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

CONCERNING

ACID BLUE 9

COLIPA n° C40

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 Blue 9 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 Blue 9 is listed as CI 42090 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.

It is allowed as a food colorant in the EU as Brilliant Blue FCF (ADI: 0-10 mg/kg bw/day).

2.1.1. Primary name

Acid Blue 9 (INCI)

2.1.2. Chemical names

- Dihydrogen (ethyl)[4-[4-[ethyl(3-sulphonatobenzyl)]amino]-2'-sulphonatobenzhydrylidene] cyclohexa-2,5-dien-1-ylidene](3-sulphonatobenzyl) ammonium, disodium salt (EU inventory)
- Disodium2-({4-[N-ethyl(3-sulfonatobenzyl]amino]phenyl} {4-[(N-ethyl(3-sulfonatobenzyl)imino]-2,5-cyclohexadien-1-ylidene}methyl)-benzenesulfonate (IUPAC)
- Benzenemethanaminium, N-ethyl-N-[4-[[4-[ethyl-[(3-sulfophenyl)-methyl]-amino]-phenyl] [2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfo, inner salt disodium salt (CAS)
- 1-((4-(ethyl-((3-sulfophenyl)methyl)amino)phenyl)-(2-sulfophenyl)-Methylen)-4(ethyl-((3-sulfophenyl)methyl)iminium-2,5-cyclohexadiene-innersalt, disodium salt

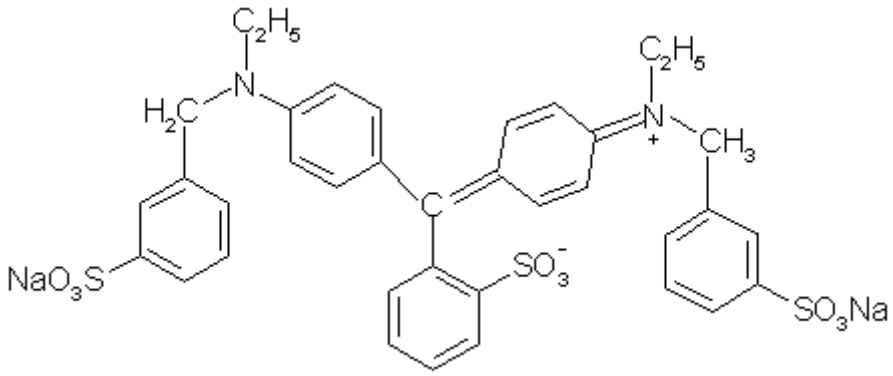
2.1.3. Trade names and abbreviations

COLIPA n°	:	C 40
Trade name	:	Covacap Bleu W 6102 (LCW)
Other names	:	Erioglaucine, Brilliant Blue FCF, Food Blue 2, E 133, Japan Blue 1, FD&C Blue No. 1

2.1.4. CAS / EINECS / COLOUR INDEX number

CAS	:	3844-45-9
EINECS	:	4223-333-98
Colour index	:	CI 42090 (the material contains 2 additional isomers with CAS numbers 2650-18-2 and 68921-42-6)

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{37}H_{34}N_2Na_2O_9S_3$
Mol weight : 792.86

2.1.7. Purity, composition and substance codes

Batch No. : 0789AG, Lot AK 0965; FDA certified Lot AK 0965; 00-2320 (R00056594); Toshiki 0809

Purity as sum of 3 isomers

Total colour content	:	82-93 %
NMR quantitative	:	75.5-84.4% (w/w)
Loss on drying	:	0.9-10% (w/w)
Water content	:	2.3-7.6% (w/w)
Sulfated ash content	:	17.3-19.7% (w/w)

Impurity

Sum of o-, m-, and p-sulfobenzaldehyde	:	< 1.5% (w/w)
N-Ethyl,N-(m-sulfobenzyl)sulfanilic acid	:	< 0.3% (w/w)
2-formylbenzosulfonic acid	:	0.06-0.9%
Leuco base	:	< 5% (w/w)
Subsidiary colours	:	< 6% (w/w)
Lead	:	< 10ppm
Arsenic	:	< 2 ppm
Iron	:	< 100 ppm
Mercury	:	<0.1 ppm
Manganes	:	5% and 6% respectively in AK0965 and R00056954

Solvent residues : <100 ppm (methanol, ethanol, isopropanol, n-propanol, acetone, ethylacetate, cyclohexane, methyl ethyl ketone and monchlorobenzene)

2.1.8. Physical properties

Appearance	:	Dark Violet powder
Melting point	:	283°C
Boiling point	:	1184 °C (calculated by QSAR) *
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	1.0 E-36 hPa (calculated by QSAR) *
Log P_{ow}	:	-0.32 (calculated by QSAR) *

* See General Comments below

2.1.9. Solubility

Water	:	> 20% (w/w) (pH 5.1)
DMSO	:	> 10% (w/w)
Acetone/water (1:1)	:	> 10% (w/w)
Ethanol/water (4:6)	:	> 10% (w/w)

2.1.10 Stability

Stability of Acid Blue 9 was studied in an approximate 10% aqueous solution. The stability in the marketed product is not reported.

General comments on analytical and physico-chemical characterisation

* Purity of all batches tested is not reported. In several tests, batch no. and purity of the test material is not reported.
* 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 ionisable groups.

2.2. Function and uses

Acid Blue 9 will be used as direct dye in semi-permanent hair dye formulations at a maximum concentration of 0.5%.

TOXICOLOGICAL CHARACTERISATION

Most of the basic toxicological data are presented in form of original articles mostly published in well reputed and peer reviewed journals. As the substance is already in use in a wide range of applications for a long time, part of the toxicological data was originated in the time between 1970 and 1980. However reviews produced by authorities, such as JECFA/FAO-WHO on Acid Blue 9 as a food colour (CI 42090) as well as a colouring agent for drugs and in cosmetic products have shown the "GRAS" character of the substance.

Moreover the applicant of the new dossier carried out a literature search using the ChemI Plus-system, a well reputed search system including most of the acknowledged data bases as MEDLINE, TOXNET NLM Gateway etc. A statement is given that all hits of relevance for risk assessment of Acid Blue 9 were included in the present safety evaluation. Thus, the description of the results obtained are sufficient for an evaluation of the general toxicology of Acid Blue 9 as for the intended use and applied concentrations.

2.3. Toxicity

2.3.1. Acute oral toxicity

LD_{50} rat: > 2.000 mg/kg/bw. (orig.: Lu, F.C. & Lavallée, A. [1964] Can. Pharm. J. 97, 30)
LD_{50} mouse s.c. 4.600 mg/kg/bw. (orig.: Gross, E. [1961] Z. Krebsforschg. 64, 287)

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

Staining and deposits in the stomach of rats were seen when Acid Blue 9 was administered orally in a concentration of 4 % in the diet (about 2 g/kg bw/day) for at least 3 weeks. No further details in the review. Original: Willheim, R. & Ivy, A.C. (1953) Gastroenterology 23, 1.
In an experiment with 13 hamsters a single s.c. or i.p.-injection of 1 mg Acid Blue 9 (about 8 mg/kg/bw) showed the kidney as a target organ (orig.: Price, P.J. et al. (1978) Int. J. Cancer 21, 361).

Ref.: 1

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 2.3.10.

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

Chronic Toxicity (lifetime) study in rats

Guideline	:	/
Species/Strain	:	rat, Charles River CD
Group size	:	60 males + 60 females F0; 70 males + 70 females F1
Test substance	:	Acid Blue 9, purity 90 %, FDA certified
Batch No.	:	/
Dose levels	:	0, 0.1, 1.0, 2.0 % in the diet
Route	:	oral; diet
Exposure	:	F1 in utero and lifetime
GLP	:	/

The study was conducted with a prenatal treatment phase in which the administration of the test compound to the F0 generation was started approximately 2 months before mating and was continued during pregnancy and lactation. After randomly selecting the F1 animals from the litters of the respective experimental groups the lifetime phase was initiated at the same dose levels of 0 %, 0.1 %, 1.0% or 2.0 % in the diet. Two control groups, which received the basal diet, were used to account for random biological variation. Maximum exposure times were 116 and 111 weeks for males and females, respectively. The purity of the test item was 90 %. The remaining 10 % consisted of subsidiary colourings, volatile chlorides and sulphates, and uncombined intermediates.

Results
No adverse effects were observed in male animals. A 15 % decrease in terminal body weight and a decreased survival was observed in the females treated with the high-dose compared with the combined control groups. Results on carcinogenicity are provided in section 3.7.
The NOAELs established in this study are dietary concentrations of 2 % for males (corresponding to 1072 mg/kg bw/day) and 1 % for females (631 mg/kg bw/day).

Ref.: 2

Chronic Toxicity (lifetime) study in mice

Guideline	:	/
Species/Strain	:	mouse, Charles River CD-1
Group size	:	60 males + 60 females
Test substance	:	Acid Blue 9, purity 90 %, FDA certified
Batch No.	:	/
Dose levels	:	0, 0.5, 1.5, 5.0 % in the diet
Route	:	oral; diet
Exposure	:	lifetime (104 weeks)
GLP	:	/

The study was part of a lifetime carcinogenicity/toxicity study. The study design in the main corresponds to OECD-Guideline 451. The study was terminated after 104 weeks. Two control groups, which received the basal diet, were included to account for random biological variation. The test item was 90 % pure. The remaining 10 % consisted of subsidiary colourings, volatile chlorides and sulphates, and uncombined intermediates. Results on carcinogenicity are provided in section 3.7.

Results
No consistent, significant compound-related adverse effects of biological relevance were noted. The NOAEL established in this study is a dietary concentration of 5.0 % (corresponding to 7354 mg/kg bw/day for male and 8966 mg/kg bw/day for female mice).

Ref.: 2

Conclusion
Both long-term toxicity studies in mice and rats are valid studies. Rats were considerably more sensitive towards Acid Blue 9 toxicity than mice. The NOAEL for chronic oral toxicity of Acid Blue 9 is 631 mg/kg bw/day. This value is used for the final risk assessment and calculation of the margin of safety. All results can be used for a conclusive evaluation, especially the lowest NOAEL of 631 mg/kg/bw in the rat two year study.

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline	:	/
Species/strain	:	Human volunteers
Group size	:	207
Test substance	:	Brilliant Blue
Batch number	:	/
Purity	:	/
Dose	:	5% aqueous solution
GLP	:	/

Moderate irritation occurred in 40 of 207 volunteers following 48-hour covered application of test substance. 14 volunteers had similar responses in identical tests with an insoluble Brilliant Blue complex (the aluminium lake). Only a summary and not the full report was available for evaluation.

Ref.: 1

2.4.2. Irritation (mucous membranes)

Chronic eye irritation in rabbits

Transient eye irritation occurred in rabbits treated (over a 4-week period) with 40 x 0.2 ml applications of a 10% aqueous suspension of either Brilliant Blue or its lake. Only a summary, and not the full report, was available for evaluation.

Ref.: 4

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

Guideline	:	/
Species/strain	:	fertilised fresh chicken eggs
Group size	:	6
Test substance	:	IT 414, Blue 1
Batch number	:	lot 0809
Purity	:	/
Dose	:	1% in water
GLP	:	in compliance

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.: 5

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

Guideline	:	/
Cell line	:	HaCaT human keratinocytes
Test substance	:	IT 414, Blue 1
Batch number	:	lot 0809
Purity	:	/
Dose	:	1% in water
GLP	:	/

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 76% in the first assay and 71% in the second, so the NRU-50 is reported > 10000 µg/ml, and hence the test item classified as non-irritant.

Ref.: 6

2.5. Sensitisation

Local Lymph Node Assay (LLNA)

Guideline	:	Local Lymph Node Assay (OECD 429)
Species/strain	:	CBA/J mice
Group size	:	50 females, divided into 10 groups of 5 animals.
Test substance	:	CI 42090, FD&C Blue 1
Batch number	:	0809
Purity	:	84.2 weight % (NMR) and 69 area% (HPLC)
Dose	:	0.5 – 4% in dimethylsulfoxide 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.: 7

Two clinical studies published in 1974 and 1978 are included in the dossier describing peroral provocation with FD&C Blue 1 in selected patients with various symptoms considered as possible allergic diseases (asthma, rhinitis, urticaria). The frequency of positive reactions was around 1-14% among challenged patients. The studies do not fulfil today's requirement of double blinded, placebo controlled provocation tests for food hypersensitivity, and the results are not relevant in relation to the use of the compound in hair dyes.

Ref.: 8, 9

2.6. Teratogenicity/Reproduction toxicity

Prenatal development in rats

Guideline	:	/
Species/Strain	:	rat, strain not stated
Group size	:	24 females
Test substance	:	Acid Blue 9
Batch No.	:	/
Dose levels	:	0, 200, 600, 2000 mg/kg bw/day
Route	:	oral; gavage
Exposure	:	day 6 to 15 of gestation
GLP	:	/

This unpublished study was cited in a review article. No further information on the experimental design was provided.

Results
An increase in kidney abnormalities (hydronephrosis) was seen in the foetuses of the mid-dose group only (suggesting this effect may not have been treatment related). No other signs of maternal or foetal toxicity were seen in examinations which included the skeleton and soft tissues.

Ref.: 1

Prenatal development study in rabbits

Guideline	:	/
Species/Strain	:	rabbit, not stated
Group size	:	15-19 females
Test substance	:	Acid Blue 9
Batch No.	:	/
Dose levels	:	0, 20, 60, 200 mg/kg bw/day
Route	:	oral; gavage
Exposure	:	day 6 to 18 of gestation
GLP	:	/

This unpublished study was cited in a review article. No further information on the experimental design was provided.

Results
No increase in foetal malformations or other maternal or foetal effects were seen.

Ref.: 1

Conclusion
The validity of the studies in rats and rabbits could not be evaluated, as the original data were not available. The data indicate that Acid Blue 9 is not teratogenic in doses up to 2000 mg/kg bw /day in rats and 200 mg/kg bw/day in rabbits, highest doses applied.

One generation reproduction study in rats

Guideline	:	/
Species/Strain	:	rat, Charles River CD
Group size	:	60 males + 60 females F0; 70 males + 70 females F1
Test substance	:	Acid Blue 9, purity 90 %
Batch No.	:	/
Dose levels	:	0, 0.1, 1.0, 2.0 % in the diet
Route	:	oral; diet
Exposure	:	premating, in utero and during lactation
GLP	:	/

This study was part of a long term carcinogenicity/toxicity study. The study design corresponds in most aspects to OECD-Guideline 415. Rats received the test item for approximately 2 months before mating and during gestation and lactation. Two control groups, which were fed the basal

diet, were included. The test item contained 90 % dye, the remaining 10 % consisted of subsidiary colourings, volatile chlorides and sulphates, and uncombined intermediates.

Results
There were no substance-related effects on fertility, gestation, parturition, lactation, pup survival through weaning, or on the numbers of live and stillborn pups. One female in the 0.1 % group, one male and one female in the 1.0 % group, and one male in the 2.0 % group died (all F0). The authors of the study concluded that these deaths were not related to treatment.

Conclusion
The data indicate that Acid Blue 9 has no adverse effect on reproduction in rats in concentrations up to 2 % in the diet (= 1000 mg/kg bw/day, assuming a standard food factor of 0.05 for rats). Not all reproduction parameters were recorded in this study.

Ref.: 2

Two generation reproduction study in rats

Guideline	:	/
Species/Strain	:	rat, not stated
Group size	:	10 males + 20 females
Test substance	:	Acid Blue 9
Batch No.	:	/
Dose levels	:	0, 10, 100, 300, 1000 mg/kg bw/day
Route	:	oral; no further information
Exposure	:	two generations
GLP	:	/

The study is not published in original. No further information on study design was available.
Results
No substance specific effects were seen regarding mortality, mating, pregnancy, fertility rate, and neonatal mortality. Body weights of F1 and F2 neonates of the highest dose group were reduced. It was not stated whether this effect was statistically significant or not.There was a slight increase in the incidence of lung and kidney abnormalities in adult males from one generation (no further information) of the highest dose group, in examinations of a fairly wide range of tissues. Rats from the next lowest dose group (300 mg/kg bw/d) and adults from other generations were not subject to detailed examinations for tissue abnormalities. The investigators considered that the incidence of abnormalities was similar to that previously detected in untreated rats, however no data were provided in support of this statement.

Conclusion
The highest dose tested in this study was 1000 mg/kg bw/day. No effects on reproductive parameters were observed. A reduction of the bodyweights of F1 and F2 neonates was observed in the highest dose group (NOAEL: 300 mg/kg bw/d). Due to the lack of information regarding details of the study design and outcome the validity of the study is limited.

Ref.: 1

General Conclusion
Acid Blue 9 elicits no reproductive toxicity up to 1000 mg/kg bw/day (highest concentration tested). Also, no developmental toxicity was observed in rats and rabbits exposed in utero to

doses up to 1000 mg/kg bw/d and 200 mg/kg bw/d, respectively. A further study describes slight developmental toxic effects in the highest dose group (1000 mg/kg bw/d: reduced bodyweights of neonates of F1 and F2; NOAEL 300 mg/kg bw/d) Detailed figures are not available. But, no concerns arise from these findings in regard to reproductive toxicity for the application of Acid Blue 9 as hair dye.

2.7. Toxicokinetics (including Percutaneous Absorption)

Percutaneous absorption *in vitro*

Guideline	:	Draft OECD Guideline (1996)
Tissue	:	400 – 900 µm thickness skin from porcine ears
Method	:	Flow through diffusion cells made of glass.
Test substance	:	FD&C Blue 1 (CI 42090) (experiment I) and formulation with SC Hair Colour Gel – containing 0.5% FD&C Blue 1 (experiment II)
Batch no	:	0789AG (colour) and C1 L371 1 (formulation)
Purity of colour	:	Certified colour content 88%
Dose levels	:	1 ml of 5 mg/ml colour (experiment I) and 1.2 g of final hair dye product (experiment II)
GLP	:	In compliance

Six cells were used. The diffusion chambers had a diameter of 1.135 cm and a volume of 1 ml in the donor chamber. Saline adjusted to pH 3.0 was used as receptor medium and pumped through the cells with a flow rate of 1-2 ml/hour. The temperature in the incubator was 32 ºC. After filling the donor chambers with test material they were covered with Parafilm® for 30 minutes and then the test substance removed and the surface washed 3 times with shampoo. The donor chambers were then filled with saline and receptor medium collected at 0.5, 1, 2, 4, 6, 8, and 24 hours. The samples were analysed by HPLC with a detection limit of 150 ng/ml. After the end of study mass balance was made by analysing the amount of test substance bound to the skin and in the washings. The mean recovery was 93.3% ± 4.05% (SD) in experiment I, and 115,2% ± 6.43% (SD) in experiment II. Appropriate controls were included.

Result
No measurable permeation through skin was detected. With the detection limit in mind the maximal possible, calculated flux of the test item across the skin barrier was 5.5 - 5.7 µg/cm^2 in the two experiments, equivalent to 0.09-0.12% of the applied total amount. When the skin extracts are added the worst case scenario gives a maximal absorption of 6.2 µg/cm^2 (0.13%) in the first experiment and 35.2 µg/cm^2 (0.60%) in the second experiment.

Ref.: 13

***In vivo* metabolic disposition, study 1**

Guideline	:	/
Species/Strain	:	rat, Wistar
Group size	:	5 males + 4 females
Route	:	oral, gavage
Test substance	:	^{14}C-labelled *Acid Blue 9*, radiochemical purity > 95 %;
Batch No.	:	not stated
Dose levels	:	30 µg/kg bw or 3 mg/kg bw in aqueous solution (5 ml/kg bw)

GLP : not reported

^{14}C-labelled colouring was administered by a single oral gavage to male (30 µg/kg or 3 mg/kg) and female (3 mg/kg) rats. Urine and faeces were collected at 24-hour intervals for 3 days, after which time the animals were killed. Additionally, in predosing experiments unlabelled substance was mixed with the diet and administered for 21 days to provide a dose level of 30 mg/kg bw/d. For the determination of biliary excretion in male rats ^{14}C-labelled dye was introduced into the stomach (3 mg/kg bw) and bile was collected for 5 hours. For studying transplacental migration pregnant rats were given a single oral dose of ^{14}C-labelled dye (3 mg/kg bw) on day 8 of pregnancy and urine and faeces were collected at 24-hour intervals for 3 days. The animals were killed on day 11 of pregnancy and the level of radioactivity in the foetuses was determined.

Results
Radioactivity was rapidly eliminated in the faeces, substantially all of the dose being accounted for within 72 hours. No radioactivity was detected in expired CO_2 and only a very small percentage of the dose was found in the urine (0.5 %). The authors discussed the possibility that the radioactivity in the urine could have been due to leaching of the water soluble dye from faecal material during separation in the metabolic chamber. Treating male rats with unlabelled colouring in the diet for 21 days prior to dosing with the ^{14}C-labelled dye had no effect on the route of excretion of radioactivity or the rate of elimination. The lack of absorption of the radioactive material from the intestinal tract was confirmed by studies using isolated loops of small intestine. Similarly, pregnant rats excreted substantially the entire administered radioactivity in the faeces within 72 hours and only 0.0004-0.0006 % of the dose of *Acid Blue 9* was detected in the total foetuses in each litter. The dye was not metabolised during its passage through the gastrointestinal tract, as proven by TLC. The total radioactivity excreted in the bile during the 5 hours following administration of an oral dose of ^{14}C-labelled dye was found to be less than 0.05 % of the radioactivity administered.

Ref.: 10

***In vivo* metabolic disposition, study 2**

Guideline	:	/
Species/Strain	:	mouse, Tuck TO
Group size	:	3 males
Route	:	oral, gavage
Test substance	:	^{14}C-labelled *Acid Blue 9*, radiochemical purity > 95 %
Batch No.	:	/
Dose levels	:	30 µg/kg bw or 3 mg/kg bw in aqueous solution (5 ml/kg bw)
GLP	:	/

^{14}C-labelled colouring was administered by a single oral gavage (30 µg/kg or 3 mg/kg). Urine and faeces were collected at 24-hour intervals for 3 days, after which time the animals were killed.

Results
Radioactivity was rapidly eliminated in the faeces, substantially all of the dose being accounted for within 72 hours. No radioactivity was detected in expired CO_2 and a very small percentage of the dose was found in the urine (about 1.0 %). The lack of absorption of the radioactive material from the intestinal tract was confirmed by studies using isolated loops of small intestine.

Ref.: 10

***In vivo* metabolic disposition, study 3**

Guideline	:	/
Species/Strain	:	Guinea-pig, Dunkin-Hartley
Group size	:	6 males
Route	:	oral, gavage
Test substance	:	^{14}C-labelled *Acid Blue 9*, radiochemical purity > 95 %
Batch No.	:	/
Dose levels	:	30 µg/kg bw or 3 mg/kg bw in aqueous solution (5 ml/kg bw)
GLP	:	/

^{14}C-labelled colouring was administered by a single oral gavage (30 µg/kg or 3 mg/kg). Urine and faeces were collected at 24-hour intervals for 3 days, after which time the animals were killed.

Results
Radioactivity was rapidly eliminated in the faeces, substantially all of the dose being accounted for within 72 hours. No radioactivity was detected in expired CO_2 and a very small percentage of the dose was found in the urine (about 1 %). The lack of absorption of the radioactive material from the intestinal tract was confirmed by studies using isolated loops of small intestine.

Conclusion
Corresponding findings of very low intestinal absorption of *Acid Blue 9* were reported for 3 species in 3 independent studies.

Ref.: 10

***In vivo* metabolic disposition, study 4**

Guideline	:	/
Species/Strain	:	rat, Sprague-Dawley
Group size	:	13 females
Route	:	oral, gavage
Test substance	:	^{14}C-labelled *Acid Blue 9*, radiochemical purity > 99 %
Batch No.	:	/
Dose levels	:	0.27 mg (1 ml aqueous solution)
GLP	:	/

Three of the rats were bile-duct cannulated 2 days before dosing. One day before dosing, five of the rats were bile-duct ligated. All the rats were fasted overnight and until 8 hours after dosing. Two rats each from the intact and bile-duct ligated groups were placed in CO_2-collection chambers immediately after dosing. The CO_2-trapping agent from these chambers was sampled after 24 and 48 hours. After 48 hours, $^{14}CO_2$ excretion was negligible, so these rats were placed in normal metabolism cages for the remainder of the study. Urine and faeces were collected from intact and bile-duct cannulated rats after 24, 48, 72 and 96 hours. After 96 hours the rats were killed and samples of internal organs, tissues and gut contents were collected. Urine and bile samples were analysed by thin-layer chromatography.
Results
Urinary, faecal and pulmonary excretion of radioactivity after oral administration of ^{14}C-labelled dye to bile-duct ligated rats was 2.02, 97.28, 0.01 % of the administered dose, respectively.

Radioactivity recovered from internal organs represented 0.02 % of that administered (99.38 % total recovery). The estimate of total intestinal absorption based upon the radioactivity in urine, internal organs and CO_2 from bile-duct ligated rats was 2.05 ± 0.35 % of the dose. Intestinal absorption in intact rats averaged only 0.27 % (91.69 % recovery), while biliary excretion in bile-duct cannulated animals averaged 1.32 % of dose. Thin-layer chromatography revealed that about 95 % of the radioactivity in the samples was unchanged dye. The remainder of radioactivity was unidentified, possibly representing a decomposition product resulting from loss of a sulphonate group.

Ref.: 11

These results are in good agreement with the data reported by others (Ref. 1). It was further reported that rats excreted more than 90 % of an intravenously administered dose in the bile within 4 hours.

Ref.: 12

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Reverse Mutation Testing Using Bacteria

Guideline	:	OECD/471 (1997)
Species/Strain	:	*S. typhimurium (*TA1535, TA1537, TA98, TA100) ; *E. coli* WP2uvrA
Test item	:	FD&C Blue 1 (CI 42090)
Batch No.	:	0789AG
Lot No.	:	AK0965
Purity	:	Certified total colour content: 88%
Replicate	:	2 experiments (pre-incubation)
Doses	:	33, 100, 333, 1000, 2500, 5000 µg/plate (± S9)
Positive controls	:	according to OECD Guideline
Metabolic Act.	:	Phenobarbital / Naphthoflavone induced rat liver homogenate
GLP	:	in compliance

Results
There is no indication of induction of revertants in all strains and under all conditions.

Conclusion
The test item is not mutagenic on bacterial cells.

Ref.: 15

Reverse Mutation Testing Using Bacteria

Guideline	:	OECD/471 (1997)
Species/Strain	:	*S. typhimurium (*TA1535, TA1537, TA98, TA100, TA102);
Test item	:	A003547 (FD&C Blue Nr.1)
Batch No.	:	002320 (BASF)
Lot No.	:	23211
Purity	:	HPLC 92.9 area % (210 nm); 97.1 area % (620 nm)
Replicate	:	2 experiments

Doses : 1, 10, 100, 1000, 5000 µg/plate (± S9)
Positive controls : according to OECD Guideline
Metabolic Act. : Aroclor 1254 induced rat liver homogenate (S9)
GLP : in compliance

Results
There was no indication of reduction of revertants with doses which were reproducible and statistically significant in all strains and in all conditions.

Conclusion
The test item is non mutagenic on bacterial cells.

Ref.: 16

Reverse Mutation Testing Using Bacteria

Published papers

Mutagenic in *S. typhimurium* (TA 1538, TA 98, +S9 from hamster liver homogenate)

Ref.: 14

Non mutagenic in *S. typhimurium* (TA 92. TA 1535, TA 100, TA 1537, TA 94, TA 98, TA 2637 + S9 from induced rat liver homogenate)

Ref.: 17

Non mutagenic in *S. typhimurium* (TA 1535, TA 1537, TA 1538, TA 98, TA 100 + S9 from induced rat liver homogenate)

Ref.: 18

Non mutagenic in *S. typhimurium* (several strains + S 9).

Ref.: 19, 20

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

Published paper

Clastogenic on CHL cell line + S 9.

Ref.: 17, 20

***In Vitro* Mammalian cell Transformation Assay**

Published paper

Negative (inadequate also for COLIPA dossier) on BHK 21/C 13 cell line.

Ref.: 18

Negative on F 1700 Fischer rat embryo cell line.

Ref.: 23

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

Guideline	:	OECD/476 (1997)
Species/Strain	:	Mouse Lymphoma L5178Y cells (forward mutation at Thymidine Kinase ($TK^{+/-}$) locus
Test item	:	FD&C Blue 1 (C.I. 42090)
Batch No.	:	0789AG
Lot No.	:	AK965
Purity	:	Certified total colour content: 88%
Doses	:	156.3, 312.5, 625, 1250, 5000 (± S9) (4h) 156.3, 312.5, 625, 1250, 5000 (- S9) (24h)
Replicate	:	1 experiment ± S9 2 cultures/each 1 experiment - S9 2 cultures/each
Metabolic Act.	:	Phenobarbital / Naphthoflavone induced rat liver homogenate (S9)
Positive controls	:	MMS (-S9); 3-MC (+S9)
GLP	:	in compliance

Results
Toxicity: no toxicity was observed at the maximum concentration in the preliminary experiment.

Mutagenicity
The results on the positive controls are the following:

TREATMENT	Small colonies		Large colonies	
	1st culture	2nd culture	1st culture	2nd culture
4h				
MMS	392	665	67	81
Control	85	80	24	21
3-MC	169	169	43	78
Control	62	79	18	23
24h				
MMS	471	209	97	43
Control	26	38	8	3

The positive controls demonstrated their ability to induce gene mutation and clastogenicity in the absence of S9 (4h and 24h) and in the presence of S9 (4h).
The test item treated cell populations did not show any induction of mutation and clastogenicity in all conditions.

Conclusions
The test item does not induce gene mutation and clastogenicity in mammalian cells.

Ref.: 21

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

Published paper

Non mutagenic on mouse lymphoma cell line + S9.

Ref.: 14

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

Published paper

Positive in primary rat hepatocytes.

Ref.: 22

2.8.2 Mutagenicity/Genotoxicity *in vivo*

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

Published paper

Negative (inadequate also for COLIPA dossier) on mice, via i.p.

Ref.: 24

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

Guideline	:	OECD/486 (1997)
Species/strain	:	Wistar Hanlbm:Wist (SPF)
Group size	:	4 animals/group dosed
Test Substance	:	BLUE 1 WR 23312
Batch no.	:	lot 0809
Purity	:	84.2 % weight (NMR spectrum)
Positive Control	:	2h: DMH (N,N'-dimethylhydrazinedihydrochloride) 40 mg/kg, orally. 16h: 2-AAF (2-Acetylamino fluorene) 100 mg/kg, orally
Treatment	:	oral, 10 ml/kg
Evaluation	:	Autoradiography
GLP	:	in compliance

Results
Toxicity: 2 animals/group were treated with 2000 mg/kg orally and observed for1, 2-4, 6, and 24 hours. No significant toxic effects were observed.

Genotoxicity: Positive controls: DMH (14.11 net grains/nucleus) and 2 AAF (27,1 net grains/nucleus) induced significant increase of UDS over the negative control (-5.68/-6.56 net grains/nucleus). C 40 at the two times did not increase the control value.

Conclusion
C 40 is not genotoxic *in vivo* for the induction of UDS in rat liver.

Ref.: 25

2.9. Carcinogenicity

Oral administration

Rat

Four groups of 15 male and 15 female Wistar rats, four to six weeks old, were fed diets containing 0 (control), 0.03, 0.3 or 3% Acid blue 9, disodium salt (food grade) for 75 weeks, at which time the experiment was terminated. The numbers of survivors at this time were 23 (control), 19, 20 and 19 rats in the four groups, respectively; tissues from five male and five female survivors in each group were examined histologically. One lymphosarcoma occurred in a male fed the 0.3% dose level [inadequate histological examination of tissues in this experiment was noted].

Ref.: 28

Groups of 12 male and 12 female three-week-old Osborne-Mendel rats received 0 (control), 0.5, 1.0, 2.0 or 5% Acid blue 9, disodium salt in their diet for two years. No significant increase in mortality or pathological changes was observed in these animals when compared with untreated control rats [inadequacy of the experiment was noted].

Ref.: 26

Male and female Charles River CD rats of the F0 generation received Acid Blue 9 (purity 90%) in a concentration of 0.1, 1.0, and 2.0 % in the diet for approximately 2 months before mating, the females subsequently during pregnancy and lactation. After randomly selecting the F1 animals the lifetime phase was initiated at the same dose levels. The groups consisted of 70 males and 70 females. Two control groups, which received the basal diet, were used. Maximum exposure times were 116 and 111 weeks for males and females, respectively. The only toxic effects observed were a 15 % decrease in terminal body weight and a decreased survival in the high-dose females compared with the combined results of the control groups. It was concluded that *Acid Blue 9* was not carcinogenic under the conditions tested.

Ref.: 2

Mice

Groups of 48 male (20-31 g) and 50 female (19-23 g) ASH/CS1 mice were fed diets containing 0 (control), 0.015, 0.15 or 1.5% Acid Blue 9, disodium salt (purity, 85%) for 80 weeks, providing intakes of approximately 0, 20, 200 or 2000 mg/kg bw/day. At 80 weeks, 16 male controls, 8, 12 and 9 treated males, 17 female controls and 13, 16 and 22 treated females had died, in the different groups, respectively. No significant increase in tumour incidence was observed in treated mice compared with that in controls. One squamous-cell carcinoma of the stomach was observed in a female treated with the highest dose; 7 kidney tumours (6 adenomas and 1 adenocarcinoma) were observed among 30 males examined that had received the 0.15% dose, compared with 1 kidney adenoma in 44 controls examined ($P<0.05$). It should be noted that no kidney tumours were found in the high dose group.

Ref.: A

Charles River CD-1 mice (groups of 60 males and 60 females) were fed Acid Blue 9 (90% pure) as a dietary admixture at levels of 0, 0 (two control groups), 0.5%, 1.5% and 5% in a lifetime carcinogenicity study. The maximum exposure time was 104 weeks for both males and females. Mean body weights for the groups of treated mice were slightly lower when compared with either control group. Statistically significant decreases in body weight ($P<0.01$) occurred at some intervals for males and females at the two highest doses. Survival was not affected by the treatment. The 5% females exhibited an increased incidence of haemangioma of the spleen (F 5%; 3/60, 1.5%; 1/60, 0.5%; 1/60 vs 0/60 in control animals). No information about historical control data regarding the spontaneous incidence of haemangiomas of the spleen was reported for this strain of mice. All three cases were found in the final killing. The relevance of these findings cannot be evaluated.

Ref.: 2

Skin painting

Mice

Skin painting studies in ICR Swiss Webster mice were carried out with a series of 14 cosmetic colours including Acid blue 9 (89% purity). The treatment groups contained 50 males and 50 females and the control groups contained 150 males and 150 females. Mice were painted twice weekly in an area that precluded oral exposure with 0.1 ml containing 1.0% Acid blue 9 to a depilated 6 cm^2 area. Survival, body weight, and palpable growth were followed for an 18 month period. Microscopic examination of the treated animals was extended to include all tumours and grossly abnormal tissues and organs. There were no significant differences between treatment and control groups.

Ref.: 3

Subcutaneous and/or intramuscular administration

Rat

Groups of 48 and 27 rats [sex and strain unspecified] received twice weekly subcutaneous injections of 1 ml of an aqueous solution of Acid blue 9, disodium salt containing 7.4 or 20 mg of the dye (37% pure). The average period of treatment was 20.5 months, and the average total dose per animal was 1.22 or 3.5 g, respectively, calculated on the basis of pure dye. Injection-site fibrosarcomas developed in 10 and 20 animals. Three of four rats given twice weekly subcutaneous injections of 20 mg Acid blue 9, disodium salt (37% pure) for nine months and observed for a further 22 months developed injectionsite sarcomas. In groups of 31 and 14 rats that served as controls and that were injected subcutaneously with 0.9% and 3.4% saline for more than eight months (maximum, 29 or 27 months), no injection-site tumours occurred.

Ref.: B

Groups of 25 and 40 rats [sex and strain unspecified] received twice weekly subcutaneous injections of 1 ml of an aqueous solution of Acid blue 9, disodium salt containing 7.4 or 20 mg of the dye (> 90% pure). The average periods of treatment were 17 and 12.5 months, and the average total dose per animal was 1.07 and 2.5 g, respectively, calculated on the basis of pure dye. Injection-site fibrosarcomas developed in 18 and 25 rats. All of seven rats given twice weekly subcutaneous injections of 20 mg brilliant blue FCF, disodium salt (>90% pure) for nine months and observed for a further 10.5 months developed injection-site sarcomas. The controls

were the same as those used in the experiment with 37% pure brilliant blue FCF, disodium salt described above

Ref.: B

Ten male and 10 female five-week-old Wistar rats received weekly subcutaneous injections of 0.5 ml of a 4% solution of Acid blue 9, disodium salt (food grade) in isotonic saline for 45 weeks (total dose, 900 mg). The experiment was terminated at 71 weeks, at which time nine of the animals were still alive. No fibrosarcomas were observed

Ref.: C

Nine male and nine female Osborne-Mendel rats, about four-weeks old, received weekly subcutaneous injections of 30 mg *Acid blue 9*, disodium salt as a 3% aqueous solution for two years; five rats were still alive at 18 months. A total of 16 rats developed injection-site sarcomas, and none occurred in 18 saline-injected control animals

Ref.: 26

Human studies

No data.

2.10. Special investigations

See also point 2.7- Kinetic studies

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

(Acid Blue 9)
(semi-permanent)

Maximum absorption through the skin	**A (µg/cm²)**	**=**	**35.5 (µg/cm²)**
Typical body weight of human	**kg**	**=**	**60 kg**
Exposed area (scalp)	**SAS (cm²)**	**=**	**700 cm²**
Systemic exposure	**A x SAS x 0.001**	**=**	**24.8 mg**
Systemic exposure dose (SED)	**A x SAS x 0.001 / 60 kg**	**=**	**0.414 mg/kg**
No observed adverse effect level (mg/kg) (rat, 2 yrs, lifetime study)	**NOAEL**	**=**	**630**
Margin of Safety	**NOAEL / SED**	**=**	**1522**

2.12. Conclusions

Incomplete physico-chemical data, including pKa and Log Pow).

Acid Blue 9 is proposed to be used as direct dye in hair colouring products. Its concentration in the finished cosmetic product will not exceed 0.5 %. It is permitted as food colorant in the European Economic Community (E133) and is listed in PAFA as a substance approved to be added to food in the USA. Further it is approved as cosmetic colorant in the EU. An Acceptable Daily Intake (ADI) of 0-12.5 mg/kg bw (*Acid Blue 9* disodium salt) has been established by the Joint FAO/WHO Expert Committee on Food Additives in 1970. The EEC Scientific Committee for Food has 1983 recommended an ADI of 10 mg/kg bw.

Only limited information on the acute toxicity of *Acid Blue 9* is available which indicates that the acute oral toxicity is low (LD_{50} (oral, rat) > 2000 mg/kg bw). Valid studies regarding the toxic effects after chronic application have been performed in rats an mice. Mice were less sensitive than rats, no adverse effects were observed in mice even in the highest concentration tested (NOAEL mice: about 9000 mg/kg bw/day (5 % *Acid Blue 9* in diet) for females). In rats a decrease in terminal body weight and decreased survival was observed in females of the highest dose group, whereas no adverse effects were seen in males (NOAEL rats: about 630 mg/kg bw/day (1 % *Acid Blue 9* in diet) for female rats; about 1000 mg/kg bw/day (2 % *Acid Blue 9* in diet) for male rats). The NOAEL of 630 mg/kg bw/day is used for the calculation of the MOS.

Acid Blue 9 in aqueous solutions up to 1 % was not irritating to skin in mice and rabbits. Reports on findings in humans, which were exposed to 5 % aqueous solutions indicate that this concentration might be moderately irritating.
1% aqueous solution of *Acid Blue 9*, assessed for eye irritation potential with HET-CAM and Neutral-Red Uptake *in vitro* assays, was considered non-irritant in both assays. These assays have limitations for use with coloured substances. Neither test has been validated. Investigations in rabbit eyes which were not in accordance with current standards indicated that even the repeated application of a 10 % aqueous solution is not irritant to mucous membranes.

A Local Lymph Node Assay which was in accordance with current standards revealed that *Acid Blue 9* is not sensitising after dermal contact. These results were confirmed by observations in humans (study of limited value). No potential for photosenitisation was detected in the same study. Reports about the induction of immediate type allergic reactions in humans by *Acid Blue 9* after oral ingestion must be doubted in their quality and significance.

The original data from studies on teratogenicity could not be evaluated concerning their validity. The data cited in a review article indicate that *Acid Blue 9* is not teratogenic in rats and rabbits in doses up to 2000 mg/kg bw /day and 200 mg/kg bw/day, respectively. A valid one-generation study reported that *Acid Blue 9* had no adverse effect on reproduction parameters in rats and on development of the F1 neonates in concentrations up to about 1000 mg/kg bw/day (2 % in diet of rats). A two-generation study which is not published reported a LOAEL of 1000 mg/kg bw/day due to reduced body weights of the F1 and F2 neonates and a slight increase in the incidence of lung and kidney abnormalities in adult males. However, no information on the statistical significance of the effects was provided.

In studies with radiolabelled substance it was shown in three species that the degree of absorption of *Acid Blue 9* after oral application is low (less than 5 %). Percutaneous absorption is also low. In vitro investigations with pig skin which were performed according to current

standards revealed penetration rates of 6.3 μg/cm^2 within 24 hours (= 0.13 % of applied dose) for the pure colour and 35.5 μg/cm^2 within 24 hours (= 0.60 % of the applied total amount) for a ready to use product which contains 0.5 % *Acid Blue 9*. The calculations of the penetration rates are based on worst case assumptions. The higher value of 35.5 μg/cm^2 will be used for the calculation of the systemic exposure dose (SED).

Several published papers reporting positive and negative mutagenic/genotoxic effects of the test item could not be evaluated, due to the lack of information for considering the adequacy of the studies, as stated also in the safety dossier.

The studies performed with a known sample of the test item and following OECD guidelines are the (1) bacterial reverse mutation test (2 studies),(2) the in vitro mammalian cell gene mutation test (1study), and (3) the *in vivo* UDS test (1 study).

The studies on bacteria indicated that the test item in non mutagenic in this assay; however in both tests induced rat liver homogenate S 9 was used. Data from the literature indicate that the test item is mutagenic in the presence of a reduced metabolic activation system (hamster liver homogenate).

The in vitro mammalian cell gene mutation test has made use of induced rat liver homogenate. The negative results may have been influenced by such metabolic condition. The *in vivo* UDS gave negative results.

Oral administration: One adequate carcinogenicity study with rats did not indicate any carcinogenic potential of Acid Blue 9. In one mice study 7 kidney tumours were found among males receiving 0.15% Acid Blue 9, while no such tumours were found among the group receiving 1.5% Acid Blue 9. In another mice study 3 haemangiosarcom of the spleen were found in the female group receiving 5% Acid Blue 9.

The sensitivity of a skin-painting test was probably too low to identify any carcinogenic potential. Injection site sarcomas have been reported in rats after subcutaneous injection. No distant metastasis was reported.

2.13. References

1. BIBRA, The British Industrial Biological Research Association. Toxicity Profile Brilliant Blue FCF. BIBRA Toxicology International, Great Britain. (1990).
2. J F Borzelleca, K Depukat, J B Hallagan. Lifetime toxicity/carcinogenicity studies of FD & C Blue No. 1 (Brilliant Blue FCF) in rats and mice. Food and Chemical Toxicology, 28 221-234 (1990).
3. S Carson. Skin painting studies in mice with 14 FD&C and D&C colors: FD&C Blue4 No. 1, Red No. 3, and Yellow No. 5, D&C Red No. 7, Red No. 9, Red No. 10, Red No. 19, Red No. 21, Red No. 27, Red No. 31, Red No. 36, Orange No. 5, Orange No. 10, and Orange No. 17. Journal of Toxicology / Cutaneous and Occular Toxicology, 3 357-370 (1984).
4. C M Burnett, D L Opdyke. Chronic eye irritation and staining properties of some organic colors and lakes. CTFA Cosmetic Journal, 3 18-22 (1971).

5. Cosmital SA. Assessment of the Eye Irritation Potential of Blue 1 (WR 23312) in the Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM). Final Report. Study No. HC 141. Marly, Switzerland. Sponsored by Wella AG Darmstadt. 2002.
6. Cosmital SA. Assessment of the Eye Irritation Potential of CI-Nr. 42090 / Colipa-No. C40 by Cytotoxicity Measurement in the Neutral Red Uptake Assay (NRU) on Human Keratinocytes (HaCaT). Final Report. Study No. NU 120. Marly, Switzerland. Sponsored by Wella AG Darmstadt. 2002.
7. MDS Pharma Services. CI 42090; FD&C Blue 1 – Local lymph node assay. MDS Study No 762/010. Sponsored by Wella AG Darmstadt. 2002.
8. M Green. Sublingual provocative testing for foods and FD&C dyes. Annals of Allergy, 33 274-281 (1974).
9. H Mikkelsen, J C Larsen, F Tarding. Hypersensitivity reactions to food colours with special reference to the natural colour Annatto Extract (Butter Colour). Archives of Toxicology, Suppl. 1 141-143 (1978).
10. J C Phillips, D Mendis, C T Eason, S D Gangolli. The metabolic disposition of ^{14}C-labelled Green and S Brilliant Blue FCF in the rat, mouse and guinea pig. Food and Chemical Toxicology, 18 7-13 (1980).
11. J P Brown, A Dorsky, F E Enderlin, R L Hale, V A Wright, T M Parkinson. Synthesis of ^{14}C-labelled FD & C Blue no. 1 (Brilliant Blue FCF) and its intestinal absorption and metabolic fate in rats. Food and Chemical Toxicology, 18 1-5 (1980).
12. T Iga, S Awazu, H Nogami. Pharmacokinetic study of biliary excretion: II. Comparison of excretion behavior in triphenylmethane dyes. Chemical and Pharmaceutical Bulletin, 19 273-281 (1971).
13. RCC Cytotest Cell Research GmbH. Skin Permeability in Vitro Absorption Through Porcine Ear Skin with FD&C Blue 1 (C.I. 42090) Report. RCC – CCR Project 641003. 2000.
14. 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. Mutation Research, 189 223-261 (1987).
15. RCC Cytotest Cell Research GmbH. Salmonella Typhmurium and Escherichia Coli Reverse Mutation Assay with FD&C Blue 1 (C.I. 42090) Report. RCC – CCR Project 641001. 1999.
16. Cosmital SA. Assessment of the Potential Mutagenicity of WR 23211 in the Ames Reversion Assay with Salmonella Typhimurium. Final Report. Study No. AT 771. Marly, Switzerland. 2002.
17. 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 and Chemical Toxicology, 22 623-636 (1984).
18. E Longstaff, DB 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 similiar materials. Dyes and Pigments, 5 65-82 (1984).
19. R B Haveland-Smith, R D Combes. Screening of food dyes for genotoxic activity. Food and Cosmetics Toxicology, 18 215-221 (1980).
20. M Ishidate, T Sofuni, K Yoshikawa. Chromosomal aberration tests in vitro as a primary screening tool for environmental mutagens and/or carcinogens. GANN Monograph on Cancer Research, 27 95-108 (1981).
21. RCC Cytotest Cell Research GmbH. Cell Mutation Assay at the Thymidine Kinase Locus ($TK^{+/-}$) in mouse Lymphoma L5178Y Cells with FD&C Blue 1 (C.I. 42090) Report. RCC – CCR Project 641002. 2000.

22. 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. Environmental Mutagenesis, 7 101-120 (1985).
23. P J Price, w A Suk, A E Freeman, W T Lane, R L Peters, M L Vernon, R J Huebner. In vitro and in vivo indicators of the carcinogenicity and toxicity of fodd dyes. International Journal of Cancer, 21 361-367 (1978).
24. M Hayashi, M Kishi, T Sofuni, M Ishidate. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food and Chemical Toxicology, 26 487-500 (1988).
25. RCC Cytotest Cell Research GmbH. In Vivo/in Vitro Unscheduled DNA Synthesis in Rat Hepatocytes with Blue 1 WR 23312 Report. RCC – CCR Study 726709. 2002.
26. W H Hansen, O G Fitzhugh, A A Nelson, J J Davis. Chronic toxicity of two food colors, Brilliant Blue FCF and Indigotine. Toxicology and Applied Pharmacology, 8 29-36 (1966).
27. IARC, International Agency for Research on Cancer. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Vol. 16. Some Aromatic Amines and Related Nitro Compounds - Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals. WHO, World Health Organization, Geneva. (1978).
28. W A Mannell, H C Grice, M G Allmark. Chronic toxicity studies on food colours. V. Observations on the toxicity of Brilliant Blue FCF, Guinea Green B and Benzyl Violoet 4B in rats. Journal of Pharmacy and Pharmacology, 14 378-384 (1962).
29. N Waterman, G O E Lignac. The influence of the feeding of a number of food colours on the occurrence of tumours in mice. Acta Physiologica et Pharmacologica Neerlandica, 7 35-55 (1958).
30. W H Hansen, O G Fitzhugh, A A Nelson, J J Davis. Chronic toxicity of two food colors, Brilliant Blue FCF and Indigotine. Toxicology and Applied Pharmacology, 8 29-36 (1966).
31. P Grasso, L Golberg. Early changes at the site of repeated subcutaneous injection of food colourings. Food and Cosmetics Toxicology, 4 269-282 (1966).
32. T S Banerjee, B R Roy. Formation of nitrosamines from food dyes and effect of additives theoren. Journal of the Institution of Chemists, 50 134-138 (1978).
33. LAN-Analysenbericht Studien Nr.A2002/343 Nov. 14, 2002. Wella AG; D-64274 Darmstadt
34. LAN-Analysenbericht Studien Nr. A2003/312 Sept. 23, 2003A2. Wella AG; D-64274 Darmstadt
35. Lan_Analysenbericht Studien Nr.A2001/357 Jan 16, 2002. Wella AG; D-64274 Darmstadt
36. LAN-Analysenbericht Studien Nr. A2001/041 May 07, 2001. Wella AG; D-64274 Darmstadt
37. LAN-Analysenbericht Studien Nr. A2002/014 Feb. 01, 2002. Wella AG; D-64274 Darmstadt
38. Food and Drug Administration; April 05, 1999. Color Additive Certificate – Certificate of analysis by Hilton Davis
39. Certificate of analysis by Toshiki Pigment
40. Certificate of analysis by BASF; July 20, 2000

SCCNFP references

A. Rowland IR, Gaunt IF, Hardy J, Kiss IS, Butterworth KR. Long-term toxicity of brilliant blue FCF in mice. Fd Cosmet Toxicol, 1977.
B. Gross E. Über die Erzeugung von Sarkomen durch die besonders gereinigten Triphenylmethanfarbstoffe Lichtgrün SF und Patentblau AE bei der wiederholten subcutanen Injection an der Ratte. Z Krebsforsch 64: 287-304, 1961.

C. Mannell WA, Grice HC. Chronic toxicity of brilliant blue FCF, blue VRS, and green S in rats. J Pharm Pharmacol 16: 56-59, 1964.

3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the use of Acid Blue 9 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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