REVIEW

The Effects of Natural and Synthetic Blue Dyes on Human Health: A Review of Current Knowledge and Therapeutic Perspectives

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ABSTRACT

Blue synthetic dyes are widely used in many industries. Although they are approved for use as food dyes and in cosmetics and some medicines, their impacts on consumer health remain unknown. Some studies indicate that 2 synthetic dyes, Blue No. 1 and Blue No. 2, may have toxic effects. It has therefore been suggested that these should be replaced with natural dyes; however, despite being nontoxic and arguably healthier than synthetic dyes, these compounds are often unsuitable for use in food or drugs due to their instability. Nevertheless, among the natural blue pigments, anthocyanins and genipin offer particular health benefits, as they are associated with the prevention of cardiovascular disease and have anticancer, neuroprotective, anti-inflammatory, and antidiabetic properties. This review summarizes the effects of blue food and drug colorings on health and proposes that synthetic colors should be replaced with natural ones. *Adv Nutr* 2021;12:2301–2311.

Statement of Significance: This review describes the effects of blue food and drug colorings on human health and their therapeutic potential.

Keywords: food and drug blue dyes, blue colorants, safety, natural dyes, synthetic dyes, anthocyanins

Introduction

In the 21st century, almost every person in developed or developing countries is familiar with the concepts of food colorants and food additives. However, the classification of natural and synthetic dyes is made far more complex by the range of their properties, uses, and chemical structures. For example, natural colorants can be classified by their chemical structures: isoprenoid derivatives (carotenoids), flavonoids (flavones, flavonols, and anthocyanins), nitrogen-heterocyclic derivatives (betalains), and pyrrole derivatives (chlorophylls) (1, 2). They can also be classified based on their sources (**Table 1**). Currently, the European Union (EU) has authorized about 43 colorings for use as food additives, and the United States has authorized about 30 (1, 3).

With the growth in reports about the harmful effects of synthetic substances, it is increasingly likely that a shopper will check the label before buying a particular food product. Many consumers prefer natural substances over their synthetic derivatives or even analogues. Carocho et al. (4) report a growing trend towards the use of naturally derived colors in food products; however, it is still more economical for manufacturers to use synthetic dyes or pigments in their products, as these may provide greater resistance to temperature, light radiation, or pH changes in the environment. Besides, in the case of blue dye, it is more likely that a synthetic analogue would be used, as natural blue dyes are scarce.

When buying a food product, consumer choice is driven not only by its price but also by its color and form. Dyes are not generally used to improve the taste or smell of the product, to extend the expiry date, nor change the nutritional value of the dish; they are used only to increase the marketing efficiency of a given product. The first food coloring substances are believed to have been used by the

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Abbreviations used: ADI, Acceptable Daily Intake; FD&C, Federal Food, Drug, and Cosmetic Act; fdw, freeze-dry weight; GTD, genipin–tyrosine derivant; HSV, herpes simplex virus; P2, purinergic; SREBP-1c, sterol regulatory element–binding protein 1c; UCP2, uncoupling protein 2; WSSV, white spot syndrome virus.

TABLE 1 Classification of natural dyes[1]

Natural dyes	
On the basis of chemical structure	On the basis of sources
Isoprenoid derivatives (carotenoids)	Plant/animal/mineral/origin
Flavonoids (flavones, flavonols, and anthocyanins)	• blue pigments
Nitrogen–heterocyclic derivatives (betalains)	• red pigments
Pyrrole derivatives (chlorophylls)	• yellow pigments
	• green pigments
	• white pigments
	• black pigments

[1] Information presented in the table is from Yusuf et al. (1)

Romans and Egyptians as early as 1500 BC to stain wines, pharmaceuticals, and food products (5–7). Nowadays, it has been estimated that a typical Indian citizen consumes 220 mg of food colorants per year (8). Several methods are currently used to extract natural colorants from fruits and other plant parts; these can be divided into conventional techniques, such as Soxhlet and maceration, or unconventional techniques or green extraction methods, such as high-pressure extraction, supercritical fluid extraction, and ultrasound-assisted extraction (2).

A blue pigment is defined as an organic molecule that absorbs red light (600 nm region) and thus appears blue to the eye. A classification of blue food dyes, with examples, is presented in **Table 2**.

Only approved dyes may be used in food in the EU and in the United States. Two key bodies regulate the use of coloring agents in food products: the European Food Safety Authority and the US FDA. The decision as to whether a compound is safe is based on its Acceptable Daily Intake (ADI): a value "covering the total amount of a substance that, according to current knowledge, can be taken from all sources throughout life without harming the body, and expressed in mg/kg body weight of man." There are only 2 natural blue colorants on the FDA list that are approved for use in food. They are grape color extract and grape skin extract. Both contain anthocyanin grape pigments (7, 9, 10).

At present, the verification of an ADI, and its dissemination, is the responsibility of the Joint FAO/WHO Expert Committee on Food Additives. Substances (dyes) for which no ADI is given should be used in accordance with the quantum satis principle: that is, use only as much as is needed (7). Blue dyes authorized for consumption in the EU are described in **Table 3**. However, it is not easy to identify natural blue colorants in databases, as they are typically organized by attributes such as composition rather than color and molecular weight. In addition, descriptions of newly identified natural products often fail to report their absorbance spectra or colors, and color may vary with factors such as solvent or pH values.

TABLE 3 Blue dyes authorized for consumption in the European Union[1]

E-number	Name of dye
E131	Patent blue V
E132	Indigotine/Indigocarmin
E133	Brilliant Blue FCF
E163	Anthocyanins

[1] Information presented in the table is from the European Union Commission (12). FCF, For Coloring Food.

Blue pigments require special criteria before being considered for use in foods. Ideally, this pigment should have a shade and brilliance similar to Federal Food, Drug, and Cosmetic Act (FD&C) Blue No. 1. For example, Newsome et al. (13) suggest that anthocyanins are the best candidates for blue colorants used in food products. This review summarizes the current state of knowledge regarding the health effects of the most popular natural and synthetic blue dyes used in food products, as indicated in various in vitro and in vivo trials.

Natural Blue Colorants

In nature, red-colored dyes and their yellow or orange derivatives, such as curcumin or carotenoids, are far more prevalent than blue colorants. This is particularly true in plants, where the blue color is only imparted by anthocyanins in an alkaline environment. In addition, blue can be observed in bacteria (blue oil slick) and fungi, as well as in cyanobacteria, algae, and even birds. However, this color is not conditioned by the dye or pigment, but by

TABLE 2 Classification of blue food dyes with examples[1]

Dye	Definition	Examples
Natural	Produced by living organisms: plants, animals, microbes (bacteria, fungi). Also, the coloring substance added to the product, in which coloration is formed as a result of transformations and interactions of colorless components during technological processing.	Anthocyanin Phycocyanin Natural indigo
Identical to natural	Obtained by chemical synthesis, identical to dyes of natural origin.	Synthetic indigo
Synthetic	Obtained by chemical synthesis, most often by introducing sulphonic or carboxyl groups into the natural dye molecule. They do not occur naturally in nature.	Indigocarmin/Indigotine Patent Blue V Brilliant Blue RS Indantren Blue
Inorganic	A group of metals and their salts, oxides and hydroxides.	—

[1] Information presented in the table is from Lis et al. (11).

TABLE 4 Stability of blue natural dyes under the influence of common environmental factors: temperature, pH, and light radiation[1]

Dye	Temperature	pH	Light
Gardenia Blue	Sensitivity to high temperatures above 80°C	Resistance to environmental pH changes	High resistance to light radiation
Phycocyanin	Very high sensitivity to elevated temperature (protein denaturing process). Above 55°C, its content dropped by more than 60% after 5 minutes of heating	Slightly sensitive to changes in the pH of the environment	Medium light resistant (after 5 h of exposure; 3.28×10^5 lux): content drop is about 30% in pH 5 and about 40% in pH 7
Anthocyanins	Not resistant at high temperatures	Very sensitive to pH changes. Changing the pH causes a change in color	In an alkaline environment, they are resistant to light rays
Natural Indigo	Stable to 90°C in MCT oil (obtained by extraction of coconut oil) for 5 days	Insensitive to pH changes (insoluble in water)	Low light–resistant (after 5 h of irradiation; 3.28×10^5 lux): the content decrease is about 70%

[1] Information used in compiling the table is from Krępska et al. (6). MCT, medium-chain triglycerides.

the cell structure or, in the case of birds, the structure of the feathers (6). Moreover, blue pigments or dyes are not often present in food; this rarity may be the reason for the color's particular attractiveness for children. The main natural blue colorants found in the literature are anthocyanins, genipin, and phycocyanin. Anthocyanins can be recovered from various plants, phycocyanins can be recovered from algae, and genipin can be recovered from *Gardenia jasminoides* Ellis and *Genipa americana*. These blue colorants can be extracted using classical techniques based on organic solvents or using more sophisticated techniques as supercritical fluid extraction, ultrasound-assisted extraction, high-pressure extraction, and pressurized liquid extraction.

Sensitivity of Natural Colorants to Certain Environmental Factors

Natural colorants are unstable coloring substances (**Table 4**). They tend to demonstrate a weaker staining force than synthetic dyes, as well as greater sensitivity to light and temperature, pH changes, or oxidizing or reducing factors. The sensitivities depend on the type of natural dye. For example, Gardenia Blue, a dye obtained from the fruits of the *Gardenia jasminoides* plant, is not resistant to heat above 80°C, but is quite resistant to light radiation or changes in pH. Anthocyanins, in comparison, are not resistant to heat at all, with their resistance depending more or less on the pH of the environment or anthocyanin type, and they are very sensitive to pH changes; however, they are highly resistant to light in the alkaline environment (5, 6).

Recently, Giusti et al. (14) have examined the hue expression and shelf-life stability of natural blue food colorings in a beverage model solution. The study used natural blue colors from various sources: the *Spirulina spp*., *Gardenia jasminoides* (gardenia) fruit, *Genipa americana* fruit, and *Clitoria ternatea* (butterfly pea) flower. The *Spirulina spp*. provides phycocyanin (protein dye), the *C. ternatea* flowers contain 9 types of anthocyanins, and the *G. jasminoides* fruit contains 3 different water-soluble pigments: flavonoids, iridoids, and crocins. In the case of *Genipa americana*, the extracted geniposide reacted with β–glucosidase to form genipin, which produced blue colors after reacting with neutral amino acids. Color expression and stability were compared to synthetic FD&C Blue No. 1 as a standard; spirulina and gardenia demonstrated precipitation during incubation.

Anthocyanins (E163)

The most common colorants in the natural world are anthocyanins: about 600 types have been identified (15). They are often found in fruits, particularly berries, where they are most concentrated in the skins (16, 17). However, they can also be freely obtained from red cabbage, red potatoes, purple sweet potatoes, and radishes (18). They are readily soluble in water, and many have been used in traditional folk medicine (19).

Anthocyanins are phenolic compounds belonging to the flavonoids family. About 5000 types of flavonoids have been identified, and they are frequently used as food colorants (20). They provide coloration for flowers and leaves and play a protective role against the harmful effects of ultraviolet (UV) radiation. They consist of 2 aromatic rings (rings A and B) linked by a 3-carbon heterocyclic ring (ring C) that contains oxygen (20). Besides, anthocyanins can be subdivided into 2 groups based on their structure: glycosides, which lack sugar, where an aglycone is responsible for color reactions, and glycosated flavonoids (or glycosidated anthocyanins). Six basic forms of anthocyanidin (aglycon) have been defined: pelargonidine, peonidine, cyanideine, malvinidine, petunidine, and dolphinidine (6, 21). For example, the major anthocyanin in the berry components of blueberries, blackberries, strawberries, and mulberries is cyanidin-3-*O*-glucoside; in fact, cyanidin-3-*O*-glucoside (38%) and cyanidin-3-*O*-rutinoside (60%) are believed to be the key natural colorants in mulberry fruits (22, 23).

Anthocyanins change color in response to environmental pH: they are red at acidic pH levels (pH $<$ 7) and blue

at alkaline pH levels (pH > 11). Coloration can also be changed by the use of certain copigments that react with anthocyanins, such as various flavonoids and polyphenols, and various alkaloids, polysaccharides, nucleotides, amino acids, and anthocyanosides. In addition, interaction with metal ions, such as aluminum, iron, manganese, and copper, is also known to influence the color (24, 25).

Anthocyanins are the least resistant to environmental factors of all natural colorants, and their resistance depends on the pH level. Therefore, blue-colored anthocyanins, which are those kept in alkaline pH, are much less resistant than red anthocyanins, which are those kept in acidic pH. Anthocyanins are not persistent at high temperatures, particularly when exposed to light radiation (26, 27). Therefore, anthocyanins have few uses as blue colorings in the industry: they are not very durable coloring agents, and few preparations in the food industry have a strongly alkaline pH, which is required for the blue color (6). Nevertheless, they are commonly consumed as part of a normal diet and are consumed in much higher amounts than other flavonoid classes; in Europe, studies have found their average daily consumption to range from 19.8 mg (Netherlands) to 64.9 mg (Italy) among men and from 18.4 mg (Spain) to 44.1 mg (Italy) among women (28, 29).

Anthocyanins are also used in medicines and food supplements. However, they demonstrate low bioavailability: the peak plasma concentration is reached 6 hours after consumption, and these concentrations remain in the micromolar range. Anthocyanins are metabolized by conjugation at the intestinal and hepatic levels (30, 31). Nevertheless, anthocyanins have a range of beneficial effects on human health, such as reducing oxidative stress, and have various anti-inflammatory and anti-cancer properties (32–34). They are also known to be powerful natural antioxidants; for example, cyanidin-, pelargonidin-, and delphinidin-tested anthocyanidins have been found to scavenge hydroxyl and superoxide radicals and to inhibit the lipid peroxidation induced by H_2O_2 in a study of lipid peroxidation (malonaldehyde and 4-hydroxyalkenol levels) and the free radical scavenging properties in rat brain homogenates. However, these compounds were not found to scavenge nitric oxide (35, 36).

Anthocyanins have also been found to demonstrate cardioprotective properties (37–48) and to have beneficial properties in controlling diabetes (49, 50) and preventing and treating neurological disorders (32). More details about their biological activities are given in review papers (42, 51).

Most significantly, toxicological studies support the view that anthocyanins pose no threat to human health (52). They are safe for consumption, even at higher doses (53). In addition, while anthocyanins are able to cross the blood-brain barrier (54), they have low bioavailability compared with other phenolic compounds (55, 56).

Gardenia Blue

Gardenia Blue is obtained from fruit from the *G. jasminoides* plant, which naturally grows in the laurel forests of China, Japan, Taiwan, and Vietnam. Fruits of *G. jasminoides* contain 3 different water-soluble pigments: flavonoids, iridoids, and crocins. This blue color was produced after geniposide turned into genipin (a water-soluble irhidoid monoterpenoid whose maximum absorbance (496 nm) does not change with the pH of the environment), through reaction hydrolyzed by the β-glucosidase. Genipin then reacts with amino acids such as glycine, lysine, or phenylalanine to obtain the dye (6, 57).

Gardenia Blue has been used as a natural food colorant for nearly 30 years in East Asia. Although it is not resistant to temperatures greater than 80°C, it does resist changes in environmental pH and it is quite resistant to light radiation; for example, its content was found to fall by approximately 20% after 5 h of irradiation at 3.28×10^5 lux in 1 experiment (6). Gardenia Blue dye can therefore be used for coloring liquid products, such as beverages, but also solid products, such as jellies and candies; however, dyeing jellies or candies brings with it a change of color to blue-green (6). Recently, Mizawa et al. (58) observed that the long-term use of gardenia fruit is not only expensive but also increases the risk of mesenteric phlebosclerosis.

A study of the genotoxicity of gardenia blue, and its precursor genipin, found that neither compound induced micronuclei in peripheral blood cells, nor did they cause DNA damage in liver, duodenum, or stomach tissues in mice. In these studies, genipin was administered at 74 and 222 mg/(kg body weight · day) for males and females, respectively, and gardenia blue was administered at 2000 mg/(kg body weight · day) for both. Following this, gardenia blue tested negative in in vitro micronucleus and chromosome aberration assays; however, genipin tested positive under some test conditions (59).

In addition to its antioxidant, anti-inflammatory, and antimicrobial properties, genipin has been found to have anticancer, anti-diabetic, neuroprotective, and antithrombotic effects. For example, a review by Li et al. (60) describes the therapeutic potential of genipin for neurodegenerative diseases. In addition, an experimental paper by Li et al. (61) found that Gardenia Blue potentially has antidepressant effects in LPS or chronic unpredictable mild stress models in rats and mice. In this experiment, Gardenia Blue was obtained from the reaction of genipin with tyrosine [genipin-tyrosine derivant (GTD)]. The results indicate that GTD treatment restored the monoamine neurotransmitter metabolism, membrane structural integrity, and mitochondrial oxidative system.

Genipin also acts as a specific inhibitor of uncoupling protein 2 (UCP2), a mitochondrial carrier protein that creates proton leaks across the inner mitochondrial membrane. In addition, UCP2 is broadly overexpressed in various cancers, including prostate and bladder cancers (62–65). Genipin appears to exert an antiproliferative effect on the growth of

glioblastoma cell lines (U87MG and A172) by inducing apoptosis through intracellular reactive oxygen species induction via the UCP2-related mitochondrial pathway; the authors tested various concentrations of genipin (2.5–100 μM) with 24-, 48- and 72-hour incubation times (63).

Genipin (50 mg/kg daily for 3 days) was also found to protect against cerebral ischemia–reperfusion injury in adult male C57BL/6 mice in vivo by regulating the UCP2–sirtuin 3 signaling pathway (32) and to have protective effects on gastrointestinal disorders, such as gastritis and gastric ulcers, in male rats when administered at 50 and 100 mg/kg (66). In addition, genipin (100 mg/kg) administration to mice 1 hour before ischemia has also been found to protect the liver from ischemia/reperfusion injury by modulating mitochondrial quality control. Furthermore, administration of genipin and geniposide at 0.1–0.25 mM appears to suppress *Helicobacter pylori* infections in gastric adenocarcinoma cells and C57BL/6 mice (67). Various other genipin glycoside derivatives have also been found to have antiviral and antifungal activities (68).

Administration of 20–500 μM of genipin also appears to enhance the therapeutic effects of oxaliplatin (10 μM) by upregulating reactive oxygen species generation, endoplasmic reticulum stress, and Bcl-2-interacting mediator of cell death pathway activity in colorectal cancer. In addition, genipin (5–200 μM) has been observed to suppress the accumulation of hypoxia-inducible factor-1 under hypoxia in different cancer cells, including colon cancer cell line HCT116, by modulating protein degradation. Finally, the tested compound reduced the expression of vascular endothelial growth factor and prevented cancer cell invasion by blocking the extracellular signal–regulated kinase signaling pathway (69, 70).

Li et al. (71) also noted that 10–200 μM of genipin also inhibits the growth of human T24 and 5637 bladder cancer cells via inactivation of the phosphoinositide-3 kinase/Akt signaling pathway. The study examined cell viability, colony formation, cell cycle progression, and apoptosis.

Liu et al. (72) also reported that the PI3k/Akt pathway appeared to be involved in the mechanism of action of genipin in acute lung injury. The same mechanism was observed in vitro, where genipin (10, 30, and 60 μM) was found to induce autophagy in oral squamous cell carcinoma via the PI3k/Akt/mechanistic target of rapamycin pathway inhibition.

Zhang et al. (45) propose that genipin (1, 1.25, and 5 mg/kg) protects against LPS-induced acute lung injury by inhibiting the NF-κB and Nod-like receptor family pyrin domain containing 3 signaling pathways. This effect was observed in 60 mice. The same authors (73) also report that genipin (2 and 5 mg/kg) appears to protect against apoptosis and inflammation in LPS-induced acute lung injury by promoting autophagy.

Seo et al. (74) note that 25, 50, and 100 mg/kg of genipin seems to prevent D-galactosamine and LPS-induced hepatic injuries in mice through suppression of necroptosis-mediated inflammasome signaling. Genipin was also found to inhibit the LPS-induced inflammatory response in BV2 microglial cells (in vitro and in vivo) by activating the Nrf2 signaling pathway; however, it did not demonstrate any cytotoxicity in the concentration range of 0 to 20 μM. They also note that it inhibited certain inflammatory mediators, such as NO and PGE_2, as well as inflammatory cytokines, such as TNF-α and IL-1β, in a concentration-dependent manner at 5, 10, and 20 μM. The levels of NO, PGE_2, TNF-α, and IL-1β were detected by ELISA.

Genipin has demonstrated therapeutic and preventive potential in blocking white spot syndrome virus (WSSV) by inhibition of Bax inhibitor-1 gene expression. In addition, 6.25–50 mg/kg of genipin has been found to inhibit WSSV replication by decreasing signal transducer and activator of transcription gene expression in crayfish and shrimp (75).

In a mouse model, genipin effectively antagonized high-fat diet–induced hyperlipidemia and hepatic lipid accumulation by regulating the miR-142a-5p/sterol regulatory element–binding protein 1c (SREBP-1c) axis. It has been found that miRNA regulates fatty acid metabolism through targeting SREBP-1c (76).

Genipin has also been extensively studied as a non-cytotoxic crosslinking compound. Liu et al. (72) report that genipin can act as an effective hemostatic agent by crosslinking microspheres, and can enhance the stability and anticancer properties of curcumin by stabilizing caseinate-chitosan nanoparticles.

An alternative source of natural blue pigments suitable for use in food products may be the genipap fruit (77). Genipap is produced by *Genipa americana* L., a member of the *Rubiaceace* family that is distributed in Central America and South America but is native to Brazil. The pigments are readily produced in both the endocarp and mesocarp of the unripe fruit; they demonstrate high stability and, due to the pH, appear blue. Genipin is characterized by the presence of 3 iridoids: genipin, geniposide, and geniposidic acid.

Genipap extract has been found to scavenge 2,2-diphenyl-1-picylhydrazil, 2,2-azino-bis-(3-ethylbenzothiazoline)-6-sulponic acid, and peroxyl radicals in vitro, and to demonstrate cytostatic effects against breast Michigan Cancer Foundation-7 (MCF-7) and glioma (U251) cell lines at concentrations of 10^{-3} to 10^{2} μg/ml (78). An ultra-high-performance LC-diode array analysis found the maximum levels of geniposide and genipin to be 89.48 mg/g freeze-dry weight (fdw) in the ripe endocarp and 60.77 mg/g fdw in the unripe endocarp. Geniposidic acid (25.04 mg/g fdw) was only found in the ripe endocarp.

Table 5 summarizes the biological properties of anthocyanins and genipin, as determined from the in vitro and in vivo studies discussed above.

Phycocyanin

Phycocyanin is a blue pigment found in cyanobacteria and various eukaryote algae of the Cryptophytes, Glaucophytes, and Rhodophytes; however, its main source is the cyanobacterium *Arthospira plantensis*, commonly named *Spirulina platensis*. Phycocyanin has also been isolated from

TABLE 5 Different biological properties of blue colorants in in vitro and in vivo models

Natural blue colorants	
Anthocyanins	**Genipin**
• prevention and treatment of cardiovascular and neurological disorders • anti-inflammatory action • anticancer properties • antioxidant properties	• antioxidant properties • anti-inflammatory action • antimicrobial agent • anticancer properties • antidiabetic action • neuroprotective properties • antithrombotic effect

Nostoc spp., *Pseudanabaena muciola*, and *Phormidium* spp. (52, 70, 79, 80). It is a photosynthetic phycobiliprotein, a light-harvesting apoprotein, bound to phycocyanobilin, an open-chain tetrapyrrole, as a prosthetic group; however, in solution, it exists as a complex mix of monomers, trimers, and various other oligomers. Despite being a protein, phycocyanin is soluble in cold and warm water; in addition, high ionic strengths, extreme pH values, alcohols, and elevated temperatures may affect its structure. Importantly, while phycocyanin is stable at room temperature and under cooling conditions, its stability decreases upon being heated above 45°C (14, 81).

Natural Indigo

One of the oldest natural colorants is indigoid. It exists in the form of a water-soluble glucoside indicant in plants. However, when it is exposed to air, it is converted into indigotin: that is, blue indigo. Good sources of natural indigo are *Isatic tinctoira* and *Indigofera tincoria* (1, 20, 82).

Synthetic Blue Dyes

The safety of the use of synthetic food colors has long been controversial; in the United States, 9 dyes are believed to have potential health concerns, despite being currently approved. It has been suggested that they should be removed and replaced with safe dyes, as they are known not to offer any nutritional benefits and few studies have been performed on their genotoxic and carcinogenic properties. It is recommended that the regulatory authorities should require independent toxicity testing and that they should be more careful when approving such dyes. Synthetic dyes have been used to color food in industrialized countries for at least a century, and are particularly used in the United States. Artificial coloring can improve the appearance of basic ingredients and food additives for consumers and mask the absence of brightly colored natural ingredients, such as fruit (83, 84).

Synthetic dyes are also often chosen because they are cheaper and more durable than most natural dyes. However, although most food dyes are organic compounds, their original source was coal tar, and now crude oil, and their use raises serious health concerns. Over the past 100 years, food colors have been found to pose a greater threat to health than any other category of food additives (85, 86). The growing interest in natural foods among consumers has resulted in some companies avoiding dyes or switching to safe, natural substances such as beta-carotene, peppers, or beet juice. In general, although many synthetic dyes are regarded as safe in acute doses, the general view is that high levels of chronic consumption throughout life are not advisable (87).

Bastaki et al. (88) proposes that the current use of food colors in the United States is safe. It is officially assumed that the amounts of dyes reported by the food industry are in line with US FDA regulations. These values are well below the maximum ADI of each color additive, as published by the WHO Joint Committee/FAO Food Additives.

FD&C Blue No. 1

One widely used blue food dye is FD&C Blue No. 1, or disodium 2-[[4-[ethyl-[(3-sulfonatophenyl)methyl] amino]phenyl]-[4-[ethyl-[(3-sulfonatophenyl)methyl]-azaniumylidene]cyclohexa-2,5-dien-1-ylidene]methyl] benzenesulfonate. It is also commonly known as Brilliant Blue FCF (For Coloring Food), acid blue 9, erioglaucine, and Patent Blue AR. It has been assigned the designation E133, with a color index of 42,090 (57).

In 1993, the FDA approved this dye for use in food generally (confections, beverages, cereals, frozen dairy desserts, popsicles, frostings, and icings), and in 1994 it was approved for use in externally applied drugs, including around the eye area, and in nontherapeutic cosmetics (10). It is believed that the greatest exposure to Blue No. 1 in the United States is through breakfast cereals, juice drinks, soft drinks, and frozen dairy desserts, as is the case with many other color additives (89).

The acceptable daily intake of Blue No. 1 for humans was established as 0–12 mg/kg body weight based on a rat intoxication study (9, 90). This compound is typically purchased as a powder. It can be dissolved in water to give a green-blue solution at a neutral pH level, a green solution at a slightly acidic pH level, or a yellow solution in strong acid. The compound is also soluble in 95% ethanol (91).

The effects of Blue No. 1 on metabolism and its genotoxicity, chronic toxicity, neurotoxicity, and carcinogenicity are discussed in detail by Kobylewski and Jacobson (91). In rats, Blue No. 1 was found to be excreted generally unchanged in the feces (96%) within 36 hours after oral administration: approximately 5% of the color dose was absorbed from the gastrointestinal tract. Although Blue No. 1 was not found to be genotoxic in terms of causing DNA damage, base pair mutations, base substitutions, or frameshift mutations, it was found to cause chromosomal aberrations in 2 studies, as cited in Kobylewski and Jacobson (91).

While industry-sponsored studies of Blue No. 1 did not find evidence of carcinogenicity or other toxicity in rats or mice, the dye was found to inhibit neurite growth and act synergistically with L-glutamic acid in vitro, suggesting the potential for neurotoxicity. This is of particular concern for fetuses and babies under the age of 6 months, whose blood-brain barrier is not fully developed (91).

Kus and Eroglu (92) report that Blue No. 1 can have cytotoxic and genotoxic effects, and advise that caution must be exercised when using it for coloring food.

Unfortunately, as the amount of dye used in commercial products is often proprietary (39), it is difficult to assess dietary intake and determine exposure in humans, which may explain the very small amount of research and publication on the subject. Nevertheless, available studies indicate that Blue No. 1 is not a stable dye, and its degradation products have been identified in vitamin jellies consumed by children.

Blue No. 1 is also used in some e-liquids for electronic cigarettes; however, the vast majority of e-liquids do not identify the formations of dyes used. Also, the FDA does not specify any ADI value for dye inhalation, as synthetic dyes have not been previously used for this purpose. Although the levels of dyes in e-liquids are well below the ADI thresholds, none of these thresholds were obtained by studies that examined their inhalation or their thermal degradation products. In addition, some degradation products of aromatic dyes and amines may be carcinogenic (93).

Blue No. 1 consumption has not been found to cause tumors in rats. However, it has been found to inhibit nerve cell development in vitro, and its use has been related to skin irritations and bronchial constriction. The dye can also inhibit purinergic (P2) receptors, which are important in maintaining cell homeostasis and controlling inflammation and apoptosis. Further studies are therefore needed to identify therapeutic or deleterious effects associated with conditions affecting the P2 receptor function (94, 95).

The dye has also been found to influence the modulation of intracellular enzymes, such as protein tyrosine phosphatases, which, together with protein tyrosine kinases, regulate the level of cellular phosphorylation. Blue No. 1 has been found to inhibit mitochondrial respiration in vitro. The dye has been observed to selectively inhibit panexin-1 in an oocyte-transfected system. Panexin-1 is associated with the P2 × 7R–induced pore formation phenomenon, and with ATP release and inflammasome formation; these may play a role in inflammatory diseases such as colitis and Crohn's disease (95).

Blue No. 1 restores an injury-induced loss of function in vascular tissues, possibly via inhibition of purinergic receptor signaling. It has been observed to inhibit platelet-derived growth factor–induced migration and proliferation of A7r5 cells, as well as the subsequent development of intimal thickening in a rabbit model during a vein graft explant. Pretreatment with Blue No. 1 also prevented the intimal thickening caused by the herpes simplex virus (HSV) in an organ culture. Its incorporation in vein graft preparation during explant procedures represents a potential therapeutic approach to mitigating intimal hyperplasia, reducing vein graft failure, and improving the outcome of the autologous transplantation of HSV (96).

Numerous studies have reported toxic effects on animals or humans, such as convulsion; gastrointestinal tumors; an increase of hepatic enzymes and bilirubin levels in an animal model; oxidation of thyroid peroxidase forming carcinogenic aromatic amines; purinergic signaling; attention deficit and hyperactivity in children; and cumulative absorption through lingual mucosa and through the skin (90). It has also been found to have the capacity for inducing allergic reactions in individuals with preexisting moderate asthma (97).

Studies on laboratory animals suggest that synthetic food dyes may also be possible triggers of ADHD, as well as of anorexia and other behavioral changes (98).

FD&C Blue No. 2

FD&C Blue No. 2—that is, disodium 5,5'-(2-(1,3-dihydro-3-oxo-2H-indazol-2-ylidene)-1,2-dihydro-3H-indol-3-one)disulfonate—is also known as Indigo Carmine and indigotine (99).

Blue No. 2 is used in dessert powders, bakery goods, cereals, snack foods, confectionery products, maraschino cherries, sausage, ice cream, sherbet, and dairy products, and as a dye for nylon, surgical sutures, foods, and ingested drugs. This dye is also used as a reagent for functional tests of the kidneys during cystoscopies and ureteral catheterization, as well as for the detection of nitrates and chlorates and in milk testing. The color is also used in drug preparations (100).

FD&C Blue No. 2 has poor pH stability: after 1 week, it will appreciably fade if kept at a pH level of 3 to 5, will considerably fade at a pH of 7, and will completely fade at a pH of 8. Complete fading occurs in alkalis, such as 10% sodium carbonate and 10% sodium hydroxide, with fading also occurring in 10% of sugar systems. It has very poor light and oxidation stability and moderate heat stability. It is the least soluble of all the food colors, with a solubility of 1.6 g in 100 ml of water at 25°C; however, it is soluble in 95% ethanol (101).

The dye is a blue-brown to red-brown powder that dissolves in water to give a solution that is blue at neutrality, blue-violet in acid pH, and green to yellow-green in base pH. When dissolved in concentrated sulfuric acid, it yields a blue-violet solution that turns blue when diluted with water. It is the only food color that has good resistance to reducing agents but has very poor compatibility with food components. Its most common use is in pet food, but it is also used in candies, confections, and baked goods. Blue No. 2 is commonly used to dye clothing, especially fireproof wear; however, much of the dye enters the wastewater during production, and its presence changes the color and odor of water, even at very low concentrations. The presence of residues in sewage poses a threat to natural water reservoirs and living organisms (102).

In 1983, the FDA permanently approved Blue No. 2 for use in foods and ingested drugs. Blue No. 2 has an ADI of 5 mg/(kg body weight · day) (9, 10, 91, 103).

Studies in rats indicate that the majority of ingested Blue No. 2 and/or its metabolites, including 5-sulfoanthranilic acid, its final breakdown product, are excreted in the feces, with smaller amounts being found in the urine. Those

studies show that 5-sulfoanthranilic acid is absorbed more readily by the gastrointestinal tract than the intact dye. The dye itself does not appear to be genotoxic, although a chromosomal aberration assay yielded positive results; however, it may be carcinogenic. In addition, Blue No. 2 is not regarded as teratogenic and does not appear to affect fertility, length of gestation, viability, or lactation indices. The no-observed-adverse-effect level for Blue No. 2 was determined as 250 mg/(kg body weight · day) in rats and rabbits (91, 103).

No chronic toxicity or carcinogenicity was detected in 2 studies on mice; however, neither study included an in utero phase, and both were shorter than 2 years. More worrisome was that a statistically significant increase in brain gliomas and malignant mammary gland tumors was observed in a rat model. Given this increased incidence of tumors, especially brain gliomas, in rats, Blue No. 2 cannot be considered safe for human consumption (91).

No incidents of allergy or intolerance have been reported after consuming Blue No. 2. (12). Even so, the dye did not protect mitochondria from injury and proved to be a potentiating agent, promoting damage to the hepatocyte membranes (104).

Discussion

With the recent growth in consumer interest in food products with beneficial health effects, there is a need to identify sources of natural blue colorants, 1 of which is the anthocyanins found in fruits and vegetables. However, there are various problems with using natural colorants in food products: they are generally less stable than synthetic colorants, and the treated food may not be acceptable to the consumer. Despite this, an increase in the concentrations of natural colorants based on anthocyanins from grapes was not found to reduce the acceptability of the attributed flavor in model juices (105). In addition, Dabas (106) reports that color should not alter the flavor profile of the food and beverage, nor should it negatively impact the stability of the flavor system. Moreover, studies, including Khoo et al.'s (36), do not often describe anthocyanin or anthocyanins as a blue color under food conditions (especially the pH). They only focus on anthocyanins in general, which are predominantly red or purple in foods, and not on blue anthocyanins.

The use of synthetic food colors remains controversial. While standards exist for manufacturers, little research exists on the potential harmfulness of these compounds on humans. In addition, some studies clearly indicate that that the use of dyes, including artificial blue dyes, can have toxic and harmful effects. For this reason, further research is needed to better understand the behavior of both natural and synthetic blue dyes, including during stabilization processes, so they can be incorporated into food products as healthier alternatives.

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