TOXICOLOGY OF FOOD COLORS[1]

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Jack L. Radomski
University of Miami, Miami, Florida

[1]Key to Chemical Names of Colors Referred to:
FD&C Blue No. 1 (Brilliant Blue FCF); disodium salt of 4-{[4-(N-ethyl-*p*-sulfobenzylamino)-phenyl]-(2-sulfoniumphenyl)-methylene}-[1-(N-ethyl-N-*p*-sulfobenzyl)-$\Delta^{2,5}$-enimine]: FD&C Blue No. 2 (Indigotine); disodium salt of 5,5'-indigotindisulfonic acid: FD&C Green No. 1 (Guinea Green B); monosodium salt of 4-[4-(N-ethyl-*p*-sulfobenzylamino)-diphenylmethylene]-[1-(N-ethyl-N-*p*-sulfoniumbenzyl)-$\Delta^{2,5}$-
FD&C Green No. 2 (Bright Green FS); disodium salt of 4-{[4-(N-ethyl-*p*-sulfobenzylamino)-phenyl] (4-sulfoniumphenyl)-methylene}-[1-(N-ethyl-N-*p*-sulfobenzyl)-$\Delta^{2,5}$-enimine]: FD&C Green No. 3 (Fast Green FCF); disodium salt of 4-{[4-(N-ethyl-*p*-sulfobenzylamino)-phenyl]-(4-hydroxy-2-sulfoniumphenyl)-methylene}-[1-(N-ethyl-N-*p*-sulfobenzyl)-$\Delta^{2,5}$
FD&C Yellow No. 4 (Yellow OB); 1-*o*-Tolylazo-2-naphthylamine: FD&C Yellow No. 5 (Tartrazine); trisodium salt of 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-*p*-sulfophenylazo-pyrazole: FD&C Yellow No. 6 (Sunset Yellow FCF); disodium salt of 1-*p*-sulfophenylazo-2-naphthol-6-sulfonic acid: FD&C Orange No. 1 (Orange 1); monosodium salt of 4-*p*-sulfophenylazo-1-naphthol: FD&C Orange No. 2 (Orange FS); 1-*o*-Toylazo-2-naphthol: FD&C Red No. 1 (Ponceau 3R); disodium salt of 1-pseudocumylazo-2-naphthol-3,6-disulfonic acid: FD&C Red No. 2 (Amaranth); trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid: FD&C Red No. 3 (Erythrosine); disodium salt of 9-*o*-carboxyphenyl-6-hydroxy-2,4,5,7-tetraiodo-3-isoxanthone: FD&C Red No. 4 (Ponceau SX); disodium salt of 2-(5-sulfo-2,4-xylylazo)-1-naphthol-4-sulfonic acid: FD&C Red No. 32 (Oil Red XO); 1-Xylylazo-2-naphthol: FD&C Violet No. 1; monosodium salt of 4-{[4-(N-ethyl-*p*-sulfobenzylamino)-phenyl]-[4-(N-ethyl-*p*-sulfoniumbenzylamino)-phenyl]-methylene}-(N,N-dimethyl-$\Delta^{2,5}$-cyclohexadienimine):
oxyphenyl-azo-2-naphthol: Ponceau MX; disodium salt of 1-(2,4-xylylazo)-2-naphthol-3,6-disulfonic acid: Ponceau 4R; trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-6,8-disulfonic acid: D&C Red No. 9; barium salt of 1-(4-chloro-*o*-sulfo-5-tolylazo)-2-naphthol: D&C Red No. 10; monosodium salt of 2-(2-hydroxy-1-naphthylazo)-1-naphthalene-sulfonic acid.

INTRODUCTION

When beginning a discussion of food colors it is customary to point out that man has been artificially coloring his food since ancient times and that color is an important characteristic of food, usually the first to be perceived. On the other hand, it appears that the use of artificial food coloring is largely the economic matter of rendering foods more attractive to the consumer.

Not much attention was paid to the safety of coloring materials for food until the early 1950s when two instances of toxicity to humans took place. The first of these was an occurrence of diarrhea in children produced by a black and orange colored Halloween taffy. Investigations at the US Food and Drug Administration of the cathartic effect of the components of this candy traced the active ingredient to FD&C Orange No. 1, an azo food color. It was soon found that this color had approximately the same cathartic potency as phenolphthalein. A similar consumer complaint was subsequently received by FDA concerning popcorn that had been colored by FD&C Red No. 32. This food coloring was also found to be a cathartic. It was then observed that FD&C Yellow No. 4 (the latter two colors at that time being used extensively in coloring butter and margarine) was also cathartic (1). As a result of these occurences, a flurry of interest in the possible harmful effects of food colors occurred, and a considerable amount of chronic toxicity testing was initiated, largely at the toxicology laboratories of FDA.

Because these colors find their way into a vast number of food products ingested by virtually everyone in the country, there is no certain way of knowing whether or not lifetime ingestion of food colors is adversely affecting the population. Outside of the above-named incidents, there is no direct evidence that the use of food coloring materials has been injurious. Use of animal testing procedures for chronic toxicity determinations allows the identification of the most potentially harmful colors.

For little apparent good reason, the specific food colors in use vary widely from country to country. Because of space considerations, this review is limited to food colors used in the United States. The reader is referred to two reviews covering food colors used in the United Kingdom (2, 3). The Food and Agriculture Association of WHO has thoroughly reviewed the toxicology and chemical composition of the food colors used around the world in hopes of achieving standardization of this chaotic situation. The results of their deliberations and their recommendations are available (4–6).

TRIPHENLYMETHANE COLORS

The triphenylmethane colors include FD&C Blue No. 1 (Brilliant Blue FCF), FD&C Green No. 1 (Guinea Green B), FD&C Green No. 2 (Bright Green FS), FD&C Green No. 3 (Fast Green FCF), and FD&C Violet No. 1. All of these colors contain sulfonic acid groups and are therefore highly water soluble. They are also all poorly absorbed from the gastrointestinal tract, undoubtedly due to their low

pKs. When these colors were given orally to rats, more than 90% of the administered dose was recovered in the feces with the exception of FD&C Green No. 2. With this color, only 68% was found in the feces, due apparently to decomposition in the GI tract. When administered to dogs, small amounts (1–3% of the dose) were found to be excreted in the bile, with the exception of FD&C Violet No. 1 where no excretion was observed (7). Thus it is apparent that these colors, because of their strongly acidic nature, are very poorly absorbed after oral administration and are largely excreted unchanged in the feces.

The triphenylmethane colors have an additional characteristic in common. It was first reported in 1937 that FD&C Green No. 2 induced sarcomas at the site of injection when a dose of 3 ml of a 2% solution was injected subcutaneously (8). The observation was later confirmed (9) and extended to the other triphenylmethane colors. In an experiment lasting 99 weeks, 76% or more of the rats developed sarcomas at the site of injection when 1 ml of a 2 or 3% solution of FD&C Blue No. 1, FD&C Green No. 2, and FD&C Green No. 3 was injected subcutaneously. FD&C Green No. 1 apparently had a much lower tendency to produce this effect, for only one tumor was observed in 18 rats tested (10). A considerable controversy over the significance of this observation exists. In a study of the mechanism of the effect, it was concluded that the production of sarcomas in rats by this technique is a reflection of the physical properties of the colors, primarily a lowering of surface tension, and not a true test for chemical carcinogenesis (11–13).

Triphenylmethane colors, for instance, gentian violet, have long been used in topical applications for controlling bacterial and fungal infections of the skin (14). Tests involving repeated application of high concentrations indicate that these substances generally are primary irritants (15).

Much more relevant to the matter of the possible harmful effects of these food colors are chronic feeding experiments. FD&C Blue No. 1 was fed to rats at concentrations up to 5% in the diet for 2 years. No effect was observed even at this high level (16). The three FD&C green colors were fed to rats, dogs, and mice for two years at concentrations up to 5% in the diet. Considering the massive amounts fed, few toxic effects were observed. The only possibly meaningful pathologic effect was an increase in hepatic tumor incidence in rats fed 5% FD&C Green No. 1. FD&C Green No. 2 produced some growth inhibition at the 2 and 5% feeding levels. FD&C Green No. 3 survived this intensive testing procedure showing no tumorigenic or toxic effects (17). It would appear that the triphenylmethane colors have a low degree of toxicity to experimental animals when fed orally. These experiments did not confirm an earlier observation of the induction of malignant lymphomas in rats fed 4% of FD&C Greens No. 1 and 3 (18).

FD&C Blue No. 2 (Indigotine) is a related sulfonated water-soluble color that does not have the triphenylmethane structure. It appears to have similar properties, producing fibrosarcomas at the site of repeated subcutaneous injection in rats. Chronic toxicity studies conducted in rats and dogs over a two year period and in pigs for 90 days indicate this substance is substantially innocuous even at the highest feeding level (5%) (16, 19).

SULFONATED NAPHTHALENE AZO COLORS

This is a group of water-soluble mono-, di-, and trisulfonated colors containing a naphthalene ring and an azo linkage to either a second naphthalene or benzene ring. It is comprised of FD&C Orange No. 1 (Orange 1), FD&C Yellow No. 6 (Sunset Yellow FCF), FD&C Red No. 1 (Ponceau 3R), FD&C Red No. 2 (Amaranth), and FD&C Red No. 4 (Ponceau SX). At the time of the investigation into the consumer complaint concerning FD&C Orange No. 1, it was observed that this color was readily split at the azo linkage by reductive fission by the bacterial flora of the gut of humans and animals (20). This phenomenon was subsequently observed to be a general one with this group of colors (21–24). This property suggests that effects observed with these colors may be due to their reduction products (25–27).

FD&C Orange No. 1 has a significant cathartic effect at 100–200 mg in dogs and beginning at 80 mg in man (28). Mice fed 15–20 mg of FD&C Orange No. 1 per week for 58 weeks did not develop a significantly increased incidence of tumors (29). In rats fed this color for 2 years at levels of 0.5, 1.0, and 2.0% of the diet, considerable toxicity was observed. At the higher feeding levels, increased mortality occurred along with splenic enlargement, leucocytosis, anemia, diarrhea, and growth suppression. Even at the 0.5% level congested kidneys, chronic nephritis, and splenic enlargement with increased pigmentation was observed. Dogs fed 0.2% in the diet survived 5 years without showing any ill effects. Higher concentrations, however, produced decreased survival though no specific pathologic effects were found (30, 31).

Of the three red colors in this group, FD&C Red No. 2 has been regarded as the least toxic. Several long-term feeding studies have been conducted on rats at concentrations of up to 5% in the diet in which no pathological effects or increase in tumors was observed (18, 32, 33). Recently, however, it was reported that this color produced liver damage including vacuolization and fatty degeneration accompanied by a rise in serum albumen and β-globulin when fed to rats for 18 months at 0.12% in the diet (34). Dogs fed 2% of the color in the diet for 7 years showed no evidence of any pathologic effect (35). Feeding studies in mice showed no tumorigenic effect (29). An embryotoxic effect was observed with FD&C Red No. 2 in female rats although no teratogenicity was found (36, 37). Studies published in the Russian literature indicated that FD&C Red No. 2 may be carcinogenic by oral administration. When this color was fed at 4% (reduced to 2.5%) in the diet for 25 months, peritoneal sarcomas were found in 11 of 18 rats (38). Of 48 animals fed FD&C Red No. 2 at 2% of the diet, 13 developed malignant tumors in another experiment lasting 33 months (39). Since carcinogenic effects were not revealed in comprehensive rat, mice, and dog experiments conducted in this country, it is possible that the effects reported above are due to an impurity (40). Colors are frequently mixtures of compounds and the composition of the same color may vary in different countries.

The other two red water-soluble azo colors, FD&C Red No. 1 and FD&C Red No. 4, appear to have somewhat greater toxicity. Although no toxic or tumorigenic effects were observed in rats and mice fed levels as high as 5% of FD&C Red No. 4 in the diet for 2 years, this color produced pathological changes in dogs when fed

at a 1% level in the diet for 7 years. There was atrophy of the adrenal zona glomerulosa and chronic follicular cystitis with hematomatous projections into the urinary bladder and small hemosiderotic foci in the liver (41). It must be remembered when considering the significance of these changes that these dogs consumed huge amounts of the color.

An isomer of FD&C Red No. 4, Ponceau MX, appears to be more toxic. Pathologic effects in rats including liver cell adenomas, tubular degeneration of the kidneys, and glomerular changes were observed at all feeding levels down to 1.2% (42–44).

The most toxic of these red colors appears to be FD&C Red No. 1. At feeding concentrations to rats of 0.5–5%, this color produced increased mortality, growth inhibition, and most significantly, malignant liver tumors (45, 46). This color also produced mortality and liver pathology in dogs (45). It would appear that the toxicity and carcinogenicity of this color may be related to its metabolic products, in particular mesidine and pseudocumidine, the trimethyl aniline derivatives produced by reductive fission of the azo linkage. Tests conducted on 2,4-, 2,5-, and 2,6-xylidine in rats and dogs indicate that these substances are hepatotoxic (25, 47–50).

Perhaps the least toxic of these water soluble colors has been found to be FD&C Yellow No. 6. This color has been extensively tested for carcinogenicity and chronic toxicity in mice, rats, and dogs. No pathologic or toxicologic effects were noted even at 2–5% of the diet. The dog studies were conducted for a total of 7 years (51, 52). FD&C Yellow No. 6 has also been tested in pigs for 98 days at levels up to 100 mg/day. No toxicological abnormalities were observed (53).

One is tempted to generalize concerning the relationship of the structure to toxicity of this series of compounds. The less toxic members of this series, FD&C Yellow No. 6, FD&C Red No. 2, and FD&C Red No. 4 (slightly more toxic) are sulfonated on both aromatic rings adjacent to the azo group. They would therefore yield only sulfonated fission products which are certain to be poorly absorbed. With the more toxic FD&C Red No. 1 and FD&C Orange No. 1, sulfonic acid groups are limited to one of the aromatic rings. The other nucleus which would be released upon reductive fission would therefore be unsulfonated and probably absorbed. This suggestion is supported by the low toxicity of Ponceau 4R (not used in this country) (54–56) and the greater toxicity of D&C Red No. 10 (57) and D&C Red No. 9 (58).

FD&C Yellow No. 5 (Tartrazine) is a water soluble azo color with similar biological properties but differing somewhat in structure due to the presence of a heterocyclic ring adjacent to the azo group. This color is quite nontoxic and has been extensively tested in mice, rats, and dogs in feeding concentrations up to 5% for periods up to 2 years. No toxic or pathologic effects or increased incidence of tumors were observed (18, 32, 33, 59, 60).

OIL-SOLUBLE AZO COLORS

The oil-soluble azo colors as a group are the most toxic. These include FD&C Orange No. 2 (Orange FS), FD&C Red No. 32 (Oil Red XO), Citrus Red No. 2,

FD&C Yellow No. 3 (Yellow AB), and FD&C Yellow No. 4 (Yellow OB). These colors all contain a naphthalene residue on one side and a benzene residue on the other side of the azo linkage, and are completely unsulfonated. Somewhat surprisingly, the oil-soluble azo colors are also reduced by the intestinal flora of rats, rabbits, and dogs although at a slower rate than the water-soluble ones (61, 62).

After the incident with popcorn colored with FD&C Red No. 32, this color was found to exhibit a cathartic action in dogs and rats (61). Chronic toxicity experiments with FD&C Red No. 32 revealed this compound to be highly toxic. Pathological changes including liver damage, increased mortality, decreased growth rate, and right side heart pathology were observed at the lowest feeding level of 0.1% over a 2 year period in rats. Similar effects were observed with dogs (63). A less extensive study with the closely related FD&C Orange No. 2 showed similar toxicity (63).

Because of the desirability of having an oil-soluble red or orange color for the purpose of coloring the skin of oranges, the dye industry developed a replacement for FD&C Red No. 32 named Citrus Red No. 2. This color has the same chemical structure as FD&C Red No. 32 except that the methyl groups on the benzene ring are replaced by methoxy groups. Chronic toxicity tests in dogs, mice, and rats, however, have not indicated that this color is significantly less toxic than FD&C Red No. 32 (64). Hyperplasia with thickening of the bladder wall and the production of a papillary carcinoma in one mouse were noted in an experiment in which rats and mice were fed diets containing 0.05 and 0.25% Citrus Red No. 2 for 24 months. Unfortunately, bladder stones were present in most of the animals and it was not possible to determine if these stones were responsible for the tumor and/or hyperplasia produced. However, the lesions in rats and mice were likened to those observed with the proven bladder carcinogen, 4-ethylsulfonylnaphthalene-1-sulfonamide (65). Perhaps undue significance was attached to this observation because of the identification of the O-glucuronide and the ethereal sulfate conjugates of 1-amino-2-naphthol in the urine of rats, rabbits, and dogs given Citrus Red No. 2 (66). Ortho-hydroxy amines were considered for years to be the active carcinogenic metabolite of the bladder cancer-producing aromatic amines. However, more recent results have indicated that the active carcinogenic metabolites are the N-hydroxy metabolites rather than ortho-hydroxy metabolites (67–69). There is no evidence of the production of N-hydroxy metabolites of Citrus Red No. 2 or any other azo color. It would seem, therefore, that the significance of this observation must stand on its own. In one experiment in which mice were given subcutaneous injections of the color, the female mice showed an increased incidence of malignant tumors, including adenocarcinomas of the lung and lymphosarcomas (70).

FD&C Yellows No. 3 and 4 (Yellows AB and OB) were formerly used in the United States for coloring oleomargarine. These colors are derived by coupling either aniline or orthotoluidine with 2-naphthylamine. This in itself would seem to be adequate justification for eliminating these dyes as food colors. In addition, it has been reported that azo colors of this type in dilute acid solution may undergo a reversal of the coupling reaction (71). This could result in the liberation of 2-naphthylamine in the acid milieu of the stomach. While no evidence of the metabo-

lism of FD&C Yellow No. 4 to 2-naphthylamine in animals' stomachs has been obtained, evidence of the formation of an imidazole resulting from the reaction of the azo color with naturally occurring aldehydes was obtained (72). FD&C Yellows No. 3 and 4 were tested for chronic toxicity in rats and dogs and found to be hepatotoxic (73). Right side cardiac atrophy and hypertrophy were observed in a 2 year feeding experiment with rats. Only at 0.05% in the diet did the rats survive without pathologic effects. In a 1 year feeding experiment, dogs suffered weight loss and toxicity at concentrations in the diet down to 0.05% (74, 75). A related color, 1-phenylazo-2-naphthol, formerly used in margarine in England, has also been found to be highly toxic and possibly a carcinogen. Kirby & Peacock found it to induce hepatomas after injection in stock mice (76), and carcinogenic changes in the bladder epithelium were observed in rabbits fed this color (77).

MISCELLANEOUS COLORS

FD&C Red No. 3 (Erythrosine) is a food color of rather unusual composition containing 4 iodine atoms. Not being an azo color, it does not undergo reductive fission in the intestine. It is one of the more nontoxic of the food colors. It has been extensively tested in rats, dogs, mice and gerbils. Dogs fed levels up to 2% in the diet for 2 years showed no toxic effects (78). In several experiments in mice, tumorigenicity tests both by feeding and by injection revealed no evidence of tumor production (59, 78). A series of feeding experiments in rats with levels up to 5% for 2 years revealed no significant pathologic changes (10, 18, 78–80). Gerbils fed levels up to 4% for 2 years were also without significant toxicologic effects (78). A slight but statistically significant mutagenic effect on *Escherichia coli* was observed at high concentrations of the color, however (81, 82). Apparently very little of the color is absorbed; a majority is excreted unchanged in the feces, which is perhaps an explanation for the lack of toxicity of the compound. This property, however, has caused some difficulty. A case was reported of a young boy passing red stools who was unfortunately subjected to extensive hospital diagnostic procedures before it was concluded that the source of the red was erythrosine used to color the cereal he was fond of (83). Another unusual property of erythrosine is its ability to produce elevated protein-bound iodine measurements (84, 85). At first this was thought to be due to an effect on thyroid function. However, careful study has demonstrated that it is merely a matter of interference of circulating erythrosine with the conventional analytical determination for protein-bound iodine (86, 87). There is evidence that ingestion of erythrosine-containing foods can contribute to dietary iodine intake (88).

FD&C Red No. 3, FD&C Yellow No. 6, and FD&C Blue No. 2 were tested for Heinz body formation in cats without finding significant effects (79).

It should be pointed out that people may become allergic to food colors. Production of asthma from FD&C Yellow No. 5 has been reported (89). Positive allergic reactions have been obtained with others (89–92). This fact should be kept in mind in the investigation of food allergies in patients.

NATURAL FOOD COLORS

Despite the reassuring connotation of the word "natural" there is little logic to the previous categoric assumption of the safety of these substances. Highly toxic and even lethal compounds abound in nature. The principal natural food colors permitted in the United States about which toxicological information is available are the following: the carotenoids, annatto extracts, chlorophyll, riboflavin, turmeric, and carbon black.

The carotenoids are widely present in both plants and animals and frequently have Vitamin A activity. Since the withdrawal of FD&C Yellows No. 3 and 4 as food colors, carotenoids have been widely used as fat-soluble colors (butter and oleomargarine). β-carotene, the most important natural precursor of Vitamin A, is now produced synthetically. Ingestion of large amounts by man and experimental animals, while it may produce hypercarotenemia (yellowing of the skin), surprisingly does not produce hypervitaminosis A (93). High dietary levels are not absorbed (94). A four-generation rat study at 1000 ppm of β-carotene in the diet for 110 weeks produced no adverse effects (95). β-apo-8'-carotenal and β-apo-8'-carotenoic acid, methyl or ethyl ester, have very similar biological properties to β-carotene (96, 97). Canthaxanthine is a closely related carotenoid that does not have provitamin A activity. It has been fed to rats for 2 years at levels up to 5% in the diet, and to dogs at 400 mg/kg daily for 15 weeks without producing toxic effects (98).

The annatto colors are obtained from the seeds of a tropical tree (*Bixa orellana* L.). The principal color present is the carotenoid bixin. Annatto extracts have been fed to mice and rats for their life span at levels up to 0.5% in the diet as well as injected subcutaneously. No toxicity or carcinogenicity was observed (99, 100). Chlorophyll and its copper complex are considered to be innocuous largely on the basis of their total failure to be absorbed after ingestion and their prolonged human usage. The copper complex has been fed to rats for their life span at dietary levels up to 3% without toxic effects (101). The vitamin riboflavin is used as a food color. Only short-term toxicity tests in dogs and rats have been carried out, but in view of the extensive experience with this substance and its role as an essential nutrient it has been judged to be harmless (102). Turmeric has been tested in 2 dogs for 1 year at 1% in the diet and for 420 days in rats at 0.5% in the diet without producing significant toxicity (103).

Many other substances such as dehydrated beets, caramel, grape skin extract, corn endosperm oil, paprika, and carbon black are used for coloring foods. With the exception of carbon black, no toxicity data have been reported; they have been assumed to be safe on the basis of long prior usage. With carbon black, the question has existed whether 3,4-benzpyrene and other polynuclear hydrocarbons were produced during the production of carbon black by combustion. Evidence indicates that carbon black produced by the low temperature impingement process is free of such substances and therefore safe. Carbon black is not absorbed after oral administration (104).

SUMMARY OF THE PRESENT STATUS OF FOOD COLORS

In the United States, at the present time the triphenylmethane colors FD&C Blue No. 1, FD&C Blue No. 2, and FD&C Green No. 3 are permitted to be used in foods on the basis of their almost complete excretion in the feces and their low chronic toxicity. The production of local sarcomas by repeated injection of large doses is not considered relevant to the use of these colors in foods. FD&C Violet No. 1, formerly a permitted color, was delisted. The water-soluble sulfonated azo colors FD&C Yellow No. 5, FD&C Yellow No. 6, and FD&C Red No. 2 are permitted food colors, as is FD&C Red No. 3. In view of its toxicity, FD&C Red No. 4 is permitted only for coloring maraschino cherries. The oil-soluble azo dyes are judged to be too toxic for use in foods except for Citrus Red No. 2, which is permitted only for coloring the skin of oranges.

The World Health Organization has reviewed the toxicology of food colors rather intensively and concurred with the judgments of the US Food and Drug Administration except for the limited special uses of FD&C Red No. 4 and Citrus Red No. 2.

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