

Blue 1

Blue 2

Citrus Red 2

Green 3

Orange B

Red 3

Red 40

Yellow 5

Yellow 6

Food Dyes

A Rainbow of Risks

The colors on the cover and the chapter headings are approximations. The exact color of dyes depends on concentration, pH, nature of the food, and other factors. The photo, taken from a Warner-Jenkinson Co. catalog, on the back cover shows a variety of natural colorings.

Food Dyes

A Rainbow of Risks

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Abbreviations

ADI: Acceptable Daily Intake

CCMA: Certified Color Manufacturers Association

CSPI: Center for Science in the Public Interest

FDA: U.S. Food and Drug Administration

FD&C: Foods, drugs, and cosmetics

GI: Gastrointestinal

HRG: Health Research Group (of Public Citizen)

IARC: International Agency for Research on Cancer

mg/kg bw/day: milligrams per kilogram of body weight per day

MTD: maximum tolerated dosage

NOAEL: No observable adverse effects level

NOEL: No observable effects level

NTP: National Toxicology Program

ppm: parts per million

RE: reticuloendothelial

µg: microgram

µM: micromolar

Summary

Food dyes, synthesized originally from coal tar and now petroleum, have long been controversial. Many dyes have been banned because of their adverse effects on laboratory animals. This report finds that many of the nine currently approved dyes raise health concerns.

Blue 1 was not found to be toxic in key rat and mouse studies, but an unpublished study suggested the possibility that Blue 1 caused kidney tumors in mice, and a preliminary *in vitro* study raised questions about possible effects on nerve cells. Blue 1 may not cause cancer, but confirmatory studies should be conducted. The dye can cause hypersensitivity reactions.

Blue 2 cannot be considered safe given the statistically significant incidence of tumors, particularly brain gliomas, in male rats. It should not be used in foods.

Citrus Red 2, which is permitted only for coloring the skins of oranges not used for processing, is toxic to rodents at modest levels and caused tumors of the urinary bladder and possibly other organs. The dye poses minimal human risk, because it is only used at minuscule levels and only on orange peels, but it still has no place in the food supply.

Green 3 caused significant increases in bladder and testes tumors in male rats. Though the Food and Drug Administration (FDA) considers it safe, this little-used dye must remain suspect until further testing is conducted.

Orange B is approved for use only in sausage casings, but has not been used for many years. Limited industry testing did not reveal any problems.

Red 3 was recognized in 1990 by the FDA as a thyroid carcinogen in animals and is banned in cosmetics and externally applied drugs. All uses of Red 3 lakes (combinations of dyes and salts that are insoluble and used in low-moisture foods) are also banned. However, the FDA still permits Red 3 in ingested drugs and foods, with about 200,000 pounds of the dye being used annually. The FDA needs to revoke that approval.

Red 40, the most-widely used dye, may accelerate the appearance of immune-system tumors in mice. The dye causes hypersensitivity (allergy-like) reactions in a small number of consumers and might trigger hyperactivity in children. Considering the safety questions and its non-essentiality, Red 40 should be excluded from foods unless and until new tests clearly demonstrate its safety.

Yellow 5 was not carcinogenic in rats, but was not adequately tested in mice. It may be contaminated with several cancer-causing chemicals. In addition, Yellow 5 causes

sometimes-severe hypersensitivity reactions in a small number of people and might trigger hyperactivity and other behavioral effects in children. Posing some risks, while serving no nutritional or safety purpose, Yellow 5 should not be allowed in foods.

Yellow 6 caused adrenal tumors in animals, though that is disputed by industry and the FDA. It may be contaminated with cancer-causing chemicals and occasionally causes severe hypersensitivity reactions. Yellow 6 adds an unnecessary risk to the food supply.

Almost all the toxicological studies on dyes were commissioned, conducted, and analyzed by the chemical industry and academic consultants. Ideally, dyes (and other regulated chemicals) would be tested by independent researchers. Furthermore, virtually all the studies tested individual dyes, whereas many foods and diets contain mixtures of dyes (and other ingredients) that might lead to additive or synergistic effects.

In addition to considerations of organ damage, cancer, birth defects, and allergic reactions, mixtures of dyes (and Yellow 5 tested alone) cause hyperactivity and other behavioral problems in some children. Because of that concern, the British government advised companies to stop using most food dyes by the end of 2009, and the European Union is requiring a warning notice on most dye-containing foods after July 20, 2010. The issue of food dyes and behavior has been discussed in a separate CSPI report and petition calling on the FDA to ban most dyes.

Because of those toxicological considerations, including carcinogenicity, hypersensitivity reactions, and behavioral effects, food dyes cannot be considered safe. The FDA should ban food dyes, which serve no purpose other than a cosmetic effect, though quirks in the law make it difficult to do so (the law should be amended to make it no more difficult to ban food colorings than other food additives). In the meantime, companies voluntarily should replace dyes with safer, natural colorings.

Overview

It is said that we “eat with our eyes as much as with our mouths,” and that’s certainly the case when we walk down the aisles of a supermarket. Fresh produce beckons us with its vivid colors and organic shapes, brightly colored packages and images seek to draw our eyes to those brands instead of competitors, and countless products—from Jell-O to Froot Loops—are colored with bright synthetic dyes that turn unattractive mixtures of basic ingredients and food additives into alluring novelties.¹

Dyes are complex organic chemicals that were originally derived from coal tar, but now from petroleum. Companies like using them because they are cheaper, more stable, and brighter than most natural colorings. However, consumers’ growing preference for natural foods is leading some companies to either not add colorings or to switch to safe natural colorings, such as beta-carotene (a precursor to vitamin A), paprika, beet juice, and turmeric (see back cover). That trend is stronger in Europe than the United States, but some U.S. companies recognize that an “All Natural” label can attract customers and may be moving in that direction.

Food Dye	Pounds of Total Dye Certified (includes lakes)	Percentage of Total
Blue 1 21 CFR 74.101	711,659	4.7
Blue 2 21 CFR 74.102	550,883	3.7
Citrus Red 2 21 CFR 74.302	1,764	0.0
Green 3 21 CFR 74.203	15,817	0.1
Orange B 21 CFR 74.250	0	0.0
Red 3 21 CFR 74.303	216,235	1.4
Red 40 21 CFR 74.340	6,205,374	41.3
Yellow 5 21 CFR 74.705	3,756,551	25.0
Yellow 6 21 CFR 74.706	3,558,351	23.7
TOTAL	15,016,634	100

Unlike other food additives, dyes are not permitted to be used unless the U.S. Food and Drug Administration (FDA) has tested and certified that each batch meets the legal specifications. One benefit of the certification process is that it provides information about the amounts of dyes sent into commerce each year for use in foods, drugs, and cosmetics (see Table 1). Just three dyes—Red 40, Yellow 5, and Yellow 6—account for 90 percent of all dyes used. The FDA’s data show a dramatic five-fold increase in consumption of dyes since 1955 (see Figure 1). That increase is a good indication of how Americans increasingly have come to rely on processed foods, such as soft drinks, breakfast cereals, candies, snack foods, baked goods, frozen desserts, and even pickles and salad dressings, that are colored with dyes.

1. For a list of all approved synthetic and natural colorings, see FDA (2007).

Carcinogenicity

Long-term animal feeding studies are done to determine whether long-term exposure to dyes causes cancer or other effects. However, most of the studies reviewed in this report suffer from several significant limitations. First, most of the studies were commissioned or conducted by dye manufacturers, so biases could influence the design, conduct, or interpretation of the studies. Ideally, the tests would have been conducted and interpreted by independent scientists. Second, most of the studies lasted no

longer than two years—and some were much shorter. Also, many studies did not include an *in utero* phase. Chronic bioassays would be more sensitive if they lasted from conception through 30 months or the natural lives of the rodents (as long as 3 years) (Huff, Jacobson *et al.* 2008).

Another consideration of unknown importance is that virtually all the studies evaluated the safety of individual dyes. Many foods, though, contain mixtures of dyes, such as the Blue 1, Blue 2, Red 40, Yellow 5, and Yellow 6 in Kellogg's Hot Fudge Sundae Pop Tarts. Dyes conceivably could have synergistic (or, indeed, antagonistic) effects with one another or with other food additives or ingredients.

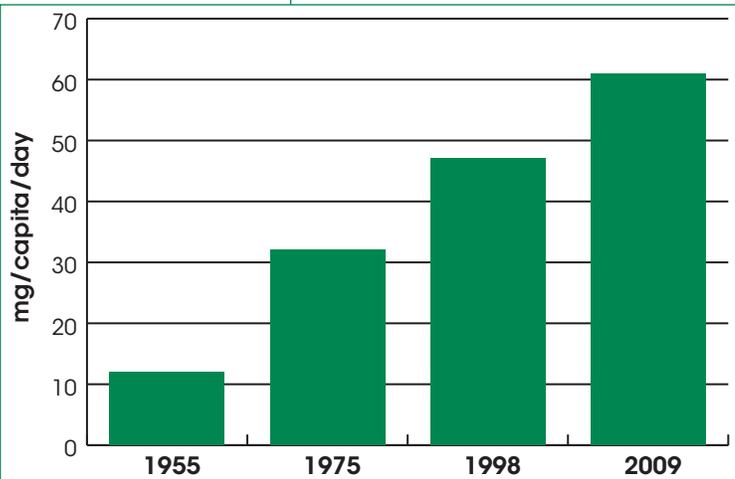


Figure 1. Food dyes marketed per capita per day (mg).

Based on FDA data of certification of straight dyes and lakes (adjusted by the authors for percentage of pure dyes) and current U.S. population.

It is worth noting that dyes are not pure chemicals, but may contain upwards of 10 percent impurities that are in the chemicals from which dyes are made or develop in the manufacturing process. For instance, Yellow 5, the second-most widely used dye, may contain up to 13 percent of a witch's brew of organic and inorganic chemicals (FDAg).²

Certain of those contaminants, such as 4-aminobiphenyl, 4-aminoazobenzene, and benzidine, are carcinogens, but are supposed to be present at safely negligible levels in the dyes (FDA 1985). Any carcinogenic effects of those low-level contaminants would not be detected in animal studies of the dyes.

2. Constituents permitted in Yellow 5: 4,4'-[4,5-Dihydro-5-oxo-4-[(4-sulfophenyl)hydrazono]-1H-pyrazol-1,3-diy] bis[benzenesulfonic acid], trisodium salt, not more than 1 percent; 4-[(4',5'-Disulfo[1,1'-biphenyl]-2-yl)hydrazono]-4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole-3-carboxylic acid, tetrasodium salt, not more than 1 percent; Ethyl or methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)hydrazono]-1H-pyrazole-3-carboxylate, disodium salt, not more than 1 percent; Sum of 4,5-dihydro-5-oxo-1-phenyl-4-[(4-sulfophenyl)azo]-1H-pyrazole-3-carboxylic acid, disodium salt, and 4,5-dihydro-5-oxo-4-(phenylazo)-1-(4-sulfophenyl)-1H-pyrazole-3-carboxylic acid, disodium salt, not more than 0.5 percent; 4-Aminobenzenesulfonic acid, sodium salt, not more than 0.2 percent; 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole-3-carboxylic acid, disodium salt, not more than 0.2 percent; Ethyl or methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole-3-carboxylate, sodium salt, not more than 0.1 percent; 4,4'-(1-Triazene-1,3-diy)bis[benzenesulfonic acid], disodium salt, not more than 0.05 percent; 4-Aminoazobenzene, not more than 75 parts per billion; 4-Aminobiphenyl, not more than 5 parts per billion; Aniline, not more than 100 parts per billion; Azobenzene, not more than 40 parts per billion; Benzidine, not more than 1 part per billion; 1,3-Diphenyltriazene, not more than 40 parts per billion; Lead (as Pb), not more than 10 parts per million; Arsenic (as As), not more than 3 parts per million; Mercury (as Hg), not more than 1 part per million.

The FDA has established legal limits for cancer-causing contaminants in dyes. Those limits are intended to ensure that a dye will not pose a lifetime risk of greater than one cancer in one million people. FDA chemists test each batch of dye to confirm that those tolerances are not exceeded. Unfortunately, the FDA's process is riddled with problems. For one thing, those tolerances were based on 1990 dye usage, but per-capita usage has increased by about 50 percent since then. Second, the FDA did not consider the increased risk that dyes pose to children, who are both more sensitive to carcinogens and consume more dyes per unit of body weight than adults (Hattis, Goble *et al.* 2005). Third, and most importantly, FDA and Canadian government scientists showed that levels of bound benzidine, a carcinogenic contaminant in at least Yellow 5 and Yellow 6 dyes, far exceeded levels of free dyes (Peiperl, Prival *et al.* 1995; Lancaster and Lawrence 1999). (Bound carcinogens have also been found in Allura Red AC, the un-certified form of Red 40 (Lancaster and Lawrence 1991).) Indeed, the Canadians found several bound carcinogens in soft drinks and hard candies (Lancaster and Lawrence 1992). Bound benzidine is largely converted to the free form in the large intestine. Large amounts of other carcinogenic contaminants might also be present in the bound form. However, the FDA generally only measures “free” contaminants and, hence, is blind to those (except possibly aniline) bound up in other molecules (FDA February 26, 2010). Fourth, the FDA should consider the cumulative risk of all dyes, rather than of each dye independently. Indeed, the Food, Drug, and Cosmetic Act requires the FDA to consider “the cumulative effect, if any, of such additive...taking into account the same or any chemically or pharmacologically related substance...”³ If the FDA considered those four factors in evaluating risks, the risks posed by the two yellow dyes—which comprise 49 percent of all dyes used—let alone all dyes taken together, would exceed the one-in-a-million standard.

Genotoxicity

A chemical's ability to cause mutations or damage chromosomes in bacterial or eukaryotic cells (ones with a nucleus) is an indication that the chemical might cause cancer in humans. While one or two positive genotoxicity studies might not ring alarm bells, a chemical like Yellow 5, which showed positive findings in 6 out of 11 genotoxicity studies deserves special attention and study—perhaps new and larger chronic feeding studies. On the other hand, only 1 of 11 genotoxicity studies on

Table 2. Numbers of positive and negative genotoxicity studies of FD&C food dyes.

FD&C Color (generic name)	Total Number of Positive Studies	Positive <i>in vivo</i> Studies*	Negative Studies
Blue No. 1 (Brilliant Blue)	2	0	7
Blue No. 2 (Indigo Carmine)	1	0	10
Green No. 3 (Fast Green)	3	0	6
Red No. 3 (Erythrosine)	4	1	8
Red No. 40 (Allura Red)	3	3	7
Yellow No. 5 (Tartrazine)	6	2	5
Yellow No. 6 (Sunset Yellow)	2	1	6

* The numbers of “Positive *in vivo* studies” are included in “Positive Studies.”

3. 21 USC 379e((b)(5)(A)(ii).

Blue 2 was positive. The discussions below of each dye summarize the results of genotoxicity tests. Table 2 lists the number of negative and positive results for genotoxicity studies on the seven main dyes covered in this report. Further details are provided in Tables A1–A7 of the Appendix.

One significant limitation of this report is that the authors were restricted to reviewing mostly published studies. Unpublished toxicology studies in the files of the FDA or companies were not always obtainable and might shed further light on the safety of the dyes.

Neurotoxicity

Note to Parents

If you believe your children are sensitive to food dyes, please file a report at www.cspinet.org/fooddyes.

A list of more than a thousand foods made with dyes is also at that Web site.

This report focuses on traditional toxicology, which considers organ damage, cancer, birth defects, and the like. We do not explore neurobehavioral toxicity. But we would be remiss if we did not note that in the early 1970s, San Francisco allergist Benjamin Feingold observed that food dyes and certain other synthetic and natural components of food could cause hyperactivity and other impaired behaviors in child and adult patients. His recommendation that parents try putting their hyperactive children on an “elimination” diet generated huge publicity and spurred dozens of scientific studies over the years.⁴ A 2004 meta-analysis of many of the studies concluded that there, indeed, was a cause-and-effect relationship between food dyes and hyperactivity. The authors stated that dyes “promote hyperactivity in hyperactive children, as measured on behavioral rating scales” and that “society should engage in a broader discussion about whether the aesthetic and commercial rationale for the use of [artificial food colorings] is justified” (Schab and Trinh 2004).

Two recent studies sponsored by the British government on cross-sections of British children found that mixtures of four dyes (and a food preservative, sodium benzoate) impaired the behavior of even non-hyperactive children (Bateman, Warner *et al.* 2004; McCann, Barrett *et al.* 2007). As a result, the British government told the food and restaurant industries to eliminate the dyes tested by the end of 2009, and the European Parliament passed a law that will require a warning notice on all foods that contain one or more of the dyes tested after July 20, 2010. The notice states that the dyed food “may have an adverse effect on activity and attention in children” (Parliament accessed February 20, 2010).

Distressingly, some products made by McDonald’s, Mars, Kraft, PepsiCo, and other major U.S. multinational companies contain dyes in the United States, but natural or no colorings in the United Kingdom. In June 2008, the Center for Science in the

4. See <http://cspinet.org/fooddyes/index.html> for more detailed information about food dyes and hyperactivity, especially “Diet, ADHD & Behavior: a quarter-century review – 2009 Update.” Jacobson MF, Schardt D. (Washington: Center for Science in the Public Interest). <http://www.cspinet.org/new/pdf/dyesreschbk.pdf>; accessed Feb. 20, 2010. Also, see CSPI’s 2008 petition to the FDA.

Public Interest (CSPI) petitioned the FDA to ban all the widely used food dyes because of their impact on children's behavior.⁵ Ideally, food dyes and all other food additives would be screened in animals and *in vitro* systems for potential behavioral effects before they are allowed into the food supply.

Food Dyes and the Law

A 1914 editorial in *The Journal of Industrial and Engineering Chemistry* stated that "America can have a coal-tar dye industry if she pays the price." (Hesse 1914) Unfortunately, America did develop a coal-tar dye industry, and we may well be paying a kind of price that the journal editors did not have in mind. Down through the years, more food dyes have been found to be risky than any other category of food additive.⁶

Prior to 1960, dyes had to be absolutely "harmless," regardless of dose—a virtual impossibility. Congress wanted to loosen the law, which, if applied strictly, could have banned every single coloring. But in passing and implementing the 1960 Color Additives Amendment, Congress and the FDA apparently had special concerns about the safety of food dyes, though enforcement of the law has not reflected those concerns. James T. O'Reilly, an adjunct professor at the University of Cincinnati College of Law, observed that "Congress felt that ...colors deserved greater regulation because of their lesser net benefit to society than such items as food preservatives and common spices." For instance,

- Congress required that each batch of food dyes, but not other colorings (such as from carrots or grape skins), be tested and certified to contain only acceptable levels of contaminants, such as lead and benzidine. Food additives, such as preservatives or flavorings, are not subject to such testing.
- Congress did not permit companies to declare that any dyes are "generally recognized as safe" (GRAS), and thereby not further regulated by the FDA. In contrast, companies are permitted to declare flavorings, emulsifiers, and other such ingredients to be GRAS.
- The FDA's definition of safety for color additives states that "safe means that there is *convincing evidence* that establishes with reasonable certainty that no harm will result from the intended use of the color additive" (FDAa). [emphasis added] The term "convincing evidence" is not in the definition of safety for non-color additives.

5. See <http://cspinet.org/new/pdf/petition-food-dyes.pdf>.

6. Banned dyes include: Green 1: liver cancer; Orange 1 and Orange 2: organ damage; Orange B (ban never finalized): contained low levels of a cancer-causing contaminant (it was used only in sausage casings, but is no longer used in the United States); Red 1: liver cancer; Red 2: possible carcinogen; Red 4: high levels damaged adrenal cortex of dog; Red 32: damages internal organs and may be a weak carcinogen (since 1956 it continues to be used as Citrus Red 2 [see discussion in this report] only to color oranges at 2 parts per million [ppm]); Sudan 1: toxic and carcinogenic; Violet 1: cancer (used to stamp USDA's inspection mark on beef carcasses); Yellow 1 and Yellow 2: high dosages caused intestinal lesions; Yellow 3: high dosages caused heart damage; Yellow 4: high dosages caused heart damage. Note, though, that in some cases companies didn't bother to re-test chemicals that might have been harmful only at high dosages and not at the lower dosages consumed in foods. <http://cspinet.org/reports/chemcuisine.htm>.

Some Members of Congress have emphasized that the safety standard for artificial colorings should be particularly high because the colorings don't offer any health benefit to offset even small risks. Representative Ted Weiss (D-NY) said, "It doesn't make any difference how much or how little (of a carcinogenic additive) a particular substance contains, especially when you've got a color additive that has no nutrient value and no therapeutic value" (Weiss July 6, 1985). Representative King said, "The colors which go into our foods and cosmetics are in no way essential to the public interest or the national security...Consumers will easily get along without (carcinogenic colors)" (D.C. Cir. 1987). [Note: It is unclear which Rep. King was quoted in the case: Rep. Cecil King, D-CA, or Rep. David King, D-Utah.]

Consumer activists have long sought to persuade the FDA to ban dyes. In the early 1970s, CSPI urged the government to ban Violet No. 1, which, ironically, was the color used in the U.S. Department of Agriculture's meat inspection stamp, because it appeared to cause cancer in animal studies (the dye was banned in 1973). Subsequently, in the 1970s and 1980s, Public Citizen's Health Research Group was the most aggressive critic of dyes, petitioning and suing the FDA to ban dyes (Burros February 13, 1985). Some of those actions were based on the 1960 law—the Delaney amendment⁷—that bans the use of colorings that cause cancer in animals or humans. Also, as noted above, in 2008 CSPI urged the FDA to ban colors because of their effects on children's behavior.

Even if color additives were all deemed to be safe, many uses of colorings, both synthetic and natural, still could be considered illegal under the Food, Drug, and Cosmetic Act. Sections 402(b)(3) and (b)(4) of that law stipulate that "A food shall be deemed to be adulterated... (3) if damage or inferiority has been concealed in any manner; or (4) if any substance has been added thereto or mixed or packed therewith so as to...make it appear better or of greater value than it is." And section 403 of the same law says that a food is misbranded "if its labeling is false or misleading in any particular."

Clearly, food colorings are added to fruit drinks, frozen desserts, gelatin desserts, salad dressings, child-oriented breakfast cereals and snack foods, and countless other products solely to conceal the absence of fruits, vegetables, or other ingredients and make the food "appear better or of greater value than it is." Defenders of colorings would say that consumers could simply plow through the list of ingredients on the back of the package to detect the presence of colorings, but it simply isn't fair to require consumers to do that plowing. Currently, the use of artificial flavorings must be declared conspicuously as part of the product names on the front labels. If nothing else, the FDA should require the same of artificially colored foods. A national Internet-based poll commissioned by CSPI and conducted by Opinion Research Corporation in January 2010 found that 74 percent of respondents favored such labeling.

7. 21 USC 379e(b)(5)(B)

As this report discusses, studies of the nine dyes currently approved by the FDA suggest, if not prove, that most of the dyes cause health problems, including cancer, hypersensitivity, or neurotoxicity (including hyperactivity). And that's the case even though most of the research was commissioned, conducted, and interpreted by the chemical industry itself and its testing labs and academic consultants. The health concerns indicate that most dyes fail the FDA's safety requirement "that there is convincing evidence...that no harm will result from the intended use of the color additive." Fortunately, numerous natural colorings could be used in place of dyes: beet juice, beta-caramel, carotene, carrot juice, chlorophyll, elderberry juice, grape juice/skin, paprika extract, purple corn, purple sweet potato, red cabbage, and turmeric.

Getting Dyes Out of Foods

CSPI has urged several major multinational companies that do not use dyes in Europe to do the same in the United States. Unfortunately, most of those companies said that they don't use dyes in Europe because government has urged them not to—but that they would continue to use dyes in the United States until they were ordered not to or consumers demanded such foods. (Starbucks and the maker of NECCO Wafers have eliminated dyes, and Frito-Lay said that it would be phasing out dyes in the coming years (Jacobson and Small 2009).)

Consumers should not have to wait decades, if not forever, for companies to voluntarily remove questionable dyes from their products. The FDA, which is charged with protecting the public from unsafe food ingredients, should ban most or all of the dyes. However, it is worth noting that the Food, Drug, and Cosmetic Act makes it even harder for the FDA to revoke previous approvals of food colors than other food additives.⁸ As one legal analyst stated,

Thanks to the foresight and effective lobbying of the cosmetics industry in the 1960s, the proponent of a color additive petition is in an excellent position if the FDA decides to remove [a coloring's] permanent listing. The burdens of proof in a complex process fall on the FDA, and the time required to pass through the procedural maze acts as a disincentive to FDA undertaking any delisting action (O'Reilly 2007).

Ideally, the law would be changed to provide greater consumer protection from unsafe dyes. We turn now to detailed assessments of the toxicology research done on the nine dyes currently permitted for use in some or all foods in the United States. Based on those assessments, the authors' conclusion is that the time has come to eliminate

8. To challenge a proposed ban on a food or color additive, companies can request that the FDA hold a formal public hearing and, if the FDA subsequently still wants to ban the substance, companies can go to court. The process for color additives, though, includes another hurdle, because, if a dye is alleged to cause cancer, companies can request that FDA create an outside advisory committee to opine on the matter. Compare 21 USC 379e(b)(5)(C) and 21 USC 371(e)(2) and (f)(2) for colorings with 21 USC 348 409(f) and (h) and 21 CFR 171.130 for other additives.

FOOD DYES

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dyes from our food supply and return to the use of natural colorings (or foods that don't require colorings to be marketable), the direction in which Europe—and some American companies—are moving.

FD&C Blue 1

Introduction

FD&C Blue No. 1 (Fig. 2), or Brilliant Blue,⁹ is a water-soluble coloring used in many baked goods, beverages, dessert powders, candies, cereals, drugs, and other products. Blue 1 received FDA approval for general use in foods and ingested drugs in 1969. In 1982, the FDA permanently approved the color for use in externally applied drugs and general use in cosmetics excluding the area of the eye. The FDA suggests a maximum Acceptable Daily Intake (ADI) for Blue 1 of 12 mg/kg bw/day (FDA 1982a). For a 30-kilogram (66-pound) child, that would equate to 360 mg/day. Current average dye production is equivalent to about 3 mg/person/day (based on the entire population, not just children). Chemically, Blue 1 is almost identical to Green 3, except for a hydroxyl group.

Metabolism

In a study of rats, Blue 1 was largely excreted unchanged in the feces (96%) within 36 hours after a 200-mg oral administration. None of the dye was excreted in the urine. In the same study, only 0.7% and 2.8% of a 200-mg oral dose was excreted in the bile of two bile-duct cannulated dogs indicating some intestinal absorption. Investigators calculated that the quantity of absorption of the color from the gastrointestinal (GI) tract was about 10 mg out of a 200-mg dose (Hess and Fitzhugh 1955). Brown *et al.* reported similar results after administering a single 0.27-mg dose of ¹⁴C-labeled Blue 1 to female Sprague-Dawley rats. Bile duct-ligated rats excreted the dye in their urine and feces at concentrations of 2.02 and 97.28%, respectively. Given the lower percentage of dye being excreted in the bile, the large amount eliminated through the feces indicates that the dye is poorly absorbed by the GI tract. In this particular study, total intestinal absorption was estimated to be about 2.05% and 0.27% of the total dose in bile duct-ligated and intact rats, respectively. Analysis of the biliary and urinary excretion showed that 95% of the recovered radioactivity was from unchanged Blue 1 while 5% was an unidentified metabolite or degradation product (Brown, Dorsky *et al.* 1980). These results indicate that in rats Blue 1 is not susceptible to breakdown by intestinal microbiota, but up to 5% is absorbed via the GI tract.

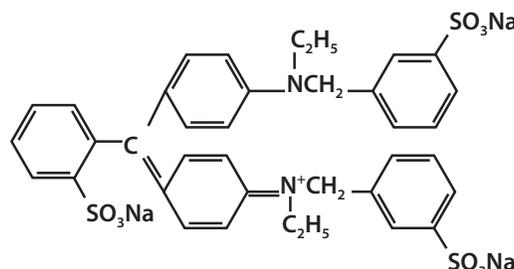


Figure 2. FD&C Blue No. 1; Brilliant Blue

9. The color of each section heading is printed in the color of the dye being discussed; the actual color in a food depends on the dye's concentration and the colors imparted by other dyes and ingredients.

Genotoxicity

Table A1 in the Appendix summarizes the genotoxicity studies performed on Blue 1. Based on those assays, the dye is not genotoxic in terms of DNA damage, base pair mutations, base substitutions, or frameshift mutations. However, Blue 1 caused chromosomal aberrations in two studies (Ishidate, Senoo *et al.* 1974; Hayashi, Matsui *et al.* 2000).

Chronic Toxicity/Carcinogenicity

Hansen *et al.* performed chronic toxicity studies using Blue 1 on rats and dogs. The rat study lasted 2 years and used 24 Osborne-Mendel rats/sex/group at doses of 0, 0.5, 1, 2, and 5% of the diet. There were no reported compound-related effects in any group on mortality, hematology, or organ weights (heart, liver, spleen, testis, kidney). There was also no reported significant growth inhibition or gross lesions. The small numbers of rats in each group renders this study quite insensitive and of marginal value (Hansen, Fitzhugh *et al.* 1964).

In Hansen *et al.*'s dog study, 12 beagles, aged 6-7 months, were fed the dye at doses of 0 (1 male, 1 female), 1 (2 males, 2 females), or 2% (4 males, 2 females) in the diet for up to one year. Investigators reported no clinical signs attributable to the color, though one dog in the highest-dose group died of intercurrent viral infections, and one dog in the 1% group was sacrificed due to its poor condition before the end of the study. Blue 1 caused no gross or microscopic lesions. The NOELs (No Observed Effect Level) for rats and dogs were 5% and 2%, respectively (Hansen, Fitzhugh *et al.* 1964). According to FDA guidelines, the dog study was inadequate because a one-year study in dogs should have equal numbers of males and females and the control group should have at least 4 animals per sex (FDA 2000). And, of course, a dog study lasting just one year cannot detect effects that occur only after years of exposure.

Rowland *et al.* performed a chronic toxicity/carcinogenicity study on Blue 1 in 48 males/group and 50 females/group of ASH/CS1 mice. The mice were administered 0, 0.015, 0.15, or 1.5% Blue 1 in their diets for only 80 weeks. Seven out of the surviving 30 male mice in the 0.15% group had kidney tumors compared to only 1 kidney tumor in the 44 surviving controls. The increase in kidney tumor rates was statistically significant ($p < 0.05$). However, no dose-response relationship was said to be found, diminishing, but not eliminating, concern about carcinogenicity (data on the 1.5% group were not provided). This study, which was conducted by the British Industrial Biological Research Association, a now-defunct industry-sponsored organization, was reviewed by the International Agency for Research on Cancer (IARC) and published in abstract form in an IARC monograph, but for unknown reasons the full study was never published.¹⁰

10. Pers. Comm. Rowland IR. June 26, 2009. Rowland did not know why the study was never published.

The highest-quality carcinogenicity/toxicity studies were performed by Borzelleca *et al.* for the Certified Color Manufacturers Association (CCMA). The 2-year studies used Charles River CD rats and CD-1 mice. The rat study included an *in utero* phase with 60 rats/sex/group. The rats were fed 0 (two control groups), 0.1, 1, and 2% Blue 1 in the chow for about two months prior to mating. Investigators reported no compound-related effects on fertility, gestation, parturition, lactation, pup survival through weaning, and number of live and stillborn pups. F₁ generation rats were randomly selected, and 70 rats/sex/group were used in the lifetime feeding study (same dosage groups, including two controls). The maximum exposure times for males and females were 116 and 111 weeks from birth, respectively. F₁ females in the 2% group had a significant decrease in terminal mean body weight (15%) and decreased survival compared to controls. No other compound-related effects were noted. The NOAEL (No Observable Adverse Effect Level) for males was 1,072 mg/kg bw/day (2% group) and 631 mg/kg bw/day for females (1% group) (Borzelleca, Depukat *et al.* 1990a).

The mouse study did not include an *in utero* phase and used 60 mice/sex/group. Mice were administered 0 (two control groups), 0.5, 1.5, and 5% Blue 1 in their food. The maximum exposure time was 104 weeks for both sexes and the NOAEL was determined to be 5%, or 7,354 and 8,966 mg/kg bw/day for males and females, respectively. No significant compound-related effects were noted in any of the groups (Borzelleca, Depukat *et al.* 1990a).

Neurotoxicity

Lau *et al.* investigated the individual and potential synergistic effects of Blue 1 and L-glutamic acid (a close relative of the food additive monosodium glutamate) on neuronal development. Investigators used NB2a neuroblastoma cells that were induced to differentiate and grow neurites (projections from a neuron) in the presence or absence of the two food additives. Neurotoxicity was measured as an inhibition of neurite outgrowth. Individually, Blue 1 was found to have an IC₅₀ (half-maximal inhibitory concentration) of 0.0514 μM, while L-glutamic acid was found to have an IC₅₀ of 48.7 μM. When cells were treated with the two additives together, rather than just seeing an additive effect, the two compounds worked synergistically (Fig. 3). A 50:50 mixture of L-glutamic acid and Blue 1 produced 46.1% neurite growth inhibition, which was significantly different from the expected value of 15.8% if the compounds acted additively. On the other hand, the effect on cell viability from the combination of the two additives was increased only in an additive fashion (Lau, McLean *et al.* 2006). Other food dyes might behave similarly.

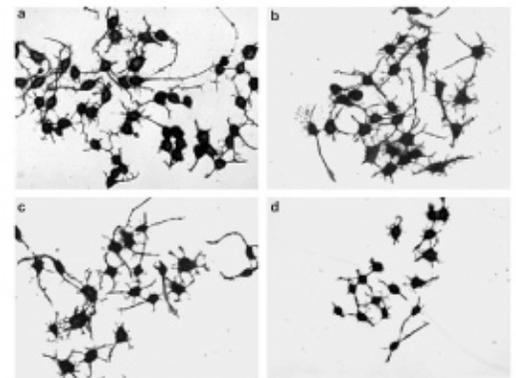


Figure 3. Images of Coomassie Blue-stained differentiating NB2a cells:

(a) in the absence of additives; (b) in the presence of 0.0001 μM Blue 1 to produce approximately 20% neurite inhibition; (c) 1 μM L-glutamic acid to produce approximately 20% neurite inhibition; (d) a mixture of Blue 1 and L-glutamic acid each at 50% of the above concentrations.

Figure used with the permission of Oxford University Press: Lau, K., W. G. McLean, *et al.* (2006). "Synergistic interactions between commonly used food additives in a developmental neurotoxicity test." *Toxicological Sciences* 90(1): 178-187.

Feingold suggested that food dyes and additives are associated with hyperactivity disorders in children (see page 4 and Feingold 1975). The developmental period of synaptogenesis (brain growth-spurt period) occurs in humans from three months before birth to several years after birth (Lau, McLean *et al.* 2006). Small amounts of Blue 1 are absorbed by the GI tract in rats, but metabolism studies in children have not been conducted. Blue 1 might possibly have potent effects, and it might take only a small absorbed amount to affect a child's brain development. The blood-brain barrier is not fully developed until 6 months in humans and even after complete development some regions of the brain are never protected by the blood-brain barrier (Brightman and Broadwell 1976; Adinolfi 1985). Further studies need to be done on Blue 1 and on the possible neurotoxicity of other dyes.

Conclusions

The most thorough studies of Blue 1, which were sponsored by industry, did not find evidence of carcinogenicity in rats or mice. On the other hand, other studies raise questions about possible harm. A review by the IARC of a study (published only in abstract form) states that male mice had a statistically significant increased incidence of kidney tumors in the mid-dose group. Also, in an *in vitro* test, Blue 1 inhibited neurite growth and acted synergistically with L-glutamic acid, suggesting the potential for neurotoxicity. That is particularly worrisome for fetuses and babies under the age of six months whose blood-brain barrier is not fully developed. Further research needs to be conducted before this dye can be considered safe.



FD&C Blue 2

Introduction

FD&C Blue No. 2 (Fig. 4) is the approved form of Indigo Carmine. In 1983, the FDA permanently listed Blue 2 for use in foods and ingested drugs (FDA 1983). It is widely used to color beverages, candies, pet foods, and many other foods and drugs. Blue 2 has an ADI of 2.5 mg/kg bw/day (FDA). That ADI is equivalent to 75 mg for a 30-kg child. The FDA certifies an amount of Blue 2 that is equivalent to about 2 mg/person/day.

Metabolism

Metabolism studies in rats demonstrated that the majority of the dye and/or its metabolites (including 5-sulfoanthranilic acid, its final breakdown product, Fig. 5) are excreted in the feces, with smaller amounts being found in the urine (Lethco and Webb 1966; Jones, Ryan, et al., 1964). In one bile-duct-cannulated rat given a 20-mg dose of Blue 2, only 0.004% of the dye was excreted in the bile—125 times as much was found in the urine. The authors concluded that the majority of the small amount of dye that is absorbed intact is excreted through the urine and not the bile, and the dye excreted in the feces is mostly from unabsorbed dye (Lethco and Webb 1966). Those studies show that 5-sulfoanthranilic acid is absorbed more readily by the GI tract than is the intact dye (Lethco and Webb 1966).

Genotoxicity

Details of the genotoxicity studies performed on Blue 2 are provided in Table A2 in the Appendix. All of the 11 tests were negative except for a chromosomal aberration assay (Ishidate, Senoo *et al.* 1974). It would be appropriate for an independent lab to repeat that study, as well as perform an *in vivo* chromosomal aberration test.

Chronic Toxicity/Carcinogenicity

Between 1984 and 1986, Borzelleca *et al.* performed a series of toxicology studies using Blue 2. One was a high-quality chronic toxicity/carcinogenicity study in rats. The study included an *in utero* phase in which 5 groups of 60 male and 60 female Charles

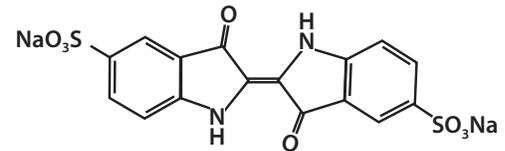


Figure 4. FD&C Blue 2; Indigo Carmine

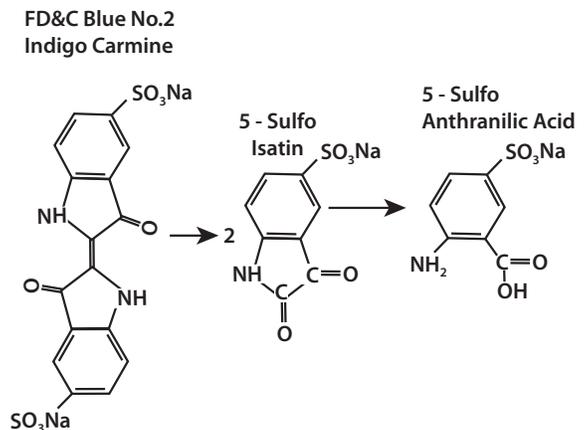


Figure 5. Natural breakdown products of FD&C Blue 2

River CD albino rats were fed 0 (two different control groups), 0.5, 1, or 2% Blue 2 starting at least 2 months prior to mating. F₁ offspring in each dosage group were randomly selected, and 70 rats/sex/group were continued on the same dosages for 29 and 30 months in males and females, respectively. Administration of the dye did not affect the number of pregnant females per group or pup viability at birth. However, there was possible evidence of carcinogenicity:

- Treated male rats showed a dose-related increase in the incidence of transitional cell neoplasms (an abnormal mass of tissue that may be benign (not cancer) or malignant (cancer)) of the urinary bladder, but the numbers of affected animals were small and the apparent increase was not statistically significant when compared to combined controls (0.8, 1.6, 2.9, and 4.5% of the animals had bladder neoplasms in the control, low-, mid-, and high-dose groups, respectively; the *trend* likely was significant).
- Male rats in the 2% group had statistically significant increases in malignant mammary-gland tumors and brain gliomas. However, the investigators concluded that the increased mammary-gland tumors were not related to Blue 2. They also concluded that the gliomas were not consistent with several criteria they said were required to classify a compound as a carcinogen. For instance, neither a dose-effect relationship nor a concurrent decrease in survival time was seen. They also reported that the incidence of gliomas in treated animals was consistent with historical controls. All too often companies (or the FDA) will resort to comparisons with historical controls when the test group has more tumors than the controls in the current study. Based on this study, the investigators estimated that the NOAEL for Blue 2 was 2.0%, or 1,282 mg/kg bw/day and 1,592 mg/kg bw/day for males and females, respectively (Borzelleca, Hogan *et al.* 1985a).

The FDA's Cancer Assessment Committee concluded that the occurrence of urinary bladder transitional cell neoplasms, though apparently dose-related, was not related to treatment with Blue 2 because (a) historical evidence suggests that this form of cancer is not rare in Charles River CD albino rats, (b) the number of neoplasms in the high-dose group was small, and (c) the number of tumors in the high-dose group was not significantly higher than in the control groups.

Regarding the malignant tumors of the mammary gland in the high-dose males, when the Committee combined malignant and benign tumors, there was no longer a statistically significant difference between the controls and high-dose male rats. The Committee concluded that Blue 2 did not cause any significant treatment-related effects in rats (FDA 1983).

Although there was a significantly higher incidence of brain gliomas in the high-dose male rats, the FDA's Cancer Assessment Committee was still reluctant to conclude that

Blue 2 was the cause because (a) of a lack of gliosis (scarring of the central nervous system) in the high-dose animals, (b) the first two observed gliomas of the brain occurred in controls animals, and (c) data were lacking on the historical incidence of brain gliomas in Charles River albino rats that survive for 30 months. The FDA concluded that “except for brains of male rats for which the data are equivocal, there is no evidence for carcinogenicity in rats or mice of either sex for all organs examined.” Upon reevaluation of the brain microslides and comparison to controls from a simultaneous study on Green No. 3, the new statistics produced p-values that were just above 0.05 (one test, the Breslow time-adjusted analysis, produced a p-value of 0.053). It is highly questionable to switch a comparison to a different control group after a study is completed. Still, the FDA stated, “. . . although statistical methods provide insight into the likelihood of being right or wrong in making specific conclusions, they do not provide for certainty as to whether an increase or decrease in tumor incidence is related to treatment.” The Board of Scientific Advisors of the National Toxicology Program concluded that Blue 2 is safe for consumption, citing (a) a lack of evidence for a dose-related trend, (b) lack of non-neoplastic cellular changes in addition to frank neoplasia (new and abnormal development of cells that may be benign or malignant), (c) no reduction in latency period, (d) no varying progression of brain tumors, (e) inability of Blue 2 to cross the blood-brain-barrier, (f) negative mutagenicity assays, and (g) lack of evidence in structure-activity analysis (FDA 1983).

Borzelleca *et al.* consulted three outside sources on the carcinogenicity issues in rats. Robert Squire, a prominent industry consultant at the Johns Hopkins University School of Medicine, found a lack of persuasive evidence for compound-related carcinogenicity/toxicity in the glioma and urinary bladder samples (Jacobson 1981). However, Aleksandar Knezevich and Geoffrey Hogan, former vice president of pathology and former vice president of toxicology, respectively, at Bio/dynamics (an industry consulting firm), concluded that the glioma findings “cannot be dismissed as accidental.” On the other hand, those men agreed with the FDA committee that the rates of urinary neoplasms in treated male rats were not clearly different from the controls and were probably not of concern (Knezevich and Hogan 1982).

After Blue 2 was approved for permanent listing in 1983, the Public Citizen Health Research Group (HRG) filed an objection on the grounds that the increase in brain tumors in rats fed Blue 2 was statistically significant. The group argued that the decision to list Blue 2 permanently violated both the Delaney Clause (which bars cancer-causing food and color additives) and the general safety clause since the dye had not been proven safe (FDA 1988).

In a statement to the HRG in 1982, Dr. William Lijinsky, a cancer specialist at the National Cancer Institute’s Frederick Cancer Research Center, wrote,

...the incidence of these (brain) tumors in the high dose group versus the controls is highly significant. . . In my own laboratory this would be considered

prima facie evidence of carcinogenicity of a treatment. This is especially so because this tumor is so rare, and my conclusion is that Blue 2 is a carcinogen, and should be regulated accordingly.

Regarding his own evaluation of the histopathology of brain/spinal cord sections in microslides, Dr. Benjamin A. Jackson, of the FDA's Division of Pathology in the Color and Cosmetics Evaluation Branch, wrote, "...the possibility cannot be outrightly excluded that the compound (Blue 2) itself, its metabolite(s) or a secondary effect induced by the high dose of the color may have acted to increase the number of brain tumors seen in this study."

An administrative law judge found that a lack of certain biological factors, such as gliosis, invasiveness of tumors, a clear dose-response relationship, and an increased latency, outweighed the statistically significant incidence of brain gliomas in the rats. Reviewing the matter, the FDA commissioner concluded that the evidence produced at the hearing supported the notion that Blue 2 was not an animal carcinogen and that the permanent listing of Blue 2 was appropriate (FDA 1987).

HRG challenged the FDA's decision, contending that the rats may not have been exposed to the maximum tolerated dosage (MTD). According to the FDA, the highest dosage used in a study "should be sufficiently high to induce toxic responses in test animals, and should not cause fatalities high enough to prevent meaningful evaluation of the data from the study." Chronic-study doses "...should be based on results from subchronic studies and other related test substance information." (FDA 2000) HRG questioned whether the MTD was used in the chronic toxicity rat study because (a) no subchronic study was conducted to establish the MTD (the FDA found it acceptable to rely on the results of a previous 1966 study by Hansen), (b) adult rats in the study did not show alterations typical of animals given the MTD according to the FDA Redbook (FDA's guide for the testing of additives), (c) 5% was used as the MTD for the chronic mouse study discussed on page 17 (as opposed to 2% in the rat study), and (d) the Hansen study used a high dose of 5%, which led to an increase in the overall number of tumors compared to other groups. HRG argued that allowing a 2% MTD was contradictory to the FDA's own guidelines (Meyer, Schultz *et al.* 1987). Notwithstanding those arguments, the court ruled in favor of the FDA (FDAc).

In another study, 30 Charles River CD-1 mice/sex/group were fed 0.2, 0.4, 0.8, or 1.6% Blue 2 for 84 weeks. Controls consisted of 60 males and 60 females. The overall death rates in treated mice did not differ significantly from that in the controls. The most common neoplasms seen in both the control and treated mice were generalized lymphoblastomas and pulmonary adenomas. The incidence of lymphoblastomas was not associated with the feeding of Blue 2. There was a significant increase in the incidence of pulmonary adenomas in the lowest-dose treatment group in males compared to controls. That increase was not seen in females or in higher-dosage groups and, therefore, was not considered by the authors to pose a risk to humans. In this study

the NOAEL was determined to be 0.4% of the diet or approximately 600 mg/kg/day. With a safety factor of 100, that translates into an intake of about 360 mg/day for a 60 kg person (Hooson, Gaunt *et al.* 1975). This study cannot be considered definitive due, in part, to its brevity—Charles River CD-1 mice often live to well over 2 years (Huff, Jacobson *et al.* 2008)—and because the mice were not exposed *in utero*.

Borzelleca *et al.* also conducted a carcinogenicity/toxicity study of Blue 2 in mice. That study did not include an *in utero* phase. Blue 2 was fed to 60 Charles River CD-1 mice/sex in 0 (two control groups), 0.5, 1.5, and 5% groups. The study lasted 22 months for males and 23 months for females—longer than the Hooson study discussed above, but still shy of 2 years, let alone the lifetime of the mice. Investigators concluded that Blue 2 did not cause any significant effects on behavior, morbidity, mortality, hematology, or physical observation and considered the NOAEL to be 5%, or 8,259 and 9,456 mg/kg bw/day in male and female CD-1 mice, respectively (Borzelleca and Hogan 1985b).

Reproductive Toxicity and Teratogenicity

Borzelleca *et al.* conducted a 3-generation reproductive study of Blue 2 in Charles River CD rats. Groups of 10 males and 20 females were fed the dye at levels of 0, 2.5, 25, 75, or 250 mg/kg bw/day. Retinoic acid, a known teratogen in rats, was used as a positive control. Treated parents and pups were normal in terms of general appearance and behavior. The compound was not teratogenic and did not affect fertility, length of gestation, viability, or lactation indices. The compound did not cause anatomical abnormalities in the uteri or ovaries of females given caesarian sections. There were also no compound-related effects on organ weights and gross and microscopic pathological lesions (Borzelleca, Goldenthal *et al.* 1986).

Borzelleca *et al.* tested the potential teratogenicity of Blue 2 in Charles River CD rats and Dutch Belted rabbits. Twenty pregnant rats/group received 0.5% methacol (a vehicle control), 7.5 mg/kg/day retinoic acid (a positive control), or 25, 75, or 250 mg/kg/day Blue 2. Ten pregnant rabbits/group followed the same regimen as the rats, except that 150 mg/kg/day thalidomide was used as a positive control in place of retinoic acid. Investigators reported no compound-related adverse effects on maternal appearance, behavior, body weight, or mortality. There were also no adverse effects on fetal body weight, viability, or abnormalities. The NOAEL for Blue 2, on the basis of this study, was determined to be 250 mg/kg/day in rats and rabbits (Borzelleca, Goldenthal *et al.* 1987a).

Conclusions

The majority of intact Blue 2 is not absorbed by rats. However, the dye is readily broken down in the GI tract, and the final breakdown product, 5-sulfoanthranilic acid, is absorbed and excreted mostly in the urine. However, there are no metabolism studies in humans.

Blue 2 did not affect reproduction or cause birth defects in rats or rabbits. Two chronic toxicity/carcinogenicity studies in mice did not find any problems, but they were flawed because they did not include an *in utero* phase and were shorter than two years. More worrisome was a chronic toxicity/carcinogenicity study in rats that found that males in the 2% group had statistically significant increases in brain gliomas and malignant mammary gland tumors. The male rats also had dose-related increased incidences of transitional cell neoplasms of the urinary bladder, but the numbers of affected animals were small and the differences from the combined controls were not statistically significant. The FDA found reasons to excuse that evidence of carcinogenesis and neoplasia and approved the continued use of the dye.

Given the statistically significant occurrence of tumors, particularly brain gliomas, in male rats, Blue 2 cannot be considered safe for human consumption. Since Blue 2 is a non-nutritive food additive that does not provide any health benefit and there is hardly “convincing evidence” of safety, it should not be permitted for human consumption.

Citrus Red 2

Introduction

Citrus Red No. 2 (Fig. 6) is an azo dye approved to color the skins of Florida oranges not used for processing. Amounts are permitted up to 2 ppm in the whole fruit (FDA 1963). Only about 1,500 pounds of this dye are certified annually, but that's enough to color about two billion oranges.

Metabolism

Radomski *et al.* administered a single oral dose of Citrus Red 2 to rats, dogs, and rabbits. Rats given a single oral dose of 2-20 mg excreted 5-7% of intact dye in their feces over 48 hours. Similar to water-soluble azo dyes, this water-insoluble dye is broken down in the GI tract by intestinal bacteria. One breakdown product is 1-amino-2-naphthol, which has been shown to cause bladder cancer in mice (Bonser, Bradshaw *et al.* 1956). At single doses higher than 5 mg, the dye accumulated in the fat of rats. Small amounts of 1-amino-2-naphthyl sulfate were found in the urine of rats, demonstrating that the 1-amino-2-naphthyl metabolite is absorbed, sulfonated, and then excreted (Radomski 1961).

Chronic Toxicity/Carcinogenicity

In one study, 50 mice/sex/group were fed Citrus Red 2 at levels of 0, 0.01, 0.03, 0.1, 0.3, 1, or 3% of their diet. The study lasted up to 80 weeks, an inadequate duration. The study was discontinued in the 0.3, 1, and 3% groups due to increased morbidity and mortality. Mice in the 0.1% group also experienced increased mortality, and females showed degeneration of the liver (Sharratt, Frazer *et al.* 1966).

The same researchers conducted a study with 50 mice/sex injected subcutaneously with 10% Citrus Red 2 for 35 weeks, followed by injections every 3 weeks for 15 weeks. The control group received only vehicle injections. Female mice showed an increase in total malignant tumors, which appeared earlier than tumors in the control group. The most common malignant tumors were adenocarcinomas of the lung and lymphosarcomas. There were no injection-site tumors (Sharratt, Frazer *et al.* 1966).

Hazleton Laboratories conducted a chronic feeding study in rats. The toxicological data were evaluated by the director of FDA's Division of Pharmacology, A. J. Lehman, who concluded that the synthetic dye is a toxic substance. In this study, 40 rats/sex/group were fed Citrus Red 2 at doses of 0, 0.05, 0.1, 0.5, 1, 3, and 5%. Rats in the two highest dosage groups were sacrificed after 31 weeks because of severe toxicity. The remainder of the rats remained in the study for 104 weeks. Rats in the 0.5 and

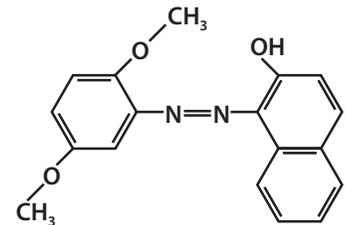


Figure 6. Citrus Red No. 2

1% groups experienced differences from controls in gross appearance, growth, organ weights, and gross and microscopic pathology. At the 0.1% levels, rats showed differences in organ weights, incidence of edema-like swelling, a possible trend toward an increased incidence of fatty metamorphosis (fat droplets in the cytoplasm of cells), and a significant difference in weight gain in females. Researchers did not report an increase in the occurrence of tumors. The NOEL was judged to be 0.05% (500 ppm) (Fitzhugh 1959).

Dacre administered Citrus Red 2 for 24 months to 20 mice and 20 albino rats per dosage group. The dye was given at dosages of 0, 0.05, and 0.25% beginning immediately after weaning, without *in utero* exposure. This study found hyperplasia (an increased number of cells, but not necessarily leading to a tumor) and a thickening of the urinary bladder wall in both treatment groups in rats and mice. Of greater concern, 2 out of 20 mice that were examined developed benign papillomas and one male mouse developed a malignant papilloma in the urinary bladder, and 4 out of 28 rats that were examined developed benign papillomas. About the same number of pathological changes were seen in the low- and high-dosage groups in both species and sexes. No problems were seen in control animals (Dacre 1965).

An internal FDA memo expressed concern about the carcinoma seen in Dacre's mouse study, because benign tumors and hyperplasia also were seen in the mice (Davis 1970). FDA veterinarian Kent J. Davis wrote, "...this becomes a level of meaningful significance to cancer research workers." He added,

Citrus Red 2 then becomes an intolerable human health hazard if only from the amounts consumed from fingers after peeling oranges treated with this dye. (Some additional dye may be ingested with peel or orange.) The continued certification and use of this color may also be a violation ...of the Federal Food, Drug, and Cosmetic Act as amended which prohibits use of any carcinogenic color additive for uses which *may* result in ingestion of *part* of such additive.

Conclusions

Citrus Red No. 2 is toxic to rats and mice at modest levels and, according to an FDA scientist and the IARC, is a bladder carcinogen (IARC 1975). The FAO/WHO Expert Committee on Food Additives stated bluntly: "This color should not be used as a food additive" (FAO/WHO 1969).

FD&C Green 3

Introduction

FD&C Green No. 3 (Fig. 7), or Fast Green FCF, is a synthetic dye approved for use in food, drugs, personal care products, and cosmetics except for in the area of the eye. It is one of the least-used dyes, but may be found in candies, beverages, dessert powders, ice cream, sorbet, and other foods, as well as ingested drugs, lipsticks, and externally applied cosmetics (FDA 1982a). The ADI for Green 3 is 2.5 mg/kg bw/day, or 75 mg/day for a 30-kg child (FDAd). Current production is equivalent to only 0.1 mg/person/day.

Metabolism

Hess and Fitzhugh studied the metabolism of Green 3 in rats and dogs. Three female and 3 male Osborne-Mendel rats were orally administered a single 200-mg dose of Green 3. An average of 94% of the dye was excreted intact in the feces. No recovery from the urine was reported. Male and female bile duct-cannulated dogs were orally administered a single 200-mg dose of Green 3. None of the color was found in the urine and about 2% of the dye was recovered in the bile of two of three dogs. Hess and Fitzhugh calculated the absorption of the dye from the GI tract of rats and dogs to be about 5% (Hess and Fitzhugh 1955).

Genotoxicity

Table 2 lists the number of negative and positive results for genotoxicity studies performed on Green 3, with Table A3 in the Appendix providing more details. The dye was not mutagenic in most assays except the *S. Typhimurium* strain TA100 Ames Assay at 10 mg/plate. That assay tests for base-pair mutations, and Green 3 only yielded positive results when tested as a mixture of several batches of dye of varying purity (Ishidate, Sofuni *et al.* 1984). Green 3 was also positive for mutagenicity in a Fischer rat embryo cell transformation assay (Price, Suk *et al.* 1978). That particular assay tests for malignant cell transformation, an indicator of carcinogenic potential. Green 3 was positive at 1 µg/ml but, surprisingly, produced negative results at higher concentrations. In summary, those tests do not raise significant concerns.

Chronic Toxicity/Carcinogenicity

In 1977, the FDA required that additional chronic toxicity studies be conducted before Green 3 could become a permanently listed food coloring (FDA 1977). To fulfill that requirement, the CCMA sponsored chronic feeding studies in mice and rats.

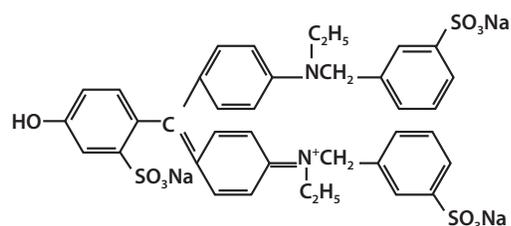


Figure 7. FD&C Green No. 3, Fast Green FCF

In the first study, Green 3 was administered to 60 Charles River albino rats/sex/group at dosage levels of 0 (two control groups), 1.25, 2.5, and 5% for at least 2 months prior to mating. After reproduction, 2, 3, or 4 pups/sex/litter/group were randomly selected for the long-term study. The same dosage levels used in the *in utero* phase were administered to 70 rats/sex/group for approximately 30 months. No significant effects were noted during the *in utero* phase except that pup mortality was increased in the mid- and high-dose groups of the F₁ generation. In the F₁ generation, a significant decrease in survivorship was seen in all treated groups of males and females, but there was no dose-response trend, making that decreased survivorship difficult to interpret. Urinalysis, hematologic parameters, physical observations, and ophthalmology did not indicate any adverse effects of Green 3 (Bio/dynamics 1982a).

Histopathological examination revealed that the high-dose group of male rats had increased incidences of urinary bladder transitional cell/urothelial neoplasms, testes Leydig's cell tumors (usually rare and benign in humans), and liver neoplastic nodules. Statistical analysis found that the increased incidences were significant for the urinary bladder transitional cell/urothelial neoplasms (p=0.04, BioDynamics analysis) and testes Leydig's cell tumors (p=0.04; FDA analysis), when compared to combined controls (Bio/dynamics 1982a). Mark Nicolich, a statistician working at the company that conducted the study, stated, "Therefore, there is statistical evidence that the high dose of the test material increases the occurrence of certain types of tumors in rats" (Bio/Dynamics 1981). Nevertheless, FDA scientists concluded that the tumors in the testes were not compound-related because (a) they are common in aged rats, and (b) the numbers of tumors in the low-dose and high-dose groups were comparable (though it is possible that the maximum rate of tumors occurred in the low-dose group). Regarding the urinary bladder neoplasms, the original report submitted by the petitioners stated that the high-dose male rats had a significantly increased incidence of those benign tumors. However, in the final submission, the petitioners submitted an addendum claiming, without any specific justification, lack of statistical significance. The FDA pathologists concluded that neither the incidence nor the severity of the transitional cell hyperplasia of the urinary bladder was treatment related (FDA 1982b).

In the CCMA-sponsored 24-month chronic toxicity/carcinogenicity study on Charles River CD-1 mice, 60 mice/sex/group were fed 0 (two control groups), 0.5, 1.5, or 5% Green 3 in their diet. The mice were not exposed to Green 3 *in utero*. No gross or microscopic neoplastic and non-neoplastic observations related to administration of the color were observed. Statistical analysis concluded that Green 3 did not have any negative effect on time-to-tumor, survivorship, or tumor incidence in mice (Bio/dynamics 1982b).

Conclusions

Green 3 did not increase tumor rates in CD-1 mice, though the only study did not include *in utero* exposure. Green 3 caused significant increases in bladder transitional cell/urothelial neoplasms and testes Leydig's tumors in high-dose male rats. Despite a last-minute assertion by the testing laboratory that the bladder neoplasms were no longer statistically significant and the FDA's dismissal (based on qualitative considerations, not statistical analysis) of the significance of the testes tumors, Green 3 must remain suspect until further testing demonstrates that it is safe.



Orange B

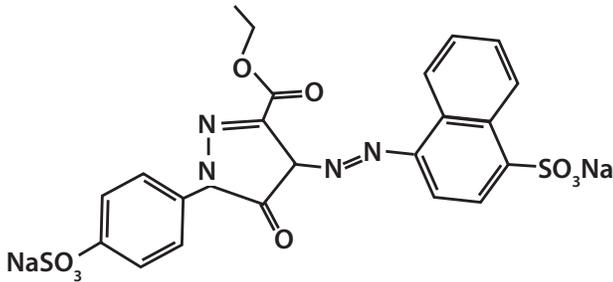


Figure 8. Orange B

Introduction

Orange B is an azo dye that is approved by the FDA for use only in frankfurter and sausage casings up to 150 ppm in the finished food (FDA 2008). Batches of Orange B have not been certified for use in the past decade or longer.

Metabolism

Orange B is poorly absorbed in rats. The color is reduced in the gut to form naphthionic acid. That metabolite appears in both the feces and the urine, indicating that some of the metabolite is absorbed (CCIC 1965).

Chronic Toxicity/Carcinogenicity

Orange B was fed to 50 Sprague-Dawley rats/sex/group at doses of 0, 0.5, 1, 2 or 5% for 2 years (an *in utero* phase was not conducted). By the end of the second year, all of the rats in the 2% and most in the remaining groups (including the control groups) were dead. Male and female rats in the two highest-dose groups showed lymphoid atrophy of the spleen and bile-duct proliferation. All examined animals in the highest-dose group experienced moderate chronic nephritis, but increased tumor rates were not reported. Investigators gave Orange B a NOAEL of 0.5% for rats (CCIC 1965).

Orange B was fed to 50 C₃H mice/sex/group and 50 C₅₇BR/cd mice/sex/group at doses of 0, 1, or 5% dietary supplement for their lifespans (an *in utero* phase was not conducted). There was no effect on tumor development or lifespan. The growth rate of the C₃H mouse was depressed in the 5% groups. Investigators assigned a NOEL of 1% to mice (CCIC 1965).

Groups of 3 beagles/sex/group were fed 0, 1, 2, or 5% Orange B as a dietary supplement in a chronic study. One dog/group was sacrificed at the end of 1 and 2 years, and the remaining dogs stayed in the study until the end of 7 years. The investigators assigned a NOEL of 1% to dogs (CCIC 1965), but the study used too few dogs for too short a time to provide meaningful information.

Conclusion

In 1978 the FDA proposed banning Orange B (Fed Reg. October 3, 1978), but, because companies stopped using it, the FDA never bothered to finalize the ban; it should do so now.

FD&C Red 3

Introduction

FD&C Red No. 3 (Fig. 9), or Erythrosine B, has been used as a food dye since its approval by the U.S. Department of Agriculture in 1907. It is a water-soluble dye with about a 58% iodine content (Lin and Brusick 1986). It is used in maraschino cherries, sausage casings, oral drugs, baked goods, and candies. The ADI for Red 3 is 2.5 mg/kg bw/day or 75 mg/day for a 30-kg child (FDAe). Annual production of Red 3 is equivalent to only about 1 mg/person/day.

Metabolism

Osborne-Mendel rats were administered 0.5-500 mg/kg bw Red 3 by stomach tube. Qualitative analysis demonstrated that the dye excreted in the urine or bile was unchanged (Webb, Fonda *et al.* 1962). In another study, 14 male rats were given one dose (0.5 mg/kg bw) of Red 3. Approximately, 55-72% was excreted unchanged in the feces within 3 days. In two bile-duct cannulated rats, 0.44 and 1.67% of the dye was excreted in the bile, indicating that a small amount is absorbed. No color was recovered in the urine. Investigators concluded that "Red 3 is metabolized to some extent in the tissue" (Daniel 1962). Rats administered Red 3 twice weekly for 3 months at doses (according to an industry petition) of 5, 10, 15, and 50 mg/200-250 gm bw had elevated serum levels of protein-bound and total iodine (Bowie, Wallace *et al.* 1966). Butterworth *et al.* also showed that rats administered Red 3 at 0-2% dietary doses over 13 weeks had a dose-related increase in serum levels of protein-bound and total iodine (Butterworth, Gaunt *et al.* 1976).

In a human study, subjects were orally administered 16 mg of Red 3 for 10 days (more than 15 times typical consumption). Subjects had about twice as much protein-bound iodine in their serum compared to levels prior to administration. Levels peaked around days 15-20 and did not return to normal until about 3 months after the beginning of the study (Anderson, Keiding *et al.* 1964).

In Vitro Effects on Neurotransmitters

Red 3 was applied to isolated frog neuromuscular synapses to test its effect on neurotransmitter release using electrophysiological techniques. Concentrations of 10 μ M and greater caused an irreversible, dose-dependent increase in acetylcholine release. Investigators concluded that Red 3 may alter the function of more complex systems, but any conclusions regarding its effects on mammalian behavior would be premature given the *in vitro* nature of the study (Augustine and Levitan 1980).

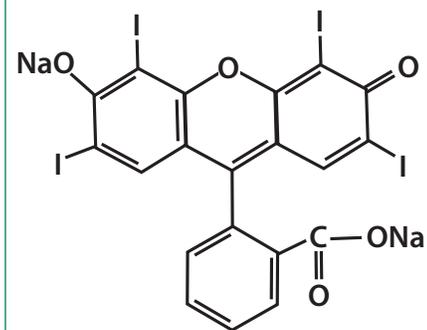


Figure 9. FD&C Red No. 3;
Erythrosine B

Genotoxicity

Table 2 lists the numbers of negative and positive results in *in vitro* and *in vivo* genotoxicity studies using Red 3. Although the majority of the tests were negative, several studies demonstrated the genotoxic potential of the dye (Ishidate, Sofuni *et al.* 1984; Matula and Downie 1984; Sasaki, Kawaguchi *et al.* 2002). Of particular concern is that the positive results were in studies using mammalian cells or an *in vivo* method (comet assay), while most of the negative results came from prokaryotic systems. Some of the key genotoxicity studies are summarized in Table A4 in the Appendix.

Chronic Toxicity/Carcinogenicity

Almost 40 years ago, Hansen *et al.* performed long-term toxicity studies on Red 3 on rats and dogs, but the numbers of animals in the study did not meet current FDA recommendations (FDA 2000). The rat study used 12 Osborne-Mendel weanling rats/sex/group that were fed diets with 0, 0.5, 1, 2, or 5% Red 3 for 2 years. No significant adverse effects were observed. In the same study, 18 rats were given weekly subcutaneous injections of Red 3 at an initial dose of 12 mg/rat (1 ml of a 2% dose that was reduced to 1.5%, then 0.75% over the 2-year period due to ulcerations in the animals) for 2 years. Other than injection site ulcerations, no significant adverse effects were observed (Hansen, Zwickey *et al.* 1973a). The dog study used only 3 dogs/sex/group.

Chronic toxicity studies focusing on the effects of Red 3 on hematology, thyroxine, and protein-bound iodide in Osborne-Mendel rats did not find any adverse effects. 25 rats/sex/group were fed 0 (the only group with 50 rats/sex), 0.5, 1, 2, or 4% Red 3 for 86 weeks or intubated twice weekly with 0, 100, 235, 750, or 1500 mg/kg Red 3 for 85 weeks. The study did not include an *in utero* phase. At the end of the treatment periods, the rats were fed the control diet until the studies reached the 2-year mark. The studies found no adverse effects in gross or microscopic pathology and no changes in thyroxine-iodide levels. The levels of protein-bound iodide increased, and it was determined that this was due to increased dye levels in the serum (Hansen, Davis *et al.* 1973b).

Borzelleca *et al.* performed a CCMA-sponsored chronic toxicity/carcinogenicity study in Charles River CD-1 mice. The maximum duration of exposure of the mice to 0 (two control groups), 0.3, 1, or 3% Red 3 was 24 months. Groups, including controls, consisted of 60 males and 60 females. Investigators reported no statistically significant compound-related effects on behavior, morbidity, mortality, hematology or general physical observations. There was a statistically significant increase in the incidence of lymphocytic lymphoma in male mice in the 0.3% low-dose group. However, that effect was not considered compound-related because there was no dose-response relationship, and the incidence of lymphomas in the high-dose group was similar to that in the controls. The NOAELs were deemed to be 3% (4,759 mg/kg/day) in males and 1% (1,834 mg/kg/day) in females (Borzelleca, Capen *et al.* 1987b).

Borzelleca *et al.* also performed two CCMA-sponsored chronic toxicity/carcinogenicity studies in Charles River CD rats. Unlike the mouse study (above), these studies included an *in utero* phase. In the F₀ generation of both studies, 60 rats/sex/group were fed 0 (two control groups), 0.1, 0.5, or 1% (original study) and 0 or 4% (high-dose study) Red 3. Random offspring were selected for the F₁ generation and 70 rats/sex/group were given the same dietary levels as the F₀ generation. The maximum exposure was 30 months. Investigators reported no compound-related effects on fertility, gestation, parturition, lactation, pup survival through weaning, or numbers of live and stillborn pups. Animals from the chronic feeding phase experienced several effects. The most notable were statistically significant increases in the incidences of thyroid follicular cell adenomas in male rats in the 4% treatment group (15 adenomas in the 4% group compared to 1 in the control groups) and non-significant increases in these tumors in female rats in the 0.5, 1, and 4% treatment groups. High-dose (4%) male rats also showed a statistically significant increase in non-neoplastic proliferative changes of the thyroid. The changes included follicular cell hypertrophy and hyperplasia and follicular cystic hyperplasia. Also, 94% of male rats in the 4% treatment group showed proliferative changes of thyroid follicular cells. Based on the results of the two studies, investigators asserted that Red 3 had NOAELs of 0.5 and 1% in male and female rats, respectively (Borzelleca, Capen *et al.* 1987b).

Reproductive Toxicity

Borzelleca and Hallagan conducted a 3-generation study on Red 3 in Sprague-Dawley rats. In each generation 25 rats/sex/group received 0, 0.25, 1, or 4% of the color in their chow. The only significant finding was a statistically significant reduction in body weights of parents and pups in all generations at the 4% dietary level. That could have been due to the large consumption of a non-nutritive compound. There were no compound-related adverse effects on reproductive indices and no gross anomalies. Investigators concluded that the NOAEL for rats was 0.25%, or approximately 149 and 255 mg/kg bw/day for males and females, respectively. That NOAEL was based on the reduced body weight in the 4% group and reduced body-weight gain during gestation in females in the 1 and 4% groups (Borzelleca and Hallagan 1990b).

Conclusions

Red 3 is genotoxic in *in vivo* and *in vitro* assays and is an animal carcinogen. The Red 3 petitioners attempted to prove that the color acts as a secondary carcinogen, a chemical that exerts its carcinogenicity via an indirect pathway, and, therefore, exempt from the Delaney Clause. However, the FDA concluded that Red 3 was not proven to be a secondary carcinogen in the industry-sponsored studies and found that “FD&C Red 3 is an animal carcinogen when administered in the diet.” In 1990, the FDA terminated the provisional listing of Red 3 for use in cosmetics and externally applied drugs; all uses of Red 3 lakes were also banned (FDA 1990).

Notwithstanding its 1990 finding that Red 3 is an animal carcinogen, the agency still permits Red 3 in ingested drugs and foods, though in 1990 it was reported to have said it would “take steps” to ban those uses, too (McLaughlin April 22, 1990). About 200,000 pounds of the dye are used annually. Red 3 is allowed for those uses because petitioners had submitted CCMA-sponsored studies after the 1960 provisional listings that showed no safety concern, and in 1969 the FDA permanently approved the dye for use in ingested drugs and foods (Blumenthal 1990). In 1984, FDA’s Acting Commissioner, Mark Novitch, said that Red 3 was “of greatest public health concern... The agency should not knowingly allow continued exposure (at high levels in the case of FD&C Red No. 3) of the public to a provisionally listed color additive that has clearly been shown to induce cancer while questions of mechanism are explored” (Burros February 13, 1985). However, around the same time, Secretary of Agriculture John R. Block was pressing his counterpart at the Department of Health and Human Services not to ban the dye (Food Chemical News May 28, 1984). He wrote, “Some segments of the agricultural community are quite dependent on Red Dye #3 in the processing and marketing of certain commodities, especially canned fruits. I have assured the affected industry that their concerns would be made known to you, as well as my own concern...” And in 1989, Congress, at the behest of growers and packers, temporarily prohibited the FDA from banning the dye (Washington Post July 19, 1989). Twenty-six years later, the FDA still has not acted.

The harm that Red 3, an acknowledged animal carcinogen, is likely causing far outweighs the minor nuisance entailed in banning the dye. It is worth noting that Red 3 has been seen as invaluable by some makers of maraschino cherries, but other brands are dyed with Red 40 or (shockingly) *no* added coloring, and some brands (Del Monte, Giant) of canned fruit cocktail contain cherries colored with natural colorings (unfortunately, the natural colorings used, carmine or cochineal extract, can cause severe allergic reactions). The food industry and public would survive a ban quite easily.

Another means of protecting the public would be through California’s law that requires warning notices on products that contain chemicals that the state’s Office of Environmental Health Hazard Assessment (OEHHA) has determined pose a certain degree of cancer risk: In 1999, the California Environmental Protection Agency concluded that while one rat study was positive for tumors and others were not, “There is a *HIGH* level of *concern over the extent of exposure* to C.I. acid red 51 [Red 3], since it is used as a dye in food, drugs and cosmetics and is likely to be consumed by the general population.” [emphasis in original] The risk posed by Red 3 might exceed OEHHA’s “safe” level (which is less strict than the FDA’s level), and California could require products that contain Red 3 to bear a cancer warning notice—though most companies likely would switch to a safer coloring. However, California considered Red 3 a lower concern than other carcinogens and has not taken any action (California OEHHA).



FD&C Red 40

Introduction

Red 40 is the FDA-approved version of Allura Red, which was first produced by Allied Chemical Corp. It is approved for use in beverages, bakery goods, dessert powders, candies, cereals, foods, drugs, and cosmetics and, in terms of pounds consumed, is by far the most-used dye. Red 40 has an ADI of 7 mg/kg bw/day (FDAf). That ADI translates into 210 mg for a 30-kg child. Companies produce the equivalent of about 25 mg of the dye per person per day, with many children probably averaging several times as much.

Metabolism

In an unpublished report, rats were fed 5.19% of the dye in their diets (White 1970). 29% of the intact dye was excreted in the feces while only 0.1% was excreted in the urine. The parent dye appears to be broken down by gut flora via azo-reduction into two metabolites, cresidine-4-sulfonic acid and 1-amino-2-naphthol-6-sulfonic acid. In another study, rats and dogs were pretreated daily with unlabeled Red 40 followed by ³⁵S-Red 40 for up to 72 hours. Within 72 hours, 92-95% and 76-92% of the radioactivity was recovered in the feces of dogs and rats, respectively. Radioactivity in the urine accounted for only 5.7-19.8% and 2.7-3.6% of the total dose in dogs and rats, respectively. There was significant retention of radioactivity in the guts of sacrificed animals (White 1970).

Genotoxicity

Table 2 lists the numbers of negative and positive results for genotoxicity studies performed on Red 40. Red 40 was negative in the majority of genotoxicity assays performed, but positive in the *in vivo* comet assay in the glandular stomach, lungs, and colon of mice (Sasaki, Kawaguchi *et al.* 2002). That indicates that Red 40 can cause DNA damage *in vivo*. Details of the genotoxicity assays are provided in Table A5 of the Appendix.

Hypersensitivity

52 patients suffering from urticaria and angiodema for more than 4 weeks were placed on a 3-week elimination diet. Red 40 administered orally in doses of 1 or 10 mg induced a hypersensitivity reaction in 15% of the patients who were generally symptom-free at the time of provocation (Mikkelsen, Larson *et al.* 1978).

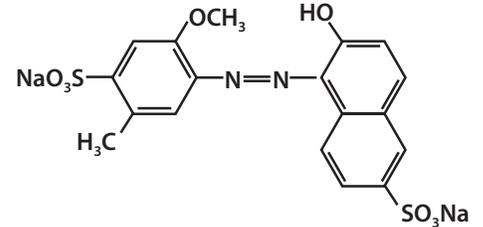


Figure 10. FD&C Red No. 40;
Allura Red

Chronic Toxicity/Carcinogenicity

In the 1970s, Hazleton Laboratories conducted chronic toxicity/carcinogenicity feeding studies in rats and mice, both of which included an *in utero* phase. Using Sprague-Dawley rats, the F₀ generation included 30 rats/sex/group that were administered 0, 0.37, 1.39, and 5.19% of Red 40 in their chow one week prior to mating, during mating, gestation, and lactation. The test of F₁ rats involved 50 rats/sex/group created by choosing random surviving offspring. The F₀ and F₁ generations were exposed to the same dosage levels. Males and females were exposed for 118 and 121 weeks, respectively. No compound-related effects were reported during the gestation and lactation periods. With the exception of a statistically significant decrease in body weight in high-dose females, investigators reported no consistent adverse effects due to Red 40. They established a NOAEL of 5.19% (2,829 mg/kg/day) for males and 1.39% (901 mg/kg/day) for females (Borzelleca, Olson *et al.* 1989).

Hazleton Laboratories also performed two chronic toxicity studies in CD-1 mice. In the first study, 50 mice/sex/group (F₀) were administered 0, 0.37, 1.39, or 5.19% Red 40 in their chow one week prior to breeding through the gestation and lactation periods. The F₁ generation was randomly selected from surviving pups and the chronic feeding study used 50 mice/sex/group. The dosages were the same in the F₀ and F₁ generations. At 42 weeks, a total of 6 reticuloendothelial (RE) tumors occurred in the males and females (0 in controls, 1 each in the low- and mid-dose groups, and 4 in the high-dose groups). (The reticuloendothelial system is a part of the immune system.) That led the investigators to sacrifice and examine 36% of the animals, reducing each group to 30 mice/sex/group. The remaining smaller groups of F₁ mice were fed Red 40 for a total of 104 weeks. By the end of the study, the investigators concluded that Red 40 did not cause acceleration of the appearance of RE tumors (Borzelleca, Olson *et al.* 1991). However, Dr. M. Adrian Gross, a senior FDA pathologist, concluded that there was clear evidence to support an acceleration effect of RE tumors because there was a decreased latency period without a corresponding increase in overall tumor incidence.

A second mouse study was conducted to address the possibility suggested by the first study that Red 40 accelerated the appearance of RE tumors, a sign of carcinogenicity (Fed. Reg. May 22, 1984). Although the second study used the same dosage groups as the first, the studies differed in several respects. First, the initial study used Ham/ICR (CD-1) mice, while the second used CD-1 outbred mice. Second, the F₀ generation in the second study used 70 mice/sex/group, and the F₁ generation consisted of 100 mice/sex/group. Third, the second study did not include a 42-week interim sacrifice. Fourth, the second study used two control groups instead of one. Finally, the mice in the second study were exposed to Red 40 for 109 weeks—five weeks longer than the first study.

The second study, according to the investigators, did not show an early appearance of or increase in RE tumors. Only the high-dose males and females experienced a signifi-

cant increase in relative and absolute thyroid weight. The investigators set a NOAEL of 5.19% in mice or 7,300 and 8,300 mg/kg/day for males and females, respectively (Borzelleca, Olson *et al.* 1991).

Limitations of the Mouse Studies

There were a number of problems with the chronic toxicity studies on Red 40. The first mouse study suggested a reduced latency period for RE tumors without a final increased incidence. Also, Hazleton Laboratories found small numbers of RE-system tumors in all treatment groups prior to the 42-week sacrifice, the highest incidence being in the high-dose group. At the time, the FDA recommended killing 36% of the mice to gain information about Red 40's ability to accelerate the occurrence of RE tumors. The sacrifices were done at week 42 of the 2-year study. However, that left a relatively small number of mice available at the end of the study and reduced the ability to analyze tumor incidence (Lagakos and Mosteller 1981).

Because of the controversial early results in the first mouse chronic-feeding study, in 1976 the FDA created a working group of scientists from the FDA, National Cancer Institute, and the National Center for Toxicological Research to monitor the rat and mouse studies being performed for Allied Chemical. Midway through the second mouse study, the working group concluded that the first study did not indicate a risk of carcinogenesis. Following controversy over that conclusion, FDA Commissioner Donald Kennedy appointed four non-governmental statisticians, including Harvard's Frederick Mosteller and Stephen Lagakos, to review the statistical methods used to analyze the mouse studies. Those statisticians were independent and not a part of the FDA working group.

Two problems found with the mouse studies included caging and litter effects (Lagakos and Mosteller 1981). Mice housed in the upper row of racks experienced a higher incidence of RE tumors than the mice in lower cages, according to the FDA consultants (Lagakos and Mosteller, 1979). That might have been because cages in both of the mouse studies were not rotated. The incidence of RE tumors was significantly correlated to the row ($p=0.0005$) and position ($p=0.02$) of the racks (Lagakos and Mosteller 1979). The working group also noted that it was impossible to know if mice were being housed with siblings (litter effect), which might have had an influence on tumor incidence (Group 1981). Confounders such as potential caging and litter effects strongly decrease the credibility of a study.

A concern regarding the second mouse experiment was the large variation in RE tumor rates compared to the first experiment, though that might have been because different mouse strains were used. Another sign that the second study could not produce definitive results is that the difference in RE death rates between the two control groups was highly statistically significant at the $p=0.008$ level (Lagakos and Mosteller 1981).

Regarding the statistical analyses of the two mouse studies, Lagakos and Mosteller commented that the difference in RE tumor rates between the two studies limited the conclusiveness of the results. They argued that the statistical methods used by the FDA Working Group were not oriented to detecting an acceleration effect (decreased latency in tumor induction) (Lagakos and Mosteller 1981). Their analysis concluded that both studies suggested a decreased latency period for, and increased incidence of, RE tumors (Lagakos and Mosteller 1979).

Carcinogenic contaminants

As discussed below with regard to Yellow 5 and Yellow 6, Red 40 might contain cancer-causing and other contaminants. Health Canada scientists, using a test method that could detect bound and free contaminants, identified small amounts of aniline, *p*-cresidine, and 1-naphthylamine in the dye (Lancaster and Lawrence 1991). The *p*-cresidine is “reasonably anticipated to be a human carcinogen,” according to the U.S. National Toxicology Program, and “possibly carcinogenic to humans,” according to the International Agency for Research on Cancer (IARC; NTP). FDA considered aniline to be weakly carcinogenic to rats (FDA 1985), though other agencies have not determined that aniline— and 1-naphthylamine—pose a risk to humans (FDA 1985; IARC).

Reproductive Toxicity/Teratogenicity

FDA scientists investigated the potential embryotoxicity and teratogenicity of Red 40. Pregnant female rats were dosed with 0, 7.5, 15, 30, 100, or 200 mg Red 40 /kg bw daily on days 0-19 of gestation through intubation or 0 or 2 mg Red 40/kg bw daily through drinking water on days 0-20 of gestation. No negative effects on maternal reproduction, embryoletality, or fetotoxicity were observed (Collins and Black 1980).

Conclusions

There is evidence, albeit controversial and inconclusive, that Red 40, the most widely used dye, accelerates the appearance of tumors of the reticuloendothelial system in mice. Also, outside consultants appointed by the FDA raised concerns about an FDA-appointed Working Group’s statistical analysis of the data. Considering the lack of published metabolism data, the positive results in comet assays, the disputed mouse studies, causation of hypersensitivity reactions, possible causation of hyperactivity in children, and the non-essentiality of the dye, Red 40 should not be used in foods.

FD&C Yellow 5

Introduction

FD&C Yellow No. 5 (Fig. 11), also known as Tartrazine, is used in numerous bakery goods, beverages, dessert powders, candies, cereals, gelatin desserts, pet food, and many other foods, as well as pharmaceuticals, and cosmetics. After Red 40, it is the most widely used dye. The ADI for Yellow 5 is 5 mg/kg/day (FDAg), which equates to 150 mg/day for a 30-kg child. Companies produce the equivalent of 15 mg of the dye per person per day, with many children consuming at least several times that much.

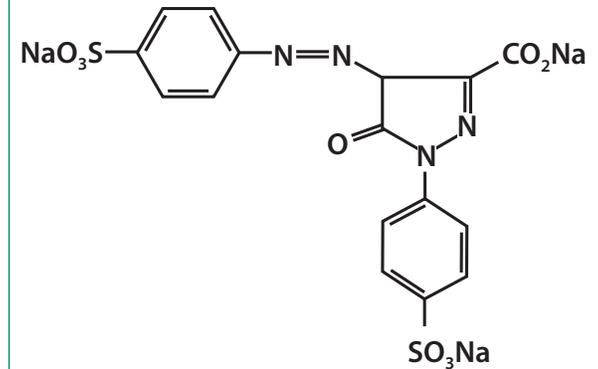


Figure 11. FD&C Yellow No. 5; Tartrazine

Metabolism and Metabolic Effects

Sulfanilic acid is a metabolite that results from the reduction of Yellow 5 at the N=N azo link. However, when Yellow 5 labeled at the phenylazo group with ^{14}C was administered intraperitoneally in rats and rabbits, no radioactive sulfanilic acid was recovered in the urine (Jones, Ryan *et al.* 1964). In the same study, when Yellow 5 was administered orally to rats, rabbits, and humans, sulfanilic acid, but little or no unchanged dye, was recovered in the urine. These results indicate that the reduction of Yellow 5 occurs via the GI flora. That is why sulfanilic acid is excreted in the urine when Yellow 5 is administered orally but not intraperitoneally. Ryan *et al.* confirmed that Yellow 5 is primarily metabolized in the guts of rats after an oral dose, where the gut microflora perform the majority of the compound's degradation (Ryan, Welling *et al.* 1969).

Apart from the metabolism of the dye, a 50-mg dose of Tartrazine led to increased or accelerated urinary excretion of zinc in hyperactive children. Whether the effect on zinc is a cause of hyperactivity is not known. Amaranth (formerly FD&C Red No. 2) had no effect (Ward 1996).

Genotoxicity

Yellow 5 caused genotoxic effects in six out of 11 studies (see Table 2 above and Table A6 in the Appendix). A 1985 report from the U.S. Department of Health and Human Services criticized two of the genotoxicity studies (Patterson and Butler 1982; Ishidate, Sofuni *et al.* 1984) and disagreed with their conclusions that Yellow 5 induces chromosomal aberrations (Flamm, Jackson *et al.* 1985). The HHS report stated, though, "If chromosome aberrations of the type reported for Tartrazine in cultured cells occurred *in vivo*, they certainly would represent a serious adverse effect." In fact, Sasaki *et al.* subsequently demonstrated that Yellow 5 does induce DNA damage *in vivo* in the

comet assay (Sasaki, Kawaguchi *et al.* 2002). At the very least, the numerous positive genotoxicity results indicate the need for further investigation.

Chronic Feeding/Carcinogenicity

The earliest chronic feeding study reported that Yellow 5 was not carcinogenic or toxic in a 2-year study using Osborne-Mendel weanling rats.¹¹ The rats were fed 0, 0.5, 1, 2, and 5% Yellow 5. However, that study used only 12 rats of each sex per dosage group (Davis, Fitzhugh *et al.* 1964). The FDA recommends a minimum of 20 rodents/sex/group for chronic toxicity studies, though many experts consider that far too small a number (FDA 2000). Also, the rats were not exposed *in utero*.

Later, Borzelleca and Hallagan also found that Charles River CD rats fed Yellow 5 did not show carcinogenic or toxic effects. In a well-designed study sponsored by CCMA, 70 rats/sex/group were exposed to 0, 0.1, 1, 2, or 5% Yellow 5 starting *in utero* for 30 months or until only 10 rats/sex/group survived (Borzelleca and Hallagan 1988a). The researchers did not find any compound-related effects on fertility, gestation, parturition, lactation, pup survival, or number of still-born pups. Investigators looked at the

hematology, clinical chemistry, and urine of 10 rats/sex at six time points. They also performed complete histopathology on 10 rats/sex/group at an interim sacrifice at 12 months, as recommended by the FDA (FDA 2000). Complete histopathology was performed on all sacrificed animals, and gross necropsies were conducted on animals that died spontaneously. No problems were seen. This group reported a NOAEL of 5% for both male and female rats.

Borzelleca and Hallagan also performed a chronic toxicity/carcinogenicity study in CD-1 mice (Borzelleca and Hallagan 1988b). Groups of 60 males and 60 females were fed 0 (two control groups), 0.5, 1.5, or 5% Yellow 5 for 104 weeks. The protocol for this study was similar to Borzelleca and Hallagan's rat study, but there was no mention of an interim necropsy of 10 mice/sex/group, as recommended by the FDA. Also, the mice were

not exposed *in utero*, but were 42 days old at the start of the study—a serious drawback, because infant animals are likely to be more susceptible to toxic or carcinogenic effects than older animals. The investigators claimed that a sufficient number of mice

Table 3. Mouse survival at termination of a 24-month study

Dose Level (%)	Survival*	
0 (control 1)	30/50	Males
0 (control 2)	28/60	
0.5	31/60	
1.5	21/60	
5.0	29/60	
0 (control 1)	20/60	Females
0 (control 2)	24/60	
0.5	18/60	
1.5	24/60	
5.0	33/60	

*No. surviving at termination of study/no. at initiation; boldface indicates inadequate numbers of mice surviving.

(Borzelleca and Hallagan 1988b)

11. Davis *et al.* also tested 3 groups of two male and two female beagles for two years at dosages of 0, 1, and 2% Yellow 5, but that small number of dogs and the brevity of the test do not permit conclusions about the long-term effects of the dye.

survived until the end of the study (24 months), however, half of the groups did not meet the FDA recommendation that in a carcinogenicity study at least 25 mice/sex/group should survive until study termination (see boldface numbers in Table 3). In any case, the investigators did not find any significant compound-related effects. The NOAEL for this study was 5% for male and female mice (indeed, the lack of any effect at the highest dosage level indicates that a higher dosage should have been used).

Carcinogenic contaminants

Yellow 5 may be contaminated with several carcinogens, including benzidine and 4-aminobiphenyl. The FDA limits free benzidine to 1 part per billion (ppb), though analytical methods can only detect 5 ppb. More importantly, FDA tests in the early 1990s found that some batches of dye contained as much as 83 ppb of free and bound benzidine, with the latter probably being liberated in the GI tract (Prival, Peiperl *et al.* 1993). As noted on page 3, the FDA does not test for bound benzidine when it certifies the purity of dyes. The FDA's 1985 risk assessment (using projections for 1990 consumption levels) calculated a risk for Yellow 5 of 4 cancers in 10 million people, which is slightly smaller than the "concern" level of 1 in 1 million (FDA 1985). However, that risk assessment failed to consider the (a) greater sensitivity of children to carcinogens (FQPA), (b) greater consumption of Yellow 5 by children than the general population, (c) substantial increase in per capita consumption of Yellow 5 since 1990, (d) possibility that some batches of dye contain large amounts of bound benzidine and other carcinogenic contaminants, and (e) the presence of similar contaminants in Yellow 6. FDA scientists found that one company eliminated benzidine contamination in 1992, suggesting that other companies could do (or might have done) the same (Peiperl, Prival *et al.* 1995). However, with more chemicals being imported from China, India, and other countries, it is important that dyes routinely be tested for bound contaminants.

Hypersensitivity

The one generally accepted concern about Yellow 5 is its hypersensitivity effects. In the 1970s, several cases of Tartrazine-sensitivity were reported, most frequently in the form of urticaria (hives) and asthma (Dipalma 1990). Neuman *et al.* reported that 26% of patients with a variety of allergic disorders had a positive allergic reaction 10-15 minutes after ingesting 50 mg of the dye. Those reactions included heat-wave, general weakness, blurred vision, increased nasopharyngeal secretions, a feeling of suffocation, palpitations, pruritus (severe itching), angioedema (swelling or welts below the skin), and urticaria (Neuman, Elian *et al.* 1978). An association between aspirin-intolerance and Tartrazine-sensitivity has been demonstrated in several studies. Stenius and Lemola separately administered aspirin and Yellow 5 to 96 patients and found that about half of the patients with positive reactions to aspirin also had positive reactions to Yellow 5, and about three-fifths of the positive Yellow 5 cases also

had positive aspirin reactions (Stenius and Lemola 1976). In a double-blind crossover study Settignano *et al.* found that 0.22 mg of Yellow 5 (much less than is used in most dyed foods) caused a positive reaction in 8% of patients with chronic urticaria and 20% of patients with aspirin intolerance (Settignano, Chafee *et al.* 1976).

These examples indicate the range and potential severity of hypersensitivity reactions to Yellow 5:

- A 42-year-old woman gradually developed chronic nasal blockage, loss of her senses of taste and smell, and asthma. She was eventually hospitalized—sometimes for weeks—three times for her asthma, which developed into a tight, unproductive cough and severe wheezing. Several years later she developed severe angioedema after taking two aspirin tablets. She also developed the same attacks from Tylenol and several other drugs, including antibiotics. She was cleared of all drugs, but her symptoms returned after she received Premarin, a menopausal drug that contained Yellow 5. The patient was finally diagnosed with an allergy to Yellow 5 and several other food additives. Her severe attacks were relieved when she stopped consuming all synthetic dyes, sodium benzoate, and drugs containing dyes (Chafee and Settignano 1967).
- A 15-year-old pregnant girl went into anaphylactic shock after she was given an enema that contained Yellow 5 and Yellow 6. Approximately 5 minutes after administration of the enema the patient became dizzy, sweaty, and hypotensive; she collapsed and was unconscious. Her blood pressure became unrecordable and her carotid pulses were “weak.” Her skin became red all over. After she regained consciousness, she was nauseous, had dull perception, and eventually developed hives, chest tightness, and shortness of breath (Trautlein and Mann 1978). Subsequent tests indicated that she was sensitive to both Yellow 5 and Yellow 6.
- A 38-year-old man experienced relapsing angioneurotic (subcutaneous) edema, giant urticaria, and a relapsing vascular purpura (purple spots). After provocation with Yellow 5, the test area became purpuric and there was purpura, swelling of the legs, and angioneurotic edema of the face (Michaelsson, Pettersson *et al.* 1974).
- A 32-year-old woman experienced recurring purpuric lesions on her lower legs. The lesions sometimes became more intense with ulcerations, pain, and swelling of the legs. She had occasional superficial thrombophlebitis (swelling of a vein caused by a blood clot). She experienced those symptoms 4-8 times a year, and they lasted about 2-3 weeks. Provocation with Yellow 5 induced purpura in the treated area (Michaelsson, Pettersson *et al.* 1974).

It was because of reactions like those that in 1986 the Joint Council of Allergy and Immunology told the FDA that just listing Yellow 5 on the label was not protective enough because reactions could be life-threatening. “Since many reactions are sudden and occur without warning,” the group urged the agency to ban Yellow 5. The joint council was established by two of the major medical organizations concerned about allergies.

Conclusions

Six out of 11 mutagenicity studies indicated potential problems, but Yellow 5 did not appear to be carcinogenic in rats. The chronic feeding study in mice was inadequate and cannot be used to support the dye’s safety. In addition, Yellow 5 may be contaminated with significant levels of carcinogens. On another front, Tartrazine (the only dye to be tested on its own, instead of in mixtures) has caused hyperactivity in children (Rowe 1988; Rowe and Rowe 1994). Yellow 5 can cause mild to severe hypersensitivity reactions. Since Yellow 5 poses some risks, has not been adequately tested in mice, and is a cosmetic ingredient that serves no nutritional or safety purpose, it should not be allowed in the food supply.

FD&C Yellow 6

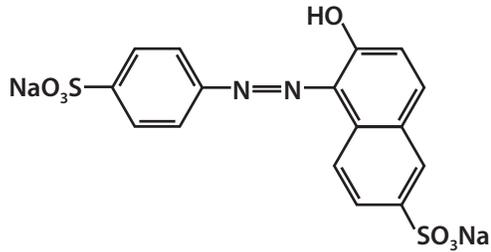


Figure 12. FD&C Yellow No. 6; Sunset Yellow FCF

Introduction

FD&C Yellow No. 6 (Fig. 13), the FDA-approved form of Sunset Yellow, is a water-soluble sulfonated azo dye that is used to color bakery goods, cereals, beverages, dessert powders, candies, gelatin desserts, sausage, and numerous other foods, as well as cosmetics and drugs. Yellow 6 has an ADI of 3.75 mg/kg bw/day, or 112.5 mg for a 30-kg child (FDAg). Current average per capita production of Yellow 6 is equivalent to about 14 mg/day, making it the third most widely used dye. Considering that the FDA estimates that an average “high user” consumes about five times as much dye as an average user over their lifetimes, some children may be consuming amounts above the ADI (FDA 1986).

Metabolism and Metabolic Effects

Several metabolites were found in the urine of rabbits given a single 0.5 mg/kg oral dose of Yellow 6. Yellow 6 is reduced at the azo linkage primarily in the gut by intestinal microflora to produce sulfanilic acid and 1-amino-2-naphthol-6-sulfonic acid, as well as the n-acetylated form of sulfanilic acid, p-acetamidobenzene-sulfonic acid. Intact Yellow 6 in the feces accounted for only about 2% of the dose (Daniel 1962). Those findings were confirmed by Honohan *et al.* who dosed 5 rats with 2.7 mg of ¹⁴C-Yellow 6 orally and found only 1-2% of the dose in the form of intact dye in the feces after 24 hours (Honohan, Enderlin *et al.* 1977). In another rat study, after a single oral dose of 100 mg, only 0.8% of intact dye was excreted in the feces, with the rest being the metabolites indicated above. Only 3.6% of the intact dye was absorbed by rats administered 50 mg of Yellow 6 orally (Radomski and Mellinger 1962).

Apart from the metabolism of the dye, a 50-mg dose of Sunset Yellow (like Tartrazine) led to increased or accelerated urinary excretion of zinc in hyperactive children. Whether the effect on zinc is a cause of hyperactivity is not known (Ward 1996).

Genotoxicity

Although Yellow 6 was negative in six genotoxicity assays, it did induce forward mutations and chromosome aberrations in two assays (McGregor, Brown *et al.* 1988; Hayashi, Matsui *et al.* 2000). As shown in Table A7 in the Appendix, Yellow 6 did not induce DNA damage in a comet assay or cause frameshift, base pair, or forward mutations; chromosomal aberrations; or cause mitotic gene conversion.

Chronic Toxicity/Carcinogenicity

The National Toxicology Program (NTP) conducted carcinogenesis studies using 50 animals/sex/group in F344 rats and B6C3F1 mice. Each group was fed a diet containing 0, 12,500, or 25,000 ppm Yellow 6 for 103 weeks. The control groups consisted of 90 rats or 50 mice of each sex. There was no *in utero* exposure in either study, a significant weakness. The rat study did not find any statistically significant color-related neoplastic or non-neoplastic lesions in any of the groups. Low-dose, but not high-dose, male mice had a significantly higher incidence of hepatocellular carcinomas and adenomas compared to controls. Partly because of the lack of a dose response relationship in the mice, the investigators concluded that Yellow 6 was not carcinogenic (NTP 1981).

In 1982, Bio/dynamics Inc., under contract to CCMA, conducted two multi-generation long-term feeding studies in Charles River Sprague-Dawley rats at doses of 0 (two control groups), 0.75, 1.5, and 3% in the first study and 0 (one control group), 0.75, 1.5, and 5% in the second study. The first study was conducted for 30 and 28.5 months for males and females, respectively, and the second study lasted for 25.6 and 27.8 months for males and females, respectively. In the F₁ generation, females in the 3% group in the first study and males in the 5% group in the second study had increased mortality. In the second study, interim sacrifice of some rats at 12 months revealed an increase in the mean absolute and relative kidney weights in females of the 5% group. At terminal sacrifice of both studies, there was an increase in mean absolute and relative kidney weights in females in the 3% groups and 5% groups, as well as an increase in the mean relative and absolute thyroid weights in males and females in the 5% groups. Females in the 3% group and both males and females in the 5% groups had statistically significant increased incidences of adrenal medullary adenomas compared to controls. Also, males in the 3% group had an increased incidence of testicular interstitial cell adenomas compared to pooled controls. Notwithstanding those findings, the investigators concluded that the studies did not find any evidence of carcinogenicity (Bio/dynamics 1982c).

After examining the results of the Bio/dynamics study, the FDA concluded that the increased incidence of the tumors was not related to Yellow 6 because of the (a) lack of dose-response in the 3% and 5% dosage groups (though that is comparing two different studies), (b) likelihood of false positives, (c) lack of precancerous lesions, (d) similar morphology of adrenal medullary lesions in control and treated animals, (e) lack of a difference in the latency periods before tumors occurred, (f) fact that the tumors seen are common spontaneous tumors in older rats, and (g) lack of other studies finding an association between Yellow 6 and this type of tumor (FDA 1986).

Bio/dynamics, again under contract to CCMA, performed a chronic toxicity/carcinogenicity study in Charles River CD-1 COBS mice, with 60 mice per sex per group. The study used dosages of 0 (two control groups), 0.5, 1.5, and 5% Yellow 6 in the

animals' chow. The study was terminated at only 20 months for the males and 23 months for the females (no *in utero* phase was conducted). Males in the 5% group had significantly higher mortality compared to controls, but that is not relevant to people, who consume far lower amounts of the dye. The laboratory concluded that the study did not indicate any concern about carcinogenicity in mice (Bio/dynamics 1982d).¹²

In the 1960s, the FDA completed a seven-year feeding study on a small number of beagle dogs. Such studies are rarely large or long enough to detect carcinogenicity, and this one was no exception. However, Kent J. Davis, an FDA veterinarian, attributed “tears, eye lid encrustations, pannus [corneal inflammation], and corneal opacity approaching blindness” to ingestion of Yellow 6 (Davis July 9, 1970). He concluded that, because of the eye lesions, “it is apparent that immediate decertification of this color is necessary in order to protect the public health at the recommended level of present safety standards.”

Carcinogenic contaminants

Yellow 6 may be contaminated with several carcinogens, including benzidine and 4-aminobiphenyl. The FDA set a limit of 1 part per billion (ppb) of free benzidine, but some batches of dye have contained a hundred or even a thousand times as much bound benzidine, which is likely liberated in the colon (Peiperl, Prival *et al.* 1995). As reported on page 3, the FDA does not test for bound benzidine. The FDA's 1986 risk assessment (using estimates for 1990 consumption levels) estimated a risk of 3 cancers in 10 million people, which is smaller than the official “concern” level of 1 in 1 million (FDA 1986). However, that assessment failed to consider the (a) greater sensitivity of children, (b) greater consumption of Yellow 6 by children than the general population, (c) substantial increase in per capita consumption of Yellow 6 since 1990, (d) possibility that some batches of dye contain bound forms of benzidine and other contaminants (FQPA), and (e) presence of similar contaminants in Yellow 5. FDA scientists found that in 1992 one company eliminated benzidine contamination of Yellow 5, suggesting that other companies could do the same for Yellow 6 (Peiperl, Prival *et al.* 1995). However, a Health Canada study found that Sunset Yellow FCF (Yellow 6 in the United States) was still contaminated in 1998 (Lancaster and Lawrence 1999). With more and more chemicals being imported, it is important that dyes routinely be tested for bound contaminants.

Hypersensitivity

Human hypersensitivity to Yellow 6 was reported as early as 1949 (Baer and Leider 1949). Since then, several cases, such as the following, of hypersensitivity to the color have been reported:

12. In the 1960s, the FDA published a 7-year study in which five female beagles were fed Yellow 6 at 2% in their diet. No abnormalities were reported, but a study involving so few animals, and only females, cannot be considered adequately sensitive FDA (1964).

- A 15-year-old pregnant girl experienced anaphylactic shock after receiving an enema that contained Yellow 5 and Yellow 6. The patient was tested via the skin-prick technique for sensitivity to all of the soluble components in the enema. Positive results were observed for both Yellow 5 and Yellow 6 (Trautlein and Mann 1978).
- A 43-year-old physician was hospitalized for stomach cramps four times over a two-year period. Double-blind tests confirmed that the cramps were caused by a hypersensitivity to Yellow 6 (Gross, Lance *et al.* 1989).
- A 53-year-old woman visited the doctor for severe skin lesions. Two days after receiving treatment she was hospitalized for distaste for food, as well as indigestion, retching, belching, severe abdominal pain, and vomiting. When the drugs (administered orally) were discontinued, the symptoms subsided, and when the drugs were administered again the symptoms reappeared. A challenge test confirmed that Yellow 6 was the causative agent (Jenkins, Michelson *et al.* 1982).

A study by Michaelsson and Juhlin involved 52 patients with, and a control group of 33 patients without, recurrent urticaria (hives). All subjects were put on a dye-free diet and were free of antihistamines prior to administration of the possible allergen. The researchers tested the effects of several food dyes (including Yellow 6) and preservatives, as well as aspirin, sulfanilic acid (a metabolite of Yellow 6), and placebo. A dose of 0.1 mg (initial dose for asthma patients) or 1 mg of Yellow 6 was administered to patients with slight or no urticaria symptoms. If no reaction was observed after the initial dose, a higher dose of 2, 5, or 10 mg was administered to the latter group of patients 1 hour after each previous dose. Symptoms of a hypersensitivity reaction included urticaria; angioedema of lips, eyes, or face; reddening of the eyes; sweating; increased tear secretion; nasal congestion; sneezing; rhinitis (runny nose); hoarseness; wheezing; and a variety of subjective symptoms. Of the 33 control patients, only two with a history of rhinitis showed signs of rhinitis when administered Yellow 5 and Yellow 6. Of the 27 patients with recurrent urticaria who were challenged with Yellow 6, ten developed urticaria and six experienced subjective symptoms; 11 were negative for symptoms. Eight out of nine patients with positive reactions to Yellow 6 also experienced a positive reaction to aspirin (people sensitive to Yellow 5 also are often sensitive to aspirin) (Michaelsson and Juhlin 1973).

Michaelsson *et al.* tested seven patients having allergic vascular purpura (purplish spots) with oral provocation by 5 mg Yellow 6. One patient had a strongly positive reaction to the dye. That patient was a 32-year-old woman who suffered for 12 years from recurring purpuric lesions. After the patient was put on a diet free from dyes and benzoates (a preservative that has been linked to allergy-like reactions) for six months, she was essentially free from lesions (Michaelsson, Pettersson *et al.* 1974).

Conclusions

The NTP study did not detect any problems in chronic feeding studies on rats and mice, though the animals were not exposed *in utero*. Bio/dynamics concluded that its studies on rats and mice showed that Yellow 6 was not an animal carcinogen, but rats in the two highest dosage groups (3%, 5%) experienced higher incidences of adrenal medullary adenomas. The FDA has given reasons for not considering those tumors significant, but those reasons are not persuasive. A Bio/dynamics mouse study did not find evidence of carcinogenicity but it did not include an *in utero* phase. It is of concern that Yellow 6 may be contaminated with significant levels of recognized carcinogens. Also, while rarely life-threatening, Yellow 6 causes mild to severe hypersensitivity reactions in a small percentage of the population and may cause hyperactivity in some children. Even if it does not cause cancer, Yellow 6 raises other, lesser concerns. Because it provides no health benefit whatsoever, Yellow 6 should be removed from the food supply.

Appendix - Genotoxicity Studies

Table A1. Summary of genotoxicity studies for Blue No. 1.

Assay	Mutation Type	S9 Activation	Dose	Results	Reference
Comet Assay	DNA damage	NA	2,000 mg/kg	Negative	(Sasaki, Kawaguchi <i>et al.</i> 2002)
Cytogenetics Assay in Chinese Hamster Cells	Chromosomal Aberrations	No	?	Positive	(Hayashi, Matsui <i>et al.</i> 2000)
S. Typhimurium TA1535 and TA100	Base Pair	Yes and No	10 mg/plate	Negative	(Auletta, Kuzava <i>et al.</i> 1977; Bonin, Farquharson <i>et al.</i> 1981)
S. Typhimurium TA1538, TA98, and TA1537	Frameshift	Yes and No	10 mg/plate	Negative	
S. Typhimurium TA1538	Frameshift	Yes and No	10 mg/ml	Negative	(Haveland-Smith and Combes 1980)
<i>E. coli</i> WP2 <i>uvrA</i>	Base Substitution	Yes and No	10 mg/ml	Negative	
S. Typhimurium TA92, TA1535, TA100	Base Pair	Yes and No	5 mg/plate	Negative	(Ishidate, Sofuni <i>et al.</i> 1984)
S. Typhimurium TA1537, TA94, TA98	Frameshift	Yes and No	5 mg/plate	Negative	
Chromosomal aberration test, CHL cells	Chromosomal aberrations	No	5 mg/ml	Positive	

Table A2. Summary of genotoxicity studies on Blue No. 2.

Assay	Mutation Type	S9 Activation	Dose	Results	Reference
Comet Assay	DNA damage	NA	2,000 mg/kg	Negative	(Sasaki, Kawaguchi <i>et al.</i> 2002)
<i>S. Typhimurium</i> TA1535 and TA100	Base Pair	Yes and No	10 mg/plate	Negative	(Auletta, Kuzava <i>et al.</i> 1977)
<i>S. Typhimurium</i> TA1538, TA98, and TA1537	Frameshift	Yes and No	10 mg/plate	Negative	
<i>S. Typhimurium</i> TA1538	Frameshift	Yes and No	1 mg/ml	Negative	(Haveland-Smith, Combes <i>et al.</i> 1979)
<i>E. coli</i> WP2 <i>uvrA</i>	Base Substitution	Yes and No	10 mg/ml	Negative	
<i>S. Typhimurium</i> TA92, TA1535, TA100	Base Pair	Yes and No	5 mg/plate	Negative	(Ishidate, Senoo <i>et al.</i> 1974)
<i>S. Typhimurium</i> TA1537, TA94, TA98	Frameshift	Yes and No	5 mg/plate	Negative	
Chromosomal aberration test, CHL cells	Chromosomal aberrations	No	12 mg/ml	Positive	
<i>S. Typhimurium</i> TA1535 and TA100	Base Pair	Yes and No	1 mg/plate	Negative	(Brown, Roehm <i>et al.</i> 1978)
<i>S. Typhimurium</i> TA1538, TA98, and TA1537	Frameshift	Yes and No	1 mg/plate	Negative	
<i>rec</i> -Assay	DNA damage	No	NA	Negative	(Kada, Tutikawa <i>et al.</i> 1972)

Table A3. Summary of genotoxicity studies on Green No. 3

Assay	Mutation Type	S9 Activation	Dose	Results	Reference
Comet Assay	DNA damage	NA	2,000 mg/kg	Negative	(Sasaki, Kawaguchi <i>et al.</i> 2002)
S. Typhimurium TA100	Base Pair	Yes	10 mg/plate	Positive (in crude sample)	(Ishidate, Sofuni <i>et al.</i> 1984)
S. Typhimurium TA92, TA1535	Base Pair	Yes and No	10 mg/plate	Negative	
S. Typhimurium TA1537, TA94, TA98	Frameshift	Yes and No	10 mg/plate	Negative	
Chromosomal aberration test, CHL cells	Chromosomal aberrations	No	4 mg/ml	Positive	
Diploid yeast <i>Saccharomyces Cerevisiae</i> (BZ 34)	Mitotic gene conversion	No	5 mg/ml	Negative	(Sankaranarayanan and Murthy 1979)
Fischer rat embryo cell transformation	Malignant cell transformation (indicator of carcinogenic potential)	No	1 µg/ml	Postive (Negative at 10 and 100 µg/ml)	(Price, Suk <i>et al.</i> 1978)
S. Typhimurium TA1535 and TA100	Base Pair	Yes and No	50 µg/plate	Negative	(Brown, Roehm <i>et al.</i> 1978)
S. Typhimurium TA1538, TA98, and TA1537	Frameshift	Yes and No	50 µg/plate	Negative	

Table A4. Summary of genotoxicity studies on Red No. 3.

Assay	Mutation Type	S9 Activation	Dose	Results	Reference
Comet Assay	DNA damage	NA	100 mg/kg in glandular stomach and colon; >100 mg/kg in urinary bladder	Positive after 3 hours; negative after 24 hours	(Sasaki, Kawaguchi <i>et al.</i> 2002)
S. Typhimurium TA1535, TA100	Base pair	Yes and No	1-10 mg/plate	Negative	(Lin and Brusick 1986)
S. Typhimurium TA1537, TA98, TA1538	Frameshift	Yes and No	1-10 mg/plate	Negative	
Mouse lymphoma assay (L5178Y/TK ^{+/+})	Gene mutation	No	100-600 µg/ml	Negative	
Mouse micronucleus Assay	Chromosomal breakage/spindle damage	Yes	24, 80, 240 mg/kg	Negative	
Rec-assay	DNA damage	NA	100-10,000 µg/ml	Negative	
<i>E. coli</i> WP2 <i>uvrA</i>	Base substitution	Yes and No	0.5 mg/ml	Negative	(Haveland-Smith, Combes <i>et al.</i> 1979)
<i>In vitro</i> chromosome aberrations in Chinese Hamster fibroblast cells	Chromosome aberrations	No	0.6 mg/ml	Positive	(Ishidate, Sofuni <i>et al.</i> 1984)
Yeast strain D7	Mitotic gene conversion	NA	0-10 mg/ml	Positive	(Matula and Downie 1984)
Yeast strain XV185-14C	Reverse mutation in eukaryotes	NA	0-10 mg/ml	Positive	
Yeast strain D5	Mitotic recombination	NA	0-5 mg/ml	Negative	

Table A5. Summary of genotoxicity studies on Red No. 40.

Assay	Mutation Type	S9 Activation	Dose	Results	Reference
Comet Assay	DNA damage	NA	10 mg/kg in colon; 100 mg/kg in glandular stomach; 1,000 mg/kg in lungs	Positive	(Sasaki, Kawaguchi <i>et al.</i> 2002)
Comet Assay	DNA damage	NA	2,000 mg/kg to pregnant mice 10 mg/kg in male mice	Positive in colon Positive in colon	(Sasaki, Kawaguchi <i>et al.</i> 2002)
<i>E. coli</i> WP2 <i>uvrA</i>	Base substitution	Yes and No	10 mg/ml	Negative	(Haveland- Smith and Combes 1980)
<i>S. Typhimurium</i> TA1535 and TA100	Base pair	Yes and No	50-500 µg/plate	Negative	(Brown, Roehm <i>et al.</i> 1978)
<i>S. Typhimurium</i> TA98, and TA1537	Frameshift	Yes and No	50-500 µg/plate	Negative	
<i>S. Typhimurium</i> TA1535 and TA100	Base pair	Yes and No	0.2-400 µg/plate	Negative	(Muzzall and Cook 1979)
<i>S. Typhimurium</i> TA98, and TA1537	Frameshift	Yes and No	0.2-400 µg/plate	Negative	
<i>S. Typhimurium</i> TA 1535 and TA 1538	Base pair (TA1535) and Frameshift (TA1538)	Yes and No	1, 10, 50, 100 and 250 µg/plate	Negative	FDA Genetic Toxicology Branch
Yeast strains D-3 and D-5	Mitotic recombination	Yes and No	10 mg/ml	Negative	

Table A6. Summary of genotoxicity studies on Yellow No. 5.

Assay	Mutation Type	S9 Activation	Dose	Results	Reference
Comet Assay	DNA damage	NA	10 mg/kg >10 mg/kg	Positive (colon) Positive (glandular stomach)	(Sasaki, Kawaguchi <i>et al.</i> 2002)
Cytogenetics Assay	Chromosomal aberrations	NA	NA	Positive	(Hayashi, Matsui <i>et al.</i> 2000)
S. Typhimurium TA94, TA1537, TA98	Frameshift	Yes and No	5 mg/plate	Negative	(Ishidate, Sofuni <i>et al.</i> 1984)
S. Typhimurium TA1535, TA100, TA92	Base pair	No	2.5 mg/ml	Positive	
Chromosomal aberration test, CHL cells	Chromosomal aberrations	No	6 mg/ml	Positive	
<i>In vitro</i> Muntiacus muntjac	Chromosomal aberrations	No	3 µg/ml	Positive	(Patterson and Butler 1982)
S. Typhimurium TA1537, TA1538, TA98	Frameshift	Yes and No	5 mg/plate	Negative	(Chung, Fulk <i>et al.</i> 1981)
S. Typhimurium TA1535, TA100	Base Pair		5 mg/plate	Negative	
S. Typhimurium TA100	Base pair	Yes and No		Negative	(Kawachi, Yahagi <i>et al.</i> 1980)
S. Typhimurium TA98	Frameshift	Yes and No		Negative	
Rec assay	DNA damage	Yes and No		Negative	
Chromosomal aberration test, CHL cells	Chromosomal aberrations	Yes and No		Negative	

Table A7. Summary of genotoxicity studies on Yellow No. 6.

Assay	Mutation Type	S9 Activation	Dose	Results	Reference
Comet Assay	DNA damage	NA	2,000 mg/kg	Negative (stomach colon, liver, kidney, bladder, lung, or brain)	(Sasaki, Kawaguchi <i>et al.</i> 2002)
Cytogenetics Assay	Chromosomal aberrations	--	--	Positive	(Hayashi, Matsui <i>et al.</i> 2000)
<i>S. Typhimurium</i> TA98	Frameshift	Yes and No	300 µg/plate	Negative	(Rafi, Hall <i>et al.</i> 1997)
<i>S. Typhimurium</i> TA100	Base Pair	Yes and No	300 µg/plate	Negative	
Bone marrow micronucleus assay	Chromosomal damage	NA	2,000 mg/kg	Negative	(Westmoreland and Gatehouse 1991)
L5178Y TK ⁺ mouse lymphoma assay	Forward mutation	Yes	1 mg/ml	Positive	(McGregor, Brown <i>et al.</i> 1988)
<i>S. Typhimurium</i> TA1537, TA1538, TA98	Frameshift	Yes and No	5 mg/plate; also tested 1 mg/plate sulfanilic acid	Negative	(Chung, Fulk <i>et al.</i> 1981)
<i>S. Typhimurium</i> TA1535, TA100	Base pair	Yes and No	5 mg/plate; also tested 1 mg/plate sulfanilic acid	Negative	
<i>Saccharomyces cerevisiae</i> BZ 34	Mitotic gene conversion	No	5 mg/ml	Negative	(Sankaranarayanan and Murthy 1979)
<i>E. coli</i> WP2 <i>uvrA</i>	Base substitution	Yes and No	10 mg/ml	Negative	(Haveland-Smith, Combes <i>et al.</i> 1979)

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