ACID BLUE 9 ALUMINIUM LAKE

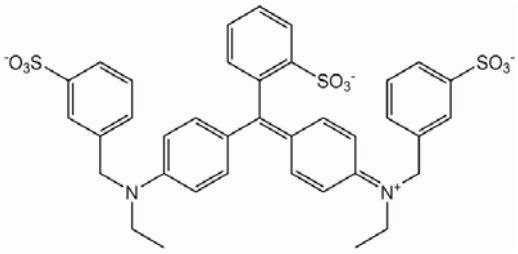
SYNONYMS

Benzenemethanaminium, N-ethyl-N-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfo-, inner salt, aluminium salt
C.I. Acid blue 9-aluminium lake
C.I. Pigment Blue 78
CI 42090:2
C.I. Food blue 2-aluminium lake
FD & C Blue no. 1 aluminium lake
Food blue 2.1
Brilliant blue FCF

CHEMICAL FORMULA

$C_{37}H_{36}N_2O_9S_3.xAl$

CHEMICAL STRUCTURE



IDENTIFIER DETAILS

CAS Number	:	68921-42-6, 53026-57-6
CoE Number	:	-
FEMA	:	-
EINECS Number	:	272-939-6
E Number	:	E133

CLP CLASSIFICATION

Ingredient CLP Classification: Yes

Endpoint	Classification	Category
Acute Oral Toxicity	conclusive but not sufficient for classification	-
Acute Dermal Toxicity	conclusive but not sufficient for classification	-
Acute Inhalation Toxicity	conclusive but not sufficient for classification	-
Skin Corrosive/irritant	conclusive but not sufficient for classification	-
Eye Damage/Irritation	conclusive but not sufficient for classification	-
Respiratory Sensitisation	conclusive but not sufficient for classification	-
Skin Sensitisation	conclusive but not sufficient for classification	-
Mutagenicity/Genotoxicity	conclusive but not sufficient for classification	-
Carcinogenicity	conclusive but not sufficient for classification	-
Reproductive Toxicity	conclusive but not sufficient for classification	-
Specific Target Organ Toxicity	conclusive but not sufficient for classification	-
Aspiration Toxicity	conclusive but not sufficient for classification	-

REACH Statement

This ingredient has been registered under REACH. Under REACH, registrants have an obligation to provide information on substances they manufacture or import. This information includes data on hazardous properties (covering various toxicological endpoints), guidance on safe use and classification and labelling. The European Chemicals Agency (ECHA) makes this information publicly available on its website: http://echa.europa.eu/.

SPECIFICATIONS

Melting Point: No information available.

Boiling point: No information available.

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 mg/kg bw	JECFA	1969	For water soluble

			form
6 mg/kg	EFSA	2010	

FDA Status: [CFR21]

Section Number	Comments
74.101	Listing of colour additives subject to certification

HUMAN EXPOSURE

Natural Occurrence: It is reported that Brilliant Blue FCF does not occur in nature. Aluminium lakes are produced by the absorption of water soluble dye onto a hydrated aluminium substrate therefore rendering the colour insoluble. Generally lakes are reported to be more stable than the corresponding water-soluble dyes, producing brighter colours and being most suitable for products containing oils and fat products,

Reported Uses: Brilliant Blue FCF, is a colorant for foods and other substances to induce a colour change. It is denoted by E number E133 and has a colour index of 42090. It has the appearance of a reddish-blue powder.

Sources other than food: As a blue colour, Brilliant Blue FCF is often found in soaps, shampoos, and other hygiene and cosmetics applications.

TOXICITY DATA

In Vivo Toxicity Status

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].

Carcinogenicity and mutagenicity

In a study reported by Grasso and Golber, (1966) Rats were injected subcutaneously with of 1 ml of 0.8 per cent aqueous solution containing Brilliant blue FCF twice a week. The authors report that this produced histological changes suggestive of subsequent sarcoma formation unassociated with chemical carcinogenic potential, [Grasso & Golber, 1966].

A group of 57 male and 43 female mice were given 1 mg colour per day in the diet. Observations extended over 500-700 days. No evidence of carcinogenic action was found (Waterman & Lignac, 1958). Subcutaneous injections of 10 doses of 4 mg followed by 50 doses of 6 mg showed no tumour production after 78 weeks (WHO, 1969).

Brilliant Blue FCF was fed in a concentration of four percent of the diet to five male and five female rats for 600 days. Gross staining the glandular stomach and some granular deposits in the stomach but no tumours were observed (WHO, 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

Brilliant blue FCF of food colour was given in the diets of mice at levels of 0 % (control), 0.08, 0.24, and 0.72 % from 5 weeks of age in the F(0) generation and continuing to 11 weeks of age in the F(1) generation and selected reproductive and neurobehavioral parameters were measured. Mice were mated at 9 weeks of age and dams were delivered offspring at 12 weeks of age. Offspring were weaned at 4 weeks of age. Regarding exploratory behaviour at 8 weeks of age in the F(0) generation, movement time (sec) displayed a significant tendency to be increased and the average time of rearing (sec) displayed a significant tendency to be decreased in females in the treatment groups in a trend test ($p = 0.019$ and 0.027, respectively). In the F(1) generation, the development of surface righting at postnatal day 4 was delayed significantly in the high-dose group (0.72 %) in male and female offspring, and those effects were significantly related to dose in a trend test ($p< 0.01$ for both). Regarding exploratory behaviour at 8 weeks of age in the F(1) generation, the number of horizontal activities exhibited a significant tendency to be decreased in females in the treatment groups in a trend test ($p = 0.015$). Regarding spontaneous behaviour, average time of movement (sec) was significantly accelerated in females in the high-dose group. The dose levels of brilliant blue FCF used in the present study produced a few significant effects on neurobehavioral parameters in multiple generations in mice [Tanaka *et al*., 2012].

Exposure to artificial food colours and additives (AFCAs) has been implicated in the induction and severity of some childhood behavioural and learning disabilities. N-methyl-D-aspartate receptors (NMDARs) and nicotinic acetylcholine receptors (nACHRs) are thought to be effective in the learning and memory-generating process. In this study, we investigated the effects of intrauterine exposure to AFCAs on subunit concentrations of NMDARs and nAChRs isoforms in rats. We administered a mixture of AFCAs (Eritrosin, Ponceau 4R, Allura Red AC, Sunset Yellow FCF, Tartrazin, Amaranth, Brilliant Blue, Azorubin and Indigotin) to female rats before and during gestation. The concentration of NR2A and NR2B subunits and nAChR α7, α4β2 isoforms in their offspring's hippocampi were measured by Western Blotting. Expressions of NR2B and nAChR β2 were significantly increased (17 % and 6.70 %, respectively), whereas expression of nAChR α4 was significantly decreased (5.67 %) in male experimental group compared to the male control group ($p < 0.05$). In the female experimental group, AFCAs caused a 14 % decrease in NR2B expression when compared to the female control group ($p<0.05$). Our results indicate that exposure to AFCAs during the foetal period may lead to alterations in expressions of NMDARs and nAChRs in adulthood. These alterations were different between male and female genders [Ceyhan *et al*., 2013].

Inhalation Toxicity

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].

The broadest toxic effect of some synthetic additives of colorants and/or flavours on different body organs and metabolic aspects in rats was investigated. In this experiment, a total of 100 male albino rats of Sprague Dawley strain were divided into 10 groups: G(1) was fed basal diet and served as control, G(2): basal diet + Brilliant blue (blue dye, No. 2, 124 mg/kg diet), G(3): basal diet + carmoisine (red dye, No. 3, 70 mg/kg diet), G(4): basal diet + tartrazine (yellow dye, FD & C yellow No. 5, 75 mg/kg diet), G(5): basal diet + trans-anethole (4.5 g/kg diet) G(6): basal diet + propylene glycol (0.25 g/kg diet), G(7): basal diet + vanillin(1.25 g/kg diet), G(8): basal diet + Brilliant blue + propylene glycol, G(9): basal diet + carmoisine + trans-anethole, G(10): basal diet + tartrazine + vanillin for 42 successive days. All food colourants mixed with or without flavour additives induced a significant decrease in body weight, haemoglobin concentration and red blood cell count. In addition, there was a significant decrease in reduced glutathione content; glutathione-S-transferase and superoxide dismutase activities in both blood and liver compared to control group. However, a significant increase in serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase

activities, bilirubin, urea, creatinine, total protein and albumin were observed in all test groups when compared to control group [El-Wahab *et al*., 2013].

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].

Artificial food colourings and additives (AFCAs) have long been suggested to adversely affect the learning and behaviour in children. In this study, we aimed to provide additional data to clarify the possible side effects of colouring additives on behaviour and memory. We administered 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) to female rats before and during gestation and then tested 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 [Doquc *et al*., 2013].

***In vitro* toxicity Studies**

Carcinogenicity & Mutagenicity

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].

Hus and Eroglu (2015) tested the genotoxicty and cytotoxicity of Brilliant Blue FCF and Sunset Yellow in an *in vitro* human lymphocyte culture. 0.4 mLblood samples were collected from ten healthy donors, and added to culture tubes alongside 5mL of culture medium. Aquatic extracts (10, 20, 30 and 40 mg/mL) of the two food dyes were then added to test tubes, which were kept, together with the control tubes, for 72 h incubated at 37℃. Cell cultures were treated

with 5µg/mL of Cytochalasin B in order to measure the amount of micronuclei and with 10µg/mL of BrdU to measure the replication index. The authors found that the frequency of the micronuclei increased as the concentrations of the two dyes increased, while the mitotic index frequencies and replication index values decreased, concluding that Brilliant Blue FCF and Sunset Yellow can have cytotoxic and genotoxic potential.

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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