# Brilliant Blue FCF

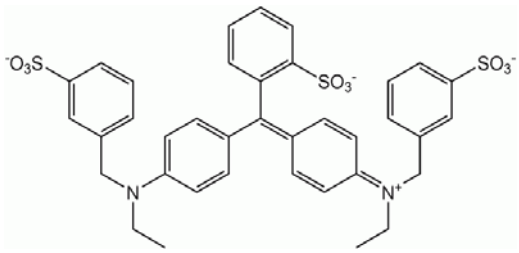
## SYNONYMS

Acid Blue 9, disodium salt;
Alphazurine FG;
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;
Bis[4-(N-ethyl-N-3-sulfophenylmethyl)aminophenyl]-2-sulfophenylmethylium disodium salt; Disodium bis[4-(N-ethyl-N-3-sulfonatophenylmethyl)aminophenyl]-2- sulfonatophenylmethylium;
Brilliant blue FCF;
Brilliant blue FCF, disodium salt;
C.I. 42090;
Erioglaucine;
Erioglaucine disodium salt;
Erioglaucine disodium salt (C.I. 42090); FD&C Blue No. 1;
Food Blue 2;
Food Blue No.1

## CHEMICAL FORMULA

$C_{37}H_{34}N_2Na_2O_9S_3$

## CHEMICAL STRUCTURE




## IDENTIFIER DETAILS

| | | |
|---|---|---|
| CAS Number | : | 3844-45-9 |
| CoE Number | : | - |
| FEMA | : | - |
| EINECS Number | : | 272-939-6 |
| E Number | : | E133 |

## CLP CLASSIFICATION

Ingredient CLP Classification: No

| Endpoint | Classification | Category |
|---|---|---|
| **Acute Oral Toxicity** | - | - |
| **Acute Dermal Toxicity** | - | - |
| **Acute Inhalation Toxicity** | - | - |
| **Skin Corrosive/irritant** | - | - |
| **Eye Damage/Irritation** | - | - |
| **Respiratory Sensitisation** | - | - |
| **Skin Sensitisation** | - | - |
| **Mutagenicity/Genotoxicity** | - | - |
| **Carcinogenicity** | - | - |
| **Reproductive Toxicity** | - | - |
| **Specific Target Organ Toxicity** | - | - |
| **Aspiration Toxicity** | - | - |

## SPECIFICATIONS

Melting Point: 283$^{o}$C

Boiling point: No information available

## PURPOSE

Dye stamp

## STATUS IN FOOD, TOBACCO AND DRUG LAWS

**CoE limits:**

| Beverages (mg/kg) | Food (mg/kg) | Exceptions (mg/kg) |
|---|---|---|
| | | |

**Acceptable Daily Intake:**

| ADI (mg/kg) | ADI Set by | Date Set | Comments |
|---|---|---|---|
| 0-12.5 | JECFA | 1969 | Unconditional acceptance |
| 6.0 | EFSA | 2010 | |

**FDA Status:** [CFR21]

| Section Number | Comments |
|---|---|
| | |

## HUMAN EXPOSURE

***Natural Occurrence:*** It is a synthetic dye produced using aromatic hydrocarbons from petroleum. It can be combined with tartrazine (E102) to

produce various shades of green. it is usually a disodium salt. The diammonium salt has CAS number [2650-18-2]. Calcium and potassium salts are also permitted. It can also appear as an aluminium lake. The chemical formation is C37H34N2Na2O9S3. The dye is poorly absorbed from the gastro-intestinal tract and 95% of the ingested dye can be found in the faeces.

***Reported Uses:*** As a blue colour, Brilliant Blue FCF is often found in ice cream, canned processed peas, dairy products, sweets and drinks. It is also used in soaps, shampoos, mouthwash and other hygiene and cosmetics applications.

## TOXICITY DATA

### *In Vivo* Toxicity Status

A colour solution of Brilliant blue FCF was orally administered to rats at a level of 200 mg per rat. The entire administered dose was excreted unchanged in the faeces within 40 hours of administration. A later experiment revealed the presence of the dye in the bile of rats, rabbits and dogs (after oral administration). The dose in the diet was not reported to exceed 5% for the dog study, [JECFA, 1969].

The administration of an aqueous solution of Brilliant blue FCF by stomach tube was reported to result in 89 % excretion in the faeces with none being found in the urine. However, the subcutaneous injection of 80-100 mg resulted in the excretion of 77 % in the faeces and 2.5 % in the urine, [JECFA, 1969].

| Route of Exposure | Species | $LD_{50}$ mg/kg b.w. |
|---|---|---|
| Oral | Wistar Rat | >2000 |
| Subcutaneous injection | Mouse | 4600 |

[Federal Register, 1988]

| Test animal | In diet / % | Exposure /d | Toxicity effect |
|---|---|---|---|
| Rats (male) | 5 | 7, 14, 21 | Growth retardation[1] |
| Rats | 5 | 21 | Reduced food intake[1] |
| Rats | 5 | 21 | No effect on liver and cecum weight[1] |
| Rats | 3 | 525 | No adverse effects o growth; no reduction of food consumption; no diminution of food efficiency.[2] |
| Rats (female) | 3 | 525 | Increase of mortality[2] |
| Rats | 5 | 730 | No mortality; no effect on hematology; no effect on weight of organs[3] |
| Rats | 2 | 900 | No effect on appearance, hematology, biochemical values, and urinalysis; no carcinogenicity[4] |
| Rats (male) | 2 | 900 | No mortality; no growth retardation[4] |

| | | | |
|---|---|---|---|
| Rats (female) | 1 | 900 | No mortality; no growth retardation[4] |
| Rats (female) | 2 | 900 | Mortality; growth retardation[4] |
| Mice | 5 | 730 | No mortality; no effect on hematology, behaviour and morbidity; no carcinogenicity[4] |
| Dogs | 2 | 365 | No microscopic lesions; no clinical signals[3] |

[1]Tsujita *et al*. (1979). [2]Mannell *et al*., (1979). [3]Hansen *et al*., (1962). [4]Borzalleca *et al (1990)*

An 11-year-old white girl with cerebral palsy was admitted for unresolving aspiration pneumonia and dehydration. Antibiotics and intravenous fluids were administered. During the hospital course, enteral nutrition containing blue food coloring FD&C Blue No. 1 also administered. Twelve hours after the start of enteral nutrition, the patient appeared cyanotic despite a regular respiratory rate and normal oxygen saturation. The pediatric code response team was called. Enteral nutrition was stopped and then restarted without blue food coloring. Over the next 24 hours, the cyanotic appearance resolved and no further complications developed. It was estimated this child ingested 780-3,940 mg of dye over a 12-hour period. This is the first known report of an adverse effect from blue food coloring, [Zillich *et al*., 2000].

This study by Lucarelli *et* al., (2004) reports two cases of abnormal systemic absorption of FD&C Blue No. 1 in critically ill patients who subsequently died of refractory shock and metabolic acidosis. Risk factors and mechanisms of FD&C Blue No. 1 toxicity are discussed, and alternate approaches to gastric aspiration detection in critically ill patients are considered, [Lucarelli *et al*., 2004].

The injection of 250 mg/kg bw (s.c.) of the colouring to five rats twice a day for three days was reported to be without oestrogenic activity with no other reported abnormalities, [JECFA, 1969].

**Short-term studies**

Daily doses of 2mg to mice for a period of 30 days were reported to be well tolerated. However, animals showed swelling of the liver and spleen if 4 mg was administered. Also, mice reported to be fed 1200 mg of dye for 19-days were reported to show ‘no damage’, [JECFA, 1969].

Beagle dogs (no numbers given) fed 1 and 2 % in the diet for a period of 1-year was reported to be without gross or microscopic changes, [JECFA, 1969].

24 weanling Osborne-Mendel rats administered the colour at levels of 0, 0.5, 1, 2 and 5 % in the diet for a period of 2-years was reported to have no effect on tumour production, (only reported unrelated incidental lesions) statistical analysis revealed no changes in organ weights mortality, growth or haematology. Similar, results were obtained for four groups of fifteen male and female rats given 0., 0.03, 0.3 and 3 % in the diet for a period of 75

weeks. Mortality was however, reported to increase in the 3 % treatment group due to an unrelated respiratory infection, [JECFA, 1969].

The s.c. injection of various amounts of colour was reported to produce fibrosarcomas at the site of injection. However, another study revealed that the s.c injection of 0.5ml of a 4% solution did not produce tumours, [JECFA, 1969].

**Carcinogenicity & Mutagenicity**

Five male and five female rats fed 4 % colour for a period of 600 days did not reveal any tumours. Similarly 85 rats fed a diet containing 0.1 % colour for their entire lifespan. With a reported daily intake of 10-15 mg also failed to reveal any tumours, [JECFA, 1969].

The subcutaneous injection of 1ml of 0.8 % solution (twice weekly, no other information provided) was reported to be associated with sarcoma formation which was reported to be unassociated with chemical carcinogenic potential, [JECFA, 1969].

The administration twice weekly (10 doses) of 20 mg per rat as a 2% solution) followed by doses of 30 g as a 6 % solution to 84-male and 84-female Wistar rats resulted in 119/168 rats developing sarcomas at the site of injection with seven animals surviving 2-years. Males did not reveal any tumours however, females reported 6, mammary, 1 ovarian, 1 uterine and 2-heptomas. 26 controls survived 2-years out of 48 and these females developed 1-mammary, one ovarian and four uterine tumours. A study in which six male and six female Slonacker rats were treated in the same manner as the Wistar rats, resulted in the death of all animals within 420 days. Tumours developed in the injection site in five male and three females. Control groups reported 10/12 animals alive at 400 days with no tumours developing in the control animals, JECFA, 1969].

The percutaneous injection of 5% aqueous solution (1g first day and 0.1 g days 9-18) in cats was not reported to be associated with methaemoglobinaemia or Heinz bodies. 57-male and 43-female mice administered 1mg of the colour a day with ‘observations extended over a period of 500-700-days’ revealed ‘no evidence of carcinogenic action’. Also, ten s.c injections of 4mg followed by 50 doses of 6 mg did not produce any tumours, [JECFA, 1969].

| Species | Strain | Route | Dose | Duration | Result |
|---|---|---|---|---|---|
| Rat | CD/Male | Oral | 0, 0.1, 1, 2 % 116wks(parents fed 0, 0.1, 1 and 2 % respectively in diet] | Lifetime | Negative |
| Rat | CD/Female | Oral | 0, 0.1, 1, 2 % 111wks(parents fed 0, 0.1, 1 and 2 | Lifetime | Negative |

| | | | | | |
|---|---|---|---|---|---|
| | | | % respectively in diet] | | |
| Mouse | CD-1/Male | Oral | 0, 0.5, 1.5 5 % in diet 104 wk | Lifetime | Negative |
| Mouse | CD-1/Female | Oral | 0, 0.5, 1.5, 5 % in diet 104 wk | Lifetime | Negative |
| Mouse | ICR/Male | Dermal | 0 and 1mg in 0.1 ml distilled water 2/wks for mean total dose of 128.8 mg | 18-Months | Negative |
| Mouse | ICR/Female | Dermal | 0 and 1mg in 0.1 ml distilled water 2/wks for mean total dose of 128.8 mg | 18-months | Negative |

[1984-1990 studies as cited from CCRIS Chemical Carcinogenisis Research Information System]

IARC reported Brilliant blue FCF, disodium salt as carcinogenic in rats after s.c injection: it produced fibrosarcomas following repeated injections, [IARC, 1978]. IARC later classified this compound as Group , [JECFA, 1969].

**Dermal Toxicity**

Brilliant blue FCF was reported to be irritating to the skin of humans and the eyes of rabbits, with individuals already suffering from allergic conditions reporting a potentiation of their symptoms following oral exposure. [BIBRA, 1990]. BIBRA have concluded that long-term feeding studies in rats and mice and the dermal application in mice has not provided evidence of carcinogenicity. Brilliant blue FCF was reported to cause DNA damage in orally treated rats and give a variety of genotoxic effects in cultured mammalian cells. However, there was reported to be no evidence of mutagenicity in the Ames assay, [BIBRA, 1990].

**Reproductive Developmental Toxicity**

No data identified.

**Inhalation Studies**

Forty-five patients with moderately severe perennial bronchial asthma were challenged by ingestion of: acetylsalicylic acid (ASA), and various food dyes, one of which includes Brilliant Blue. The findings suggest that reactions to dyes and preservatives are an uncommon cause of clinically significant bronchoconstriction in moderately severe perennial asthmatics, [Weber *et al*., 1979].

**Other relevant studies**

In 2006, the Korea Food and Drug Administration reported that combinations of dietary colors such as allura red AC (R40), tartrazine (Y4), sunset yellow FCF (Y5), amaranth (R2), and brilliant blue FCF (B1) are widely used in food manufacturing. Although individual tar food colors are controlled based on acceptable daily intake (ADI), there is no apparent information available for how combinations of these additives affect food safety. In the current study, the potencies of single and combination use of R40, Y4, Y5, R2, and B1 were examined on neural progenitor cell (NPC) toxicity, a biomarker for developmental stage, and neurogenesis, indicative of adult central nervous system (CNS) functions. A combination of Y4 and B1 at 1000-fold higher than average daily intake in Korea significantly decreased numbers of newly generated cells in adult mouse hippocampus, indicating potent adverse actions on hippocampal neurogenesis. Evidence indicates that single and combination use of most tar food colors may be safe with respect to risk using developmental NPC and adult hippocampal neurogenesis. However, the response to excessively high dose combination of Y4 and B1 is suggestive of synergistic effects to suppress proliferation of NPC in adult hippocampus. Data indicated that combinations of tar colors may adversely affect both developmental and adult hippocampal neurogenesis; thus, further extensive studies are required to assess the safety of these additive combinations, [Park *et al*., 2009].

## BEHAVIOURAL DATA

In this study, Doguc *et al*., (2012) aimed to provide additional data to clarify the possible side effects of colouring additives on behaviour and memory. Acceptable daily intake values of AFCAs as a mixture (Eritrosin, Ponceau 4R, Allura Red AC, Sunset Yellow FCF, Tartrazin, Amaranth, Brilliant Blue, Azorubin and Indigotin) were administered to female rats before and during gestation to test their effects on behaviour and on spatial working memory in their offspring. Effects on spatial learning and memory were evaluated by Morris water maze, behavioural effects were evaluated by open-field test and forced swim test. Our results showed that commonly used artificial food colourings have no adverse effects on spatial working memory and did not create a depressive behaviour in offspring. But they showed a few significant effects on locomotor activity as AFCAs increased some parameters of locomotor activity, [Doguc *et al*., 2012].

### *In Vitro* Toxicity Status

### Carcinogenicity & Mutagenicity

Results below are for the Ames assay using the strains reported:

| Test Strain | Metabolic activation | Method | Dose ug/plate | Result |
|---|---|---|---|---|
| TA98 | No | Pre-incubation | 750-3000 | Negative |
| TA98 | With and | Pre-incubation | 750-3000 | Negative |

| | | | | |
|---|---|---|---|---|
| | without activation | | before or after uv radiation for 14-50 days | |
| TA100 | No | Pre-incubation | 750-3000 before or after uv radiation for 14-50 days | Negative |
| TA100 | Yes | Pre-incubation | 750-3000 before or after uv radiation for 14-50 days | Negative |
| TA100 | Yes | Pre-incubation | 750-3000 | Negative |
| TA100 | No | Pre-incubation | 750-3000 | Negative |
| TA98 | Yes | Pre-incubation | 750-3000 | Negative |
| TA98 | With and without activation | Pre-incubation | 750-3000 before or after uv irradiation for 6-days | Negative |
| TA100 | With and without activation | Pre-incubation | 750-3000 before or after uv irradiation for 6-days | Negative |

Data obtained from CCRIS (2004).

Five synthetic food colours, including Food Blue No 1, and their UV irradiated products were tested for mutagenic activity by means of the Ames test using Salmonella typhimurium strains TA98 and TA100. Food colours were irradiated with UV light for 14 days. Food Blue No. 1 were non-mutagenic before and after irradiation, [Ozaki *et al*., 1998].

A series of synthetic food colorants used in India, including Tartrazine (E102), were tested either alone or in combination, at different concentrations, using the CBMN (Cytokinesis Block Micronucleus Assay). The study showed that, even at the allowed concentration of 100 ppm (as per Prevention of Food Adulteration – PFA), all these colorants and their combinations could cause genotoxicity to human lymphocytes. Colorant combinations showed more toxicity than the individual colorants; the toxicity varied between individual dyes, proportional to their concentration. The authors mention that a drastic reduction in toxicity can be obtained by reducing the colorant concentration to at least 50% below the permissible limit. In addition to this, the authors also suggest the need to redefine the permissible levels of food dyes (based on ADIs) [Swaroop, Roy *et al.,* 2011]

A study by HE Eroglu *et al.* (2015) investigated the genotoxic and cytotoxic effects of Brilliant Blue. Genotoxic and cytotoxic activities of the food additive were evaluated in lymphocyte cell cultures using mitotic index, replication index and micronucleus assay. Mitotic index frequencies and replication index values were decreased and micronucleus frequency was increased with increasing concentrations of Brilliant Blue. The changes in mitotic index and

micronucleus were statistically significant ($p<0.05$). The results showed that Brilliant Blue can have cytotoxic and genotoxic potential. It care must be taken when using as a food additive.

**Other Relevant Studies**

Synthetic or natural food dyes are typical xenobiotics, as are drugs and pollutants. After ingestion, part of these dyes may be absorbed and metabolized by phase I and II drug-metabolizing enzymes and excreted by transporters of phase III enzymes. However, there is little information regarding the metabolism of these dyes. It was investigated whether these dyes are substrates for CYP2A6 and UDP-glucuronosyltransferase (UGT). The in vitro inhibition of drug-metabolizing enzymes by these dyes was also examined. The synthetic food dyes studied, amongst others, included brilliant blue FCF (food blue no. 1). The natural additive dyes studied were extracts from purple sweet potato, purple corn, cochineal, monascus, grape skin, elderberry, red beet, gardenia, and curthamus. Data confirmed Brilliant Blue FCF was not a substrate for CYP2A6, UGT1A6, and UGT2B7, [Kuno *et al*., 2005].

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