cells with FD&C Yellow 5 (C.I. 19140) (Modified Test Design for Azo-Dyes). RCC - CCR Project 641202. Unpublished Report. August 2000
23. M Ishidate. Data Book of Chromosomal Aberration Test in Vitro. Elsevier Amsterdam, New York, Oxford. (1988).
24. G Rontó, P Gróf, J-P Buisson, J Einhorn, P Demerseman. Genotoxic effectivity - comparison of 36 nitrated furan and arenofuran derivatives on a quantitative scale. Statistical comparison of T7 and other short-term tests. Mutagenesis, 7 243-249 (1992).
25. R M Patterson, J S Butler. Tartrazine-induced chromosomal aberrations in mammalian cells. Food Chem. Toxicol., 20 461-465 (1982).
26. W Au, T C Hsu. Studies on the clastogenic effects of biologic stains and dyes. Environ. Mutagen., 1 27-35 (1979).
27. D Kornbrust, T Barfknecht. Testing of 24 food, drug, cosmetic, and fabric dyes in the in vitro and the in vivo/in vitro rat hepatocyte primary culture/DNA repair assay. Environ. Mutagen., 7 101-120 (1985).
28. H W Renner. Letter to the editor: Tartrazine - a reinvestigation by in vivo cytogenetic methods. Food Chem. Toxicol., 22 327 (1984).
29. J F Borzelleca, J B Hallagan. Chronic toxicity/carcinogenicity studies of FD&C Yellow no. 5 (tartrazine) in mice. Food Chem. Toxicol., 26 189-194 (1988b).
30. M Ishidate, M C Harnois, T Sofuni. A comparative analysis of data on the clastogenicity af 951 chemical substances tested in mammalian cell cultures. Mut. Res., 195 151-213 (1988).

31. M A C Thierbach, A M Geursen-Reitsma, T van Joost. Sensitization to azo dyes: negative patch tests to yellow and red azo dyes in printed paper. Contact Dermatitis, 27 22-26 (1992).
32. T Sugai, Y Takahashi, T Takagi. Pigmented cosmetic dermatitis and coal tar dyes. Contact Dermatitis, 3 249-256 (1977).
33. K H Mavournin, D H Blakley, M C Cinimo, M F Salamone, J A Heddle. The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mut. Res., 239 29-80 (1990).
34. F K Zimmermann, R C von Borstel, E S von Halle, J M Parry, D Siebert, G Zetterberg, R Barale, N Loprieno. Testing of chemicals for genetic activity with Saccharomyces cerevisiae: a report of the U.S. Environmental Protection Agency Gene-Tox Program. Mut. Res., 133 199-244 (1984).
35. Color Additive Certificate; Certificate of analysis by Hilton Davis. Food and Drug Administration, November 30, 1998
36. LAN- Analysenbericht Studien-Nr. A2002/340 December 03, 2002. Wella AG; D-64274 Darmstadt
37. LAN-Aanalysenbericht Stzudien-Nr. A2003/317 September, 23 2003. Wella AG; D-64274 Darmstadt
38. Stability in formulation; September 22, 2003. Wella AG; D-64274 Darmstadt

3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the use of Acid Yellow 23 as 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

/

5. Minority opinions

/