CENTER FOR SCIENCE IN THE PUBLIC INTEREST, BREAST CANCER PREVENTION PARTNERS, CENTER FOR ENVIRONMENTAL HEALTH, CENTER FOR FOOD SAFETY, CHEF ANN FOUNDATION, CHILDREN'S ADVOCACY INSTITUTE, CONSUMER FEDERATION OF AMERICA, CONSUMER REPORTS, DEFEND OUR HEALTH, ENVIRONMENTAL DEFENSE FUND, ENVIRONMENTAL WORKING GROUP, FEINGOLD ASSOCIATION OF THE UNITED STATES, FOOD & WATER WATCH, HEALTHY BABIES BRIGHT FUTURES, LIFE TIME FOUNDATION, MOMSRISING, PREVENTION INSTITUTE, PUBLIC CITIZEN, PUBLIC HEALTH INSTITUTE, PUBLIC INTEREST RESEARCH GROUP, REAL FOOD FOR KIDS, LISA Y. LEFFERTS, LINDA S. BIRNBAUM, AND PHILIP J. LANDRIGAN

24 October 2022

Kristi Muldoon Jacobs, PhD, Acting Director
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Re: Color additive petition pursuant to 21 U.S.C. §§ 379e, 721(b)(1) to remove FD&C Red No. 3 from the permanent list of color additives approved for use in food and dietary supplements, 21 C.F.R. § 74.303, and for use in ingested drugs, 21 C.F.R. § 74.1303, because the FDA has found that the additive induces cancer and is unsafe.

Dear Dr. Muldoon Jacobs:

Petitioners submit this pursuant to section 721 (b)(1) of the Federal Food, Drug, and Cosmetic Act and 21 C.F.R. Part 71 to remove FD&C Red No. 3 from the permanent list of color additives approved for use in food and dietary supplements, 21 C.F.R. § 74.303, and for use in ingested drugs, 21 C.F.R. § 74.1303, because the U.S. Food and Drug Administration (FDA) has already found that this color additive causes cancer in laboratory animals[1] and subsequent studies and reviews have reinforced that conclusion.[2] Furthermore, we urge that the FDA take immediate action to prohibit use of this carcinogen, as there is widespread exposure to U.S. consumers, particularly children, and new information indicates that very young children have the highest exposures.[3] This petition and all associated files have been submitted to you via CD-ROM.

Since 1960, federal law has stated that a "color additive shall be deemed unsafe, and shall not be listed, for any use which will or may result in ingestion of all or part of such additive, if the additive is found by the Secretary to induce cancer when ingested by man or animal, or if it is

[1] Final Rule: Termination of Provisional Listings of FD&C Red No. 3 for Use in Cosmetics and Externally Applied Drugs and of Lakes of FD&C Red No. 3 for All Uses, 55 Fed. Reg. 3516 (Feb. 1, 1990). (Stating, "Having concluded that FD&C Red No. 3 causes cancer in rats, the agency hereby terminates the provisional listing of FD&C Red No. 3 for use in cosmetics and externally applied drugs and the provisional listing of the lakes of FD&C Red No. 3 for use in food, drug, and cosmetic products, effective January 29, 1990.)
[2] *See* Section II.C & Appendix D Part 3 of this petition.
[3] *See* Section IV & Appendix E of this petition.

found by the Secretary, after tests which are appropriate for the evaluation of the safety of additives for use in food, to induce cancer in man or animal . . . ."[4] Similarly, since 1958, federal law has required that FDA cannot find as safe the use of any food additive that has been found "to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal."[5] This requirement, known as the Delaney Clause in honor of its Congressional author, is an absolute bright line drawn by Congress prohibiting carcinogenic additives in the food supply.[6]

FDA concluded in 1990, in response to a petition to permanently approve (list) certain uses of FD&C Red No. 3 that were only provisionally listed:

> …because FD&C Red No. 3 has been shown to induce cancer in appropriate tests, under the color additive Delaney clause … FD&C Red No. 3 is unsafe for use in externally applied drugs and externally applied cosmetics and cannot be listed.[7]

As a result, the agency appropriately terminated the provisional listings of FD&C Red No. 3 for use in cosmetics, including lipsticks and other ingested cosmetics, externally applied drugs, and for all uses of the lakes[8] of FD&C Red No. 3.[9]

At the time, FD&C Red No. 3 had already been permanently approved for use in food and ingested drugs, so the scope of the petition was limited to the provisionally approved uses. As such, FDA limited their response to those uses, but stated it would "take steps" to ban the use of FD&C Red No. 3 in food and ingested drugs, and that "[b]ecause large amounts of the color have been shown to cause cancer in rats, FDA…plans to end the remaining uses" of FD&C Red No. 3.[10] In fact, the tests that FDA relied upon to conclude that FD&C Red No. 3 caused cancer were feeding studies, and so are particularly relevant to ingested uses of FD&C Red No. 3. However, the agency has yet to delist FD&C Red No. 3 for these uses, and this carcinogen can still be found in foods, dietary supplements, and ingested drugs.[11]

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[4] 21 U.S.C. § 379e(b)(5)(B).
[5] 21 U.S.C. § 348(c)(3)(A).
[6] *Public Citizen v. Young*, 831 F.2d 1108, 1122 (D.C. Cir. 1987); *Les v. Reilly*, 968 F.2d 985, 989 (9th Cir. 1992) (providing that "[t]hroughout its 30-year history, the Delaney clause has been interpreted as an absolute bar to all carcinogenic food additives" and that ". . . Congress has repeatedly ratified a strict interpretation of the Delaney clause" (internal citations omitted)).
[7] Color Additives; Denial of Petition for Listing of FD&C Red No. 3 for Use in Cosmetics and Externally Applied Drugs; Withdrawal of Petition for Use in Cosmetics Intended for Use in the Area of the Eye, 55 Fed. Reg. 3520, 3523 (Feb. 1, 1990) [hereinafter Color Additives Notice].
[8] A lake is a pigment (i.e., water-insoluble coloring agent) derived from a dye (i.e., a water-soluble coloring agent). Lakes are used to add color to fatty foods and low-moisture foods. FDA & IFIC. Food Ingredients and Colors. April 2010. Available at: https://www.fda.gov/food/food-ingredients-packaging/overview-food-ingredients-additives-colors.
[9] Termination of Provisional Listings of FD&C Red No. 3 for Use in Cosmetics and Externally Applied Drugs and of Lakes of FD&C Red No. 3 for All Uses, 55 Fed. Reg. 3516-1 (February 1, 1990).
[10] McLaughlin, P. (April 22, 1990). Seeing Red Dye No. 3. Chicago Tribune. Chicago: 5 Available at: https://www.chicagotribune.com/news/ct-xpm-1990-04-22-9002030888-story.html; and Blumenthal D (May 1990). Red No. 3 and Other Colorful Controversies. FDA Consumer 24(4):18-21. Available: https://web.archive.org/web/20070809080710/https://www.fda.gov/bbs/topics/CONSUMER/CON00063.html.
[11] 21 C.F.R. §§ 74.303; 74.1303.

As required by the Delaney Clause, petitioners urge the FDA to continue what it started over three decades ago and completely delist FD&C Red No. 3 for any use.

Specific Action Requested

Petitioners submit this pursuant to sections 379e and 721(b)(1) of the Federal Food, Drug, and Cosmetic Act requesting the repeal by the Commissioner of regulations 21 C.F.R. § 74.303 and § 74.1303 listing certification of the color additive FD&C Red No. 3 (hereinafter "delisting"), because it is not suitable and safe for use in food, dietary supplements, and ingested drugs.[12]

I. Background

FD&C Red No. 3 is a color additive currently approved for use in food generally, including dietary supplements, and in ingested drugs.[13] This color additive was first approved in 1907 for use in food as "erythrosine"[14] and then in 1939 as "FD&C Red No. 3" for use in food, drugs, and cosmetics.[15]

[12] As required under 21 C.F.R. § 71.1, attached hereto, and constituting a part of this petition is the following:

A. The name and all pertinent information concerning the color additive, including chemical identity and composition of the color additive, its physical, chemical, and biological properties, and specifications prescribing its component(s) and identifying and limiting the reaction byproducts and other impurities (Appendix A);
B. The amount of the color additive proposed for use (zero) (Appendix B);[12]
C. Methods (Appendix C). FDA has developed methods for batch certification of FD&C Red No. 3 and for analysis of foods containing FD&C Red No. 3.
D. Full reports of investigations made with respect to the safety of the color additive (Appendix D Parts 1-3);
E. Proposed tolerances and other limitations on the use of the color additive, if required (Appendix E)
F. Complete data which will allow the Commissioner to consider, among other things, the probable consumption of, and/or other relevant exposure from the additive and of any substance formed in or on food, drugs, or cosmetics because of such additive (Appendix E);
G. If exemption from batch certification is required (Appendix G), None required.
H. Full information on each proposed change that is to be made in the original regulation (Appendix H);
I. Request for Fee Waiver (Appendix I and Section V)
J. Claim for categorical exclusion under 25.32 (Environmental review component). An environmental assessment is not required because the proposed action is categorically excluded pursuant to 21 C.F.R. § 25.32(m) as an "action to prohibit or otherwise restrict or reduce the use of a substance in food, food packaging, or cosmetics." (Appendix J)

[13] 21 C.F.R. §74.303(a)(1) identifies the color additive FD&C Red No. 3 as "principally the monohydrate of 9 (o-carboxyphenyl)–6–hydroxy–2,3,5,7, –tetraiodo–3H–xanthem–3–one, disodium salt, with smaller amounts of lower imdinated [sic] fluoresceins." Prior to its use, each batch of the color additive must be certified by FDA to be in compliance with specifications outlined in 21 C.F.R. § 74.303(b). FD&C Red No. 3 refers specifically to certified batches of the colorant. When uncertified, it is commonly called erythrosine, and there are other synonyms used. See Appendix I.

[14] In 1907, the Agriculture Department listed the coloring as "erythrosine" for use in food, and in 1939 expanded its allowable uses to drugs and cosmetics, calling it "FD&C Red No. 3." Color Additives Notice, *supra* note 7, at 3520, 3521.

[15] Color Additives Notice, *supra* note 7, at 3521.

In 1960, in response to the Color Additives Amendment, which required the FDA to determine whether color additives were "suitable and safe" for a given use,[16] the FDA placed FD&C Red No. 3 on a provisional list for all uses with tentative approval for all uses pending evaluation by FDA.[17] This provisional listing allowed for use on "an interim basis" until the agency either permanently listed or terminated the use of the additive.[18]

In 1969, in response to a 1968 petition from the Certified Color Industry Committee (in 1990 called the Certified Color Manufacturers Association, and now called the International Association of Color Manufacturers), the FDA permanently listed FD&C Red No. 3 (excluding lakes of FD&C Red No. 3) for use in food, including dietary supplements, and ingested drugs,[19] based on studies available at the time.

Later in 1969, the Toilet Goods Association Inc. (in 1990 called the Cosmetic, Toiletry and Fragrance Association, Inc., and now the Personal Care Products Council) submitted a color additive petition requesting permanent listing of FD&C Red No. 3 for coloring cosmetics, including lipsticks and other ingested cosmetics, and externally applied drugs.[20]

Thus, although the use of FD&C Red No. 3 for use in food and ingested drugs was permanently listed in 1969, the external drug uses, all cosmetic uses, and all uses of lakes of FD&C Red No. 3 remained provisionally listed pending an FDA decision, while petitioners conducted safety tests appropriate for external use.[21]

Then in 1977, FDA published revised provisional listing regulations requiring new chronic toxicity studies on 31 color additives including FD&C Red No. 3 as a condition of their continued provisional listing (in cosmetics and externally applied drugs in the case of FD&C Red No. 3). FDA required the new chronic toxicity studies because previously submitted studies were deficient in several respects (e.g., too few animals, tumors not examined microscopically). FDA postponed the closing date for the provisional listing of FD&C Red No. 3 until 1981 to allow for the studies to be completed. Over the years the closing date for the provisional listing

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[16] Barros, J.N. et al., *Color Additives: FDA's Regulatory Process and Historical Perspectives*, Food Safety Magazine (2003). Also reprinted by FDA at http://www.fda.gov/ForIndustry/ColorAdditives/RegulatoryProcessHistoricalPerspectives/.
[17] Meadows, M., Food & Drug Admin., *A Century of Ensuring Safe Foods and Cosmetics*, FDA Consumer Magazine (2006). Available at: https://www.fda.gov/files/A-Century-of-Ensuring-Safe-Foods-and-Cosmetics.pdf.
[18] Barros, J.N. et al., *Color Additives: FDA's Regulatory Process and Historical Perspectives*, Food Safety Magazine (2003), http://www.fda.gov/ForIndustry/ColorAdditives/RegulatoryProcessHistoricalPerspectives/#authors.
[19] 21 C.F.R. §§ 74.303, 74.1303 (1969); Color Additives Notice, *supra* note 7, at 3521; International Association of Color Manufacturers. About IACM. At https://iacmcolor.org/about-iacm/.
[20] Color Additives Notice, *supra note 7* at 3521; Personal Care Products Council, "CTFA Changes Name to the Personal Care Products Council, Launches Consumer Information Web Site on Product Safety," November 29, 2007, at https://www.personalcarecouncil.org/news-release/ctfa-changes-name-to-the-personal-care-products-council-launches-consumer-information-web-site-on-product-safety/.
[21] Lipman, A.L. Safety of Xanthene Dyes According to the U.S. Food and Drug Administration. Chapter 4 of Light Activated Pest Control, [pp. 34-53,] ACS Symposium Series, Vo. 616. 5 May 1995. See page 39. https://pubs.acs.org/doi/10.1021/bk-1995-0616.ch004.

would be extended many times to allow for studies and reviews to be performed, completed, or evaluated.[22]

In the early 1980s, the new chronic studies became available and indicated that FD&C Red No. 3 could cause cancer in lab rats.[23] While reviewing that evidence and collecting additional data, including data intended to determine if the mechanism for the observed cancers was an iodine excess from the sodium iodide constituent of FD&C Red No. 3, the FDA allowed FD&C Red No. 3 to continue to be used provisionally in cosmetics, and externally applied drugs and in lake form in food, drugs, and cosmetics, reasoning that "[p]ublic exposure to this color additive results largely from its permanently listed uses" in food and ingested drugs.[24]

In 1984, FDA's Acting Commissioner, Mark Novitch, wrote that FD&C Red No. 3 was "of greatest public health concern … The agency should not knowingly allow continued exposure (at high levels in this 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."[25]

In 1990, after providing numerous extensions of the provisional listing and a lengthy period of time to amass the scientific data to establish safety, the FDA concluded "FD&C Red No. 3 is an animal carcinogen when administered in the diet" and "[t]he studies showing FD&C Red No. 3 to be a carcinogen when ingested are relevant and appropriate to the evaluation of the safety of this color additive for noningested uses."[26] In fact, the agency concluded that the evidence was robust enough to "firmly establish" that FD&C Red No. 3 causes thyroid cancer in male rats.[27] As a result, it terminated the provisional listings of FD&C Red No. 3 for use in cosmetics, including lipsticks and other ingested cosmetics, and externally applied drugs, and for all uses of the lakes of FD&C Red No. 3 in 1990.[28] Illogically, although these external uses were deemed unsafe, ingested uses continued.

In addition to FDA's conclusions, other safety reviews conducted over the past three decades have confirmed that this color additive induces cancer in animals, as we discuss below.

There is no scientific or public health justification for permitting the use of FD&C Red No. 3 dye in food while prohibiting FD&C Red No. 3 lake in food and both the dye and the lake in cosmetics and externally applied drugs.

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[22] Color Additive Notice, *supra* note 7 at 3521-3523.
[23] Color Additive Notice, *supra* note 7 at 3524. The final reports of both rat studies (showing carcinogenicity) were submitted to the agency in 1982.
[24] Provisional Listing of Certain Color Additives; Proposal to Extend Closing Date, 50 Fed. Reg. 26,377, 26,380 (June 26, 1985).
[25] Burros, M. (February 13, 1985). The Saga of a Food Regulation: After 25 Years, Still No Decision. The New York Times. Available at: https://www.nytimes.com/1985/02/13/garden/the-saga-of-a-food-regulation-after-25-years-still-no-decision.html.
[26] Color Additives Notice, *supra* note 7, at 3542. FDA also acted on this conclusion in Termination of Provisional Listings of FD&C Red No. 3 for Use in Cosmetics and Externally Applied Drugs and of Lakes of FD&C Red No. 3 for All Uses, 55 Fed. Reg. 3516-1 (February 1, 1990).
[27] Color Additives Notice, *supra* note 7, at 3526.
[28] Termination of Provisional Listings of FD&C Red No. 3 for Use in Cosmetics and Externally Applied Drugs and of Lakes of FD&C Red No. 3 for All Uses, 55 Fed. Reg. 3516-1 (February 1, 1990).

II. FD&C Red No. 3 Causes Cancer in Animals.

a. The FDA Has Already Determined that Animal Feeding Studies "Firmly Establish" that FD&C Red No. 3 Causes Cancer.

As FDA itself already concluded in 1990, there is scientific evidence that FD&C Red No. 3 causes cancer in animals.[29] The FDA found in 1990 that long-term feeding studies of FD&C Red No. 3 in rats showed that treated animals developed adenomas and carcinomas of the thyroid and "provided convincing evidence that FD&C Red No. 3 is an animal carcinogen,"[30] leading the agency to conclude that FD&C Red No. 3 is "an animal carcinogen when administered in the diet."[31] Applying the Delaney clause, the FDA denied the Toilet Goods Association's petition to permanently list FD&C Red No. 3 for use in cosmetics and externally applied drugs and banned FD&C Red No. 3 for use in "all cosmetics and externally applied drugs, and for uses of lakes . . . in food, drugs, and cosmetics."[32] That same logic should apply to the permanently listed uses.

These long-term studies were sponsored by the Certified Color Manufacturers Association (CCMA) and conducted at the International Research and Development Corp. (IRDC). Recall that the studies were performed in response to FDA's requirement in 1977 for additional long-term feeding studies in rats and mice[33] as one of the conditions for the continued provisional listing of several color additives, including FD&C Red No. 3, as noted in the previous section. FDA had previously determined that earlier studies of FD&C Red No. 3 were not adequate under then-current standards to establish the safety of the color additive for the uses then provisionally listed.

In the first study in rats, CCMA contended that there were no significant results, but FDA conducted its own microscopic examination of the animals for neoplastic lesions, and its own statistical evaluations, which showed that FD&C Red No. 3 produced statistically significant increases in follicular cell adenomas plus carcinomas in males at all doses tested ($p = 0.016$, 0.0007, and 0.03, respectively using doses of 0.1, 0.5, and 1% of the diet) compared to control animals (6/64 or 9.4% at the lowest dose, 8/66 or 12.1% at the mid-dose, and 4/57 or 7.0% at the high dose, compared with 0/65 or 0% in control group 1 and 1/61 or 1.6% in control group 2). In the second study, which used a higher dose (4%), FDA again disagreed with CCMA's conclusions. FDA conducted its own histopathology review and statistical analysis and found a

[29] Color Additives Notice, *supra* note 7, at 3525; See Appendix D Part 1, Certified Color Manufacturers Association, International Research and Development Corporation (IRDC) Study No. 410-002 and Study No. 410-011, August 31, 1981; CAP No. 96.
[30] Color Additives Notice, *supra* note 7, at 3522.
[31] Color Additives Notice, *supra* note 7, at 3542.
[32] Color Additives Notice, *supra* note 7, at 3520.
[33] FDA concluded that long-term exposure of mice to FD&C Red No. 3 did not produce a carcinogenic or other deleterious effect in the chronic feeding study of mice conducted by the IRDC. This study did not include in utero exposure, unlike the study in rats. Color Additives Notice, *supra* note 7, at 3523, 3524.

statistically significant increase ($p < 0.0007$) in combined incidence of adenomas and carcinomas (18/68 or 26.5% in treated male animals compared with 2/68 or 2.9% in the controls).[34]

b. The National Toxicology Program Board of Scientific Counselors, Technical Reports Review Subcommittee Determined that There Is "Convincing Evidence of Carcinogenicity" for FD&C Red No. 3 in Rats.

The National Toxicology Program (NTP) Board of Scientific Counselors Technical Reports Review Subcommittee also reviewed data on the carcinogenicity of FD&C Red No. 3 in 1983. The NTP Subcommittee concluded that there was "convincing evidence" that FD&C Red No. 3 is an animal carcinogen, and that new data presented by consultants for the sponsor did not change its conclusions.[35]

c. Evaluations Conducted by Other Authorities and Studies Published Since 1990 Support FDA's Determination that Animal Feeding Studies Establish that FD&C Red No. 3 Causes Cancer in Animals.

Several evaluations by European authorities and the international Joint Expert Committee on Food Additives (JECFA) have occurred over the years, and these have also concluded that erythrosine (the name given to the uncertified form of FD&C Red No. 3) causes tumors in animals.

Importantly, while FDA conducted its own microscopic evaluation of the tumors and statistical analysis, which reported more tumors than CCMA's, European and international authorities did not conduct their own independent microscopic evaluation of the tumors and instead relied on results reported by CCMA. FDA's evaluation of the number and types of tumors and their significance is thus more robust than that by European and international authorities.

Despite this, two reviews of the evidence[36] were conducted approximately concurrently with FDA's evaluation and reached similar conclusions regarding the ability of erythrosine to cause tumors in animals:

> 1) The European Commission's Scientific Committee for Food (SCF) published a report in 1989 that states, "Erythrosine has been shown to cause an increase in the incidence of thyroid follicular adenomas in male rats when fed at high doses in a 2-generation long-

[34] Color Additives Notice, *supra* note 7, at 3524-3525; and FDA Memorandum dated August 11, 1989, from David G. Hattan, Ph.D., Deputy Director, Division of Toxicological Review and Evaluation, HFF-152, to Ronald Lorentzen, Ph.D., Assistant to Director for Carcinogenicity Assessment, HFF-100, "Evaluation of Data Concerning Possible Mechanism(s) Mediating Rat Thyroid Tumorigenesis by FD&C Red No. 3," (p. 3, Table 1). For follicular cell adenomas, the agency found 14/68 or 20.6% in the 4% group, compared with 1/68 or 1.5% in the controls, and for carcinomas, it found 5/68 or 7.4% in the 4% group, compared with 1.68 or 1.5%. In contrast, for example, CCMA reported 3/69 or 4.3% carcinomas in treated animals, vs. 2/69 or 2.9% in controls.

[35] Color Additives Notice, *supra* note 7, at 3521-3522, 3525.

[36] The Chair of the European Commission's Scientific Committee for Food reviewing erythrosine, C. van der Heijden, also served on the 1990 JECFA panel reviewing erythrosine.

term study. There is also some equivocal evidence for an increased incidence of thyroid carcinoma."[37]

2) The Joint Expert Committee on Food Additives of the World Health Organization and the Food and Agriculture Organization of the United Nations (JECFA) evaluated erythrosine in 1990.[38,39] JECFA states, "an increase in the incidence of thyroid follicular-cell adenomas in male rats was demonstrated at a level of 40 mg/kg of erythrosine in the diet. When thyroid follicular-cell adenomas and carcinomas were combined in the statistical analysis, significant (but not clearly dose-related) increases in the incidence of thyroid tumours in male rats given 1, 5, 10 and 40 mg/kg of erythrosine in the diet were found. Effects in females were significant only at one dose level. The Committee agreed that it was appropriate to combine thyroid follicular-cell adenomas and carcinomas in the statistical analysis, in view of evidence that adenomas are an earlier stage of carcinomas in the thyroid."[40]

The review by FDA published in its 1990 decision, although contemporaneous with these two reviews, was more rigorous because it used additional information provided by the proponents[41] of FD&C Red No. 3 as well as other independent assessments (e.g., that by NTP Board of Scientific Counselors Technical Reports Review subcommittee), and critically, its own microscopic and statistical analyses, as discussed above.

In 2011, the European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS), which replaced the SCF, re-evaluated the evidence since the 1987 decision by the SCF, but reached the same conclusion as the SCF (i.e., erythrosine has an oncogenic effect in the thyroid gland of rats).

Importantly, EFSA concluded, "[s]ince the JECFA and SCF evaluations were completed, no new data are available on chronic toxicity/carcinogenicity."[42]

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[37] Commission of the European Communities. Reports of the Scientific Committee for Food, Twenty-first series, Report EUR 11617, p. 11, 1989, http://aei.pitt.edu/40830/1/21st_food.pdf.

[38] Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Thirty-seventh report. WHO Technical Report Series 806, 1991, p. 19. http://apps.who.int/iris/bitstream/handle/10665/40288/WHO_TRS_806.pdf.

[39] The Toxicological Monograph for the 1990 meeting cites the 1990 Federal Register notice announcing the termination of certain uses of FD&C Red No. 3. Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of certain food additives and contaminants. Thirty-seventh report. WHO Food Additive Series 28, 1991. https://inchem.org/documents/jecfa/jecmono/v28je12.htm.

[40] Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Thirty-seventh report. WHO Technical Report Series 806, 1991, pp. 19-20. http://apps.who.int/iris/bitstream/handle/10665/40288/WHO_TRS_806.pdf.

[41] FDA stated in its 1990 Color Additives Notice (supra, p. 3520) that it considered the proponents of FD&C Red No. 3 to include the Cosmetic, Toiletry and Fragrance Association (CTFA) and the Certified Color Manufacturers Association (CCMA), now the Personal Care Products Council and the International Association of Color Manufacturers, respectively. CCMA was not the petitioner for the permanent listing but submitted much of the safety data to the agency.

[42] European Food Safety Authority (EFSA). Scientific Opinion on the Re-evaluation of Erythrosine (E 127) as a Food Additive. *EFSA Journal 9*(1): 1854, 2011. https://doi.org/10.2903/j.efsa.2011.1854.

Similarly, in 2018, JECFA re-evaluated the evidence since its 1990 decision, and saw no reason to revise its previous decision.[43]

We also reviewed all studies cited by the 2018 JECFA evaluation and identified no new data on carcinogenicity. Appendix D Part 3 reviews other potentially relevant studies published since FDA's 1990 decision. None of the studies since FDA's 1990 decision are chronic carcinogenicity studies and none alter the conclusion that FD&C Red No. 3 induces cancer. The FDA's 1990 conclusion that FD&C Red No. 3 causes cancer in animals is unrefuted.[44]

III. FDA Is Statutorily *Obligated* to Delist FD&C Red No. 3 Because It Has Been Shown to Cause Cancer in Animals.

a. The FDA Cannot Approve Color Additives that Cause Cancer, Regardless of the Cancer Risk Posed.

FDA is required by statute to delist FD&C Red No. 3 for all uses because the agency found, three decades ago, that the color additive induces cancer in animals.[45] Section 721(b)(4) [previously 706(b)(4)] of the Food, Drug, and Cosmetic Act (FDCA) prohibits the FDA from listing a color additive for a particular use unless the data presented to the FDA establishes that the color additive is safe for such use.[46] Since 1960, the Delaney Clause has raised the safety standard for color additives by requiring that a "color additive shall be deemed unsafe, and shall not be listed, for any use which will or may result in ingestion of all or part of such additive, if the additive is found by the Secretary to induce cancer when ingested by man or animal, or if it is found by the Secretary, after tests which are appropriate for the evaluation of the safety of additives for use in food, to induce cancer in man or animal . . ."[47]

The Delaney Clause requires the FDA to prohibit the use of any additive shown to be a carcinogen regardless of its level of exposure.[48] Thus, if a color additive is found to induce cancer in humans *or* animals, even if the risk of cancer could be "exceedingly small," it is unsafe and cannot be approved for any use.[49]

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[43] Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants. Eighty-sixth report. WHO Technical Report Series 1014, 2019, p. 32. Available at: https://apps.who.int/iris/bitstream/handle/10665/279832/9789241210232-eng.pdf.

[44] FDA noted back in 1990 (Color Additives Notice, *supra* note 7, at 3523) that all of the data submitted subsequent to the chronic rat feeding study were designed to elucidate the mechanism of action of FD&C Red No. 3's carcinogenic process and were not designed to dispute the carcinogenic response observed, and that thus its conclusion that FD&C Red No. 3 causes cancer in animals is "unrefuted." FDA's conclusion remains unrefuted.

[45] Color Additives Notice, *supra* note 7, at 3516. (Stating, "In particular, the color additive [FD&C Red No. 3] causes a carcinogenic response in rats.).

[46] 21 U.S.C. § 379e(b)(5).

[47] 21 U.S.C. § 379e(b)(5)(B); *Public Citizen v. Young*, 831 F.2d 1108, 1122 (D.C. Cir. 1987); *Les v. Reilly*, 968 F.2d 985, 989 (9th Cir. 1992) (providing that "[t]hroughout its 30-year history, the Delaney clause has been interpreted as an absolute bar to all carcinogenic food additives" and that ". . . Congress has repeatedly ratified a strict interpretation of the Delaney clause" (internal citations omitted)).

[48] 21 U.S.C. § 379e(b)(5)(B) and 21 C.F.R § 70.50(a); *Public Citizen v. Young, 831 F.2d* at 1121 footnote 18 (D.C. Cir. 1987), Id.

[49] 21 U.S.C. § 379e((b)(5)(B) and 21 C.F.R. § 70.50(a); *Public Citizen v. Young, 831F.2d* at 1113 (D.C. Cir. 1987), Id.

FDA recognized this in 1990 in regard to FD&C Red No. 3, stating that "even if CTFA's [the Cosmetic, Toiletry and Fragrance Association, Inc.] risk assessments are valid and accurate, the fact that the risks from exposure to FD&C Red No. 3, when used in externally applied drugs and cosmetics, are insignificant or trivial does not exempt the color additive from the operation of the Delaney clause U.S.C. 376(b)(5)(B) under the principle of de minimis."[50] Furthermore, the risks from ingested uses are probably larger than from external uses.

This was made clear in *Public Citizen v. Young* where the Court of Appeals for the D.C. Circuit held that Congress barred the FDA from employing a *de minimis* exception to the Delaney Clause.[51] In this case, the FDA allowed the use of D&C Orange No. 17 and D&C Red No. 19 despite the fact that these substances caused cancer in the test animals.[52] The agency concluded that the two color additives were safe based on assessments that characterized their risk as "so trivial as to be effectively no risk."[53] However, the court determined that the statutory language itself as well as its legislative history allowed for no administrative discretion to permit a carcinogenic color additive's use even when the risk of cancer was "exceedingly small."[54] It concluded that the FDA did not have the authority to list a color additive once the agency found it to induce cancer in animals.[55] Thus, in 1983, having concluded that D&C Orange No. 17 and D&C Red No. 19 cause cancer in laboratory animals when ingested, the FDA terminated the provisional listings of those color additives, as well as that for D&C Red No. 37 (chemically related to D&C Red No. 19) for ingested drugs and ingested cosmetics.[56]

Similarly, in 1976, the FDA revoked the provisional listing of FD&C Red No. 2 over statistically significant increases of tumors in animal studies,[57] despite then-Commissioner Alexander Schmidt's statements regarding FD&C Red No. 2 that "there was no evidence of a public health hazard."[58] Although the agency's Toxicology Advisory Committee was about evenly divided as to whether FD&C Red No. 2 was carcinogenic, it was unanimous that FD&C Red No. 2 could not be approved as safe. The agency found that the data before it did not establish that FD&C Red No. 2 was safe under the FDCA and denied a petition to permanently list FD&C Red No. 2.[59] The agency's decision was upheld in court, which stated that the burden of establishing safety was placed on the industry.[60]

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[50] Color Additives Notice, *supra* note 7, at 3517.
[51] *Public Citizen v. Young, 831 F.2d* 1108 (D.C. Cir. 1987).
[52] *Id*. at 1110.
[53] *Id.*at 1111.
[54] *Id*. at 1113.
[55] *Id.* at 1122.
[56] 21 C.F.R. § 81.10(q), (s).
[57] Color Additives: Denial of Petition for Permanent Listing of FD&C Red No. 2, 41 Fed. Reg. 15053 (April 9, 1976).
[58] Death of a Dye, Time, (Feb. 2, 1976).
[59] Color Additives: Denial of Petition for Permanent Listing of FD&C Red No. 2, 41 Fed. Reg. 15053 (April 9, 1976).
[60] *Certified Color Mfrs. Ass'n v. Mathews,* 543 F.2d 284, 296 (D.C. Cir. 1976) (observing that Congress gave the agency "broad authority" to remove color additives from the food supply, given that they have, "literally and accurately, a cosmetic value").

Therefore, the Delaney clause, case law, and legislative history commands the FDA to prohibit all carcinogenic color additives from use in food, drug, and cosmetic products, and FDA is compelled to delist the remaining uses of FD&C Red No. 3.

b. The FDA Cannot Approve Color Additives that Cause Cancer, Whether Through a Primary or Secondary Mechanism.

The industry has long asserted that FD&C Red No. 3 does not cause cancer through a direct mechanism and has used this to argue against restricting the substance. We first consider the legal issues raised, and in subsequent sections consider the scientific ones.

Prior to its 1990 decision, FDA considered the hypothesis put forward by proponents of FD&C Red No. 3 that the color additive might cause cancer through a secondary mechanism. As FDA describes it, "the proponents hypothesized that hormonal imbalances that resulted from the ingestion of high levels of FD&C Red No. 3 hyperstimulated the thyroid. The proponents further contended that, if a secondary mechanism exists, a threshold or 'no effect' level for the hormonal effects could be established that would permit the determination of a safe dose of the color additive."[61]

FDA extended the closing date for the provisional listed uses of FD&C Red No. 3 and its lakes multiple times in order to allow the agency to receive and evaluate reports and studies relevant to this question. For example, in 1983, the NTP Subcommittee reviewed a study designed to determine if the tumorigenic effects of FD&C Red No. 3 were due to an iodine excess from the sodium iodide constituent of FD&C Red No. 3 and considered whether the response was mediated through a secondary mechanism. It concluded that no determination could be made as to the mechanism (primary or secondary) of carcinogenic effects for FD&C Red No. 3 in the thyroid of male rats, and recommended additional studies be designed to elucidate the carcinogenic mechanisms.[62]

Even if there were data that suggest FD&C Red No. 3 causes cancer through secondary mechanisms—a hypothesis which FDA considered and rejected in 1990, as discussed in the next section—the agency recognizes that substances that cause cancer, including those that do so via secondary mechanisms, are prohibited under the Delaney clause. In a 2018 decision by FDA to no longer allow seven synthetic flavorings determined to be carcinogenic, the FDA described a comment it had received from the public:

> One comment said that the Delaney Clause applies only to food additives that induce cancer in test animals through a direct, genotoxic mechanism of carcinogenicity. The comment further stated that there are numerous examples of food ingredients that produce increased incidence of tumors in high dose rodent studies through a threshold secondary mechanism.

The FDA responded:

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[61] Color Additives Notice, *supra* note 7, 3523
[62] Color Additives Notice, *supra* note 7, 3522, 3523, 3525.3

> We disagree. The Delaney Clause does not differentiate between non-genotoxic and genotoxic carcinogens. Nor does it permit FDA to find a food additive safe for human consumption if the food additive has induced cancer in animal [sic].[63]

Therefore, evidence showing that a substance may cause cancer, regardless of mechanism, would be evidence for delisting under the Delaney Clause. In any event, as discussed in the next section, the evidence does not establish that FD&C Red No. 3 operates through a secondary mechanism of action.

i. Industry Failed to Persuade FDA in 1990 that FD&C Red No. 3 Caused Cancer in Rats Through a Secondary Mechanism.

In its 1990 decision, FDA rejected the proponents' assertions that (1) the available evidence demonstrated that FD&C Red No. 3 itself, or iodine released by the color additive, caused a thyroid hormone imbalance that leads to an increased incidence of tumors, and (2) that there is a threshold level for the effects that lead to this hormonal imbalance, implying the potential existence of a safe level of use. The proponents sponsored two 60-day studies in rats and submitted an absorption, distribution, and metabolism study of FD&C Red No. 3 in rats, as well as other data and information in support of their secondary mechanism hypothesis. Reviewing these studies, the agency concluded:

> After a full evaluation of the data submitted in support of the petition and of the other pertinent data that relate to the use of FD&C Red No. 3, FDA finds:
>
> 1. FD&C Red No. 3 is an animal carcinogen when administered in the diet.
>
> 2. The studies showing FD&C Red No. 3 to be a carcinogen when ingested are relevant and appropriate to the evaluation of the safety of this color additive for noningested uses.
>
> 3. The proponents have failed to established [sic] their hypothesis that the observed carcinogenic effect of FD&C Red No. 3 is a result of a hormonally induced secondary mechanism.[64]

The agency noted that the proponents of FD&C Red No. 3 had

> …the obligation since 1960 to establish the safety of the use of this color additive. Moreover… these proponents have been aware, at least since 1983, of the evidence that FD&C Red No. 3 is an animal carcinogen and, by virtue of the numerous extensions of the provisional listings for FD&C Red No. 3, have had a lengthy period of time in which to amass the scientific data to establish the safety of the color additive, including its mechanism of carcinogenic actions. The proponents have not provided such data.[65]

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[63] 83 FR 50490, 2018. Food Additive Regulations; Synthetic Flavoring Agents and Adjuvants, pp. 50500-50501.
[64] Color Additives Notice, *supra* note 7, at 3542.
[65] Color Additives Notice, *supra* note 7, at 3523.

Furthermore, the agency stated, "if the proponents of this color additive developed new data that they believe support the safety of the color, the proponents may submit a new petition for listing the color which FDA will evaluate."[66]

However, in the decades since, no new data or color additive petitions were submitted to the agency, to the best of our knowledge.[67]

An internal FDA memorandum in 1989 notes that short-term studies cannot establish the mechanism of action. It quotes the Division of Pathology:

> While these short term studies can provide evidence suggestive of one or another mechanism, the establishment of mechanism would require longterm [sic] studies which demonstrate either enhancement or elimination of carcinogenic effect by modifying components of a putative mechanism.[68]

Since FDA's review, no such long-term studies have been conducted, to the best of our knowledge.

The memorandum concludes:

> In summary, while the sponsors of FD&C Red No. 3 have amassed certain evidence that supports their theory of the mechanism of action of this compound … there are inadequacies in other aspects of their support for their hypothesis of an indirect mechanism for FD&C Red No. 3's thyroid tumorigenesis. …
>
> With the data provided thus far by the sponsors of FD&C Red No. 3, it is equally feasible (as an alternative to their hypothesis of an indirect effect of FD&C Red No. 3) to interpret their test results as indicating an early hormonally-mediated effect on the rat thyroid, followed by thyroid compensation and a subsequent return of the gland to a normal hormonally responsive state and much later by the expression of thyroid tumors in a separate series of events that are unrelated to the hormonal perturbations shown by the rat thyroid early on in its exposure to FD&C Red No. 3. This latter scenario would assume that the final occurrence of tumors was due to a primary carcinogenic effect of FD&C Red No. 3 independent of its ability to mediate hormonal changes of the thyroid/pituitary axis.

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[66] Color Additives Notice, *supra* note 7, at 3529.

[67] Proponents filed citizen petitions in 1990 expressing disagreement with FDA conclusion, but new data were never submitted. These petitions were later withdrawn (Personal Care Products Council letter to FDA re: Docket No. 1990P-0092, June 22, 2010; and International Association of Color Manufacturers Association, Inc. letter to FDA re: Docket No. FDA-1990-P-0322, December 22, 2011).

[68] FDA Memorandum dated August 11, 1989, from David G. Hattan, Ph.D., Deputy Director, Division of Toxicological Review and Evaluation, HFF-152, to Ronald Lorentzen, Ph.D., Assistant to Director for Carcinogenicity Assessment, HFF-100, "Evaluation of Data Concerning Possible Mechanism(s) Mediating Rat Thyroid Tumorigenesis by FD&C Red No. 3."

ii. Further Research Since 1990 Has Not Established that FD&C Red No. 3 Causes Cancer in Rats Through a Secondary Mechanism.

A 1998 EPA science policy document that FDA scientists reviewed[69] provides further insight into what data are needed to establish that a substance that causes thyroid tumors acts through a secondary mechanism.[70]

In it, EPA lays out its policy that chemicals that produce rodent thyroid tumors may pose a carcinogenic hazard for the human thyroid. Five types of data are required to move away from a presumption that chemicals that produce rodent thyroid follicular cell tumors also pose a carcinogenic risk to humans.[71] These data are not available for FD&C Red No. 3.[72]

The document also notes (pp. 20-21) that hormone levels may return to normal over time, despite continuous exposure to the chemical agent, because of homeostatic compensatory increases in thyroid activity and mass—a possibility that FDA articulated with regards to FD&C Red No. 3, as discussed above. In such cases, thyroid tumors cannot be attributed to a secondary, hormonally mediated mechanism. In its 1990 decision, the Agency noted that there are no data on thyroid hormone changes beyond seven months. This is still the case. Thus, the data do not demonstrate that FD&C Red No. 3 results in long-term hormonal changes necessary to support the hypothesis that the tumors are secondary to hormonal changes.

The EPA document also states (pp. 2, 16), "[i]n the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid-pituitary disruption." Arguments that the rat is more sensitive than humans to effects on the thyroid, including thyroid cancer, as the EFSA Panel has argued, appear to be based on the premise that rats lack thyroid binding globulin (TBG), unlike humans.[73] However, multiple scientific articles

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[69] W. Gary Flamm PhD (CFSAN), Ronald Lorentzen PhD (CFSAN), David Hattan PhD (CFSAN), Margaret Ann Miller PhD (CVM), David Gaylor PhD (NCTR), Robert J. Scheuplein PhD (CFSAN).

[70] U.S. Environmental Protection Agency Risk Assessment Forum. Assessment of Thyroid Follicular Cell Tumors. EPA/630/R-97/002, March 1998. Available at: https://www.epa.gov/osa/assessment-thyroid-follicular-cell-tumors.

[71] Required data includes: information on increases in follicular cell size and number; changes in thyroid and pituitary hormones; knowledge of where the chemical affects thyroid functioning; correlations between doses producing thyroid effects and cancer; and reversibility of effects when chemical dosing ceases. Desirable information consists of knowledge of progression of lesions over time; chemical structure-activity relationships; and various other investigations (e.g., initiation-promotion studies).

[72] For example, data showing increases in cell growth, such as increased thyroid gland weight or histologic indications of cellular hypertrophy and hyperplasia, are required; yet FDA determined that "there is inconsistent evidence of cellular hypertrophy," that there was no evidence of increased thyroid weights, and that the data "fail to establish cellular hyperplasia" (Color Additive Notice, at 3538). The EPA document further states that a margin of exposure dose-response procedure based on nonlinearity (i.e., where there is a threshold below which the chemical would not cause tumors) should be used when thyroid-pituitary disruption is judged to be the sole mode of action of the observed thyroid and related pituitary tumors. This is clearly not the case; as noted by FDA in 1990, several studies suggested genotoxic potential; and since FDA's decision, additional studies suggest that FD&C Red No. 3 may be genotoxic, as described later in this section; genotoxicity is a plausible mechanism by which FD&C Red No. 3 causes tumors.

[73] EFSA provides no citations for its assertion that the high-affinity binding protein TBG is present in humans, other primates, and dogs, and is missing in rodents, birds, amphibians, and fish. This information appears to be derived

run counter to that assumption and report evidence that the rat does possess a major high affinity thyroid hormone binding protein, with properties similar to those of human TBG, that is actively synthesized in postnatal developing pups and present in adult serum in decreased amounts.[74-85] As such, it is inappropriate to dismiss the human relevance of the evidence that FD&C Red No. 3 causes thyroid cancer in rats.

While the SCF, the EFSA Panel, and JECFA have opined on the mechanism by which erythrosine causes tumors, considering it "likely" to be due to, or that the "weight of the evidence" favored, a secondary, non-genotoxic mechanism, they did not conclude that the mechanism had been established.[86] In addition, JECFA in 2018 considered that the rat was not

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from this 1985 article that examined thyroid hormone binding in serum of 15 vertebrate species, including the same taxa mentioned by EFSA: Larsson M, Pettersson T, Carlstrom A. Thyroid Hormone Binding in Serum of 15 Vertebrate Species: Isolation of Thyroxine-binding Globulin and Prealbumin Analogs. Gen Comp Endocrinol 58(3):360-75, 1985. https://doi.org/10.1016/0016-6480(85)90108-X. See European Food Safety Authority (EFSA). Scientific Opinion on the Re-evaluation of Erythrosine (E 127) as a Food Additive. *EFSA Journal 9*(1): 1854, 2011, p. 32. https://doi.org/10.2903/j.efsa.2011.1854.

[74] Savu L., Vranckx R., Maya M., Nunez E.A. A Thyroxine Binding Globulin (TBG)-Like Protein in the Sera of Developing and Adult Rats. Biochem Biophys Res Commun 148(3):1165-73, 1987. https://doi.org/10.1016/S0006-291X(87)80255-3.

[75] [Article in French]. Savu L., Vranckx R., Maya M., Nunez E.A. [Demonstration and Ontogenesis in the Rat of a Serum Protein Analogous to Human Thyroxine Binding Globulin.] C R Acad Sci III 305(17):627-32, 1987. https://pubmed.ncbi.nlm.nih.gov/2448017/.

[76] Savu L., Vranckx R., Maya M., Nunez E.A. Binding Activities of Thyroxine Binding Globulin Versus Thyroxine Binding Prealbumin in Rat Sera: Differential Modulation by Thyroid Hormone Ligands, Oleic Acid and Pharmacologic Drugs. Biochem Biophys Res Commun 159(3):919-26, 1989. https://doi.org/10.1016/0006-291X(89)92196-7.

[77] Vranckx R,. Savu L., Nunez E.A. The Microheterogeneity of Rat TBG. FEBS Lett 244(2):343-6, 1989. https://doi.org/10.1016/0014-5793(89)80559-9.

[78] Emerson C.H., Cohen J.H. 3rd, Young R.A. et al. Gender-Related Differences of Serum Thyroxine-binding Proteins in the Rat. Acta Endocrinol (Copenh) 123(1):72-8, 1990. https://doi.org/10.1530/acta.0.1230072.

[79] Vranckx R., Rouaze M., Savu L. et al. The Hepatic Biosynthesis of Thyroxine Binding Globulin (TBG): Demonstration, Ontogenesis, and Up-regulation in Experimental Hypothyroidism. Biochem Biophys Res Commun 167(1):317-22, 1990. https://doi.org/10.1016/0006-291X(90)91767-M.

[80] Imamura S,. Mori Y., Murata Y. et al. Molecular Cloning and Primary Structure of Rat Thyroxine-binding Globulin. Biochemistry 30(22):5406-11, 1991. https://doi.org/10.1021/bi00236a012.

[81] Rouaze-Romet M. et al. Structural and Functional Microheterogeneity of Rat Thyroxine-binding Globulin During Ontogenesis. Biochem J 286(Pt 1):125-30, 1992. https://doi.org/10.1042/bj2860125.

[82] Savu L., Vranckx R., Rouaze-Romet M., Nunez E.A.. The Pituitary Control of Rat Thyroxine Binding Globulin. Acta Med Austriaca 19 Suppl 1:88-90, 1992. https://pubmed.ncbi.nlm.nih.gov/1519464/.

[83] Emerson C.H., Seiler C.M., Alex S. et al. Gene Expression and Serum Thyroxine-binding Globulin are Regulated by Adrenal Status and Corticosterone in the Rat. Endocrinology 133(3):1192-6, 1993. https://doi.org/10.1210/endo.133.3.8365361.

[84] Tani Y., Mori Y., Miura T. et al. Molecular Cloning of the Rat Thyroxine-binding Globulin Gene and Analysis of its Promoter Activity. Endocrinology 135(6):2731-6, 1994. https://doi.org/10.1210/endo.135.6.7988464.

[85] Duan J., Kang J., Deng. T. et al. Exposure to DBP and High Iodine Aggravates Autoimmune Thyroid Disease Through Increasing the Levels of IL-17 and Thyroid-binding Globulin in Wistar Rats. Toxicol Sci 163(1):196-205, 2018. https://doi.org/10.1093/toxsci/kfy019.

[86] The SCF speculated in 1989 that "the oncogenic effects seen in the long-term studies were likely to be secondary to the effects of erythrosine on thyroid and pituitary function." Commission of the European Communities. Reports of the Scientific Committee for Food, Twenty-first series, Report EUR 11617, p. 11, 1989, http://aei.pitt.edu/40830/1/21st_food.pdf. Similarly, JECFA stated in 1990 that, "the Committee considered that the occurrence of thyroid tumours in rats was most likely secondary to hormonal effects." Joint FAO/WHO Expert

"a suitable model for potential effects on the thyroid in humans,"[87] contrary to EPA's policy, as noted above, that, in the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid-pituitary disruption. Part 3 of Appendix D provides a more detailed summary and analysis of the EFSA and JECFA reviews addressing the extrapolation of thyroid effects from rat studies to humans.

The International Agency for Research on Cancer (IARC) of the World Health Organization recently updated its procedures to incorporate the use of "key characteristics" of carcinogens for evaluating mechanistic evidence on carcinogenicity.[88] In addition to genotoxicity, other characteristics include the ability to modulate receptor-mediated effects and alter DNA repair or cause genomic instability. In fact, FD&C Red No. 3 exhibits some of these characteristics. A 2011 study found that FD&C Red No. 3 was the only color additive certified for use in food that inhibited important receptor-ligand type protein-protein interactions in the tumor necrosis factor (TNF) superfamily, which play a role in the immune system and inhibiting tumorigenesis. FD&C Red No. 3 was found to be a non-specific, promiscuous, and relatively potent inhibitor of such protein-protein interactions.[89] In addition, FD&C Red 3 was the only color additive of several tested that stimulated the growth of estrogen receptor-positive human breast cancer cells, which it did in a dose-response manner.[90] These are described more in Appendix D Part 3 and lend support to the conclusion that FD&C Red No. 3 induces cancer.

Our understanding of the genotoxic potential of FD&C Red No. 3 does not appear to have substantially changed since FDA's Genetic Toxicology Branch reviewed the evidence in 1989. FDA's Carcinogenicity Assessment Committee stated in 1989 that, "[w]hile FD&C Red No. 3 produced negative findings in many assays … the more recent studies indicate an apparent capacity for the color additive to induce chromosomal effects, in vitro and in vivo, and gene mutation in cultured mammalian cells. On the basis of these mixed findings, the Committee

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Committee on Food Additives. Evaluation of certain food additives and contaminants. Thirty-seventh report. WHO Technical Report Series 806, 1991, p. 20. http://apps.who.int/iris/bitstream/handle/10665/40288/WHO_TRS_806.pdf. In 2011 EFSA said, "Two studies have shown that Erythrosine has an oncogenic effect in the thyroid gland of rats. The weight-of-evidence is that these tumours are elicited by a non-genotoxic mechanism. European Food Safety Authority (EFSA). Scientific Opinion on the Re-evaluation of Erythrosine (E 127) as a Food Additive. *EFSA Journal 9*(1): 1854, 2011, p. 32. https://doi.org/10.2903/j.efsa.2011.1854. Similarly, in 2018, JECFA said, "Two long-term feeding studies with erythrosine found an increase in the incidence of thyroid follicular cell adenomas in male rats…The previous Committee considered the occurrence of thyroid follicular tumours in rats secondary to hormonal effects based on results from studies on thyroid function and morphology…The present Committee … confirmed that the overall weight of evidence indicates that erythrosine is not genotoxic." Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants. Eighty-sixth report. WHO Technical Report Series 1014, 2019, p. 29. https://apps.who.int/iris/bitstream/handle/10665/279832/9789241210232-eng.pdf.

[87] Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants. Eighty-sixth report. WHO Technical Report Series 1014, 2018, p. 29. https://apps.who.int/iris/bitstream/handle/10665/279832/9789241210232-eng.pdf.

[88] Samet J.M., Chiu W.A., Cogliano V. et al. The IARC Monographs: Updated procedures for modern and transparent evidence synthesis in cancer hazard identification. J Natl Cancer Inst 112(1):30-37, 2020. https://doi.org/10.1093/jnci/djz169.

[89] Ganesan L., Margolles-Clark E., Song Y., Buchwald P. The food colorant erythrosine is a promiscuous protein-protein interaction inhibitor. Biochem Pharm 81(6):810-818, 2011. https://doi.org/10.1016/j.bcp.2010.12.020.

[90] Dees C., Askari M., Garrett S. et al. Estrogenic and DNA-damaging activity of Red No. 3 in human breast cancer cells. *Environ Health Perspect 105*(Suppl 3):625-632, 1997. https://doi.org/10.1289/ehp.97105s3625.

cannot conclude that FD&C Red No. 3 is not mutagenic."[91] Since 1990, six additional studies suggest that FD&C Red No. 3 may be genotoxic (Chequer et al. (2014 and 2017), Hagiwara et al. (2006), Mekkawy et al. (2000), Metwaly et al. (2018), Sasaki et al. (2002)). The remaining genotoxicity studies conducted since 1990 were either inconclusive, or the results were negative under the conditions of the study.[92] These studies are described in more detail in Appendix D Part 3.

In sum, a secondary carcinogenesis mechanism has not been established for FD&C Red No. 3, and even if it had, that would not excuse FDA from acting to delist the additive.

IV. Delisting FD&C Red No. 3 Should Be a High Priority as the Carcinogenic Substance Is Widely Used in Many Foods, Drinks, Dietary Supplements, and Drugs, Resulting in High Exposure to Young Children.

Petitioners urge immediate action in delisting FD&C Red No. 3 as many Americans are being exposed to this carcinogenic substance. According to FDA, FD&C Red No. 3 is in baby foods, breakfast cereal, cakes and cupcakes, chewing gum, cookies, decoration/chips for baking, dried fruit, frostings and icings, frozen breakfast foods, hard candy, ice cream/frozen yogurt/sherbet, ice cream cones, ice pops, meal replacement drinks and bars, soft candy/gummies, and toaster pastries.[93] FD&C Red No. 3 is also used in dietary supplements and oral drugs.[94] We obtained 2,555 results using the term RED 3 (no quotes) as an inactive ingredient in human drugs (including both prescription and over the counter drugs) in DailyMed, a database sponsored by the National Library of Medicine which contains labeling submitted to FDA by companies.[95] In 2016, FDA estimated that 84% of the U.S. population aged two years and older were exposed to FD&C Red No. 3 through food alone (excluding drugs), based on 10-14 day food consumption data.[96] The food and drug industries used 215,780.42 pounds of FD&C Red No. 3 in 2021 alone.[97]

The FDA's most recent exposure estimates from food were published in 2016 and indicate that individuals in the U.S. aged two years and older consume anywhere from 0.7 mg per person per day (mean intake under a lower-exposure scenario) to 3.2 mg per person per day of FD&C Red No. 3 (both the mean and 90th percentile, under a high-exposure scenario), using 10-14 day food

[91] Document obtained via FOIA, "FDCRED3-198090421_FinalMemo-CAC_Redacted" containing "Memorandum of Conference" dated April 21, 1989, Meeting of the Cancer Assessment Committee.
[92] See Appendix D Part 3. For example, Kawaguchi et al. (2001) found dose-related DNA damage in mice sacrificed 3 hours after exposure but not in groups sacrificed 24 hours after exposure.
[93] Doell D.L., Folmer D.E., Lee H.S., *et. al*. Exposure Estimate for FD&C Colour Additives for the US Population. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2016 May; 33(5):782-797. https://doi.org/10.1080/19440049.2016.1179536.
[94] Drugs.com. FD&C Red No. 3 Excipient. Top Medications with this Excipient. https://www.drugs.com/inactive/fd-c-red-no-3-247.html.
[95] National Library of Medicine. DailyMed. [Note: Search does not display on all browsers]. Search completed September 13, 2022. DailyMed - Search Results for INACTIVE_INGREDIENT:(RED 3) (nih.gov).
[96] Doell D.L., Folmer D.E., Lee H.S., *et. al*. Exposure Estimate for FD&C Colour Additives for the US Population. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2016 May; 33(5):782-797. https://doi.org/10.1080/19440049.2016.1179536.
[97] FDA., Color Certification Reports for January1 2021 through December 31 2021 (through first quarter FY 2020), https://www.fda.gov/industry/color-certification/color-certification-reports .

consumption data (eaters only).[98,99] On a body weight basis, children two to five years old consume about twice as much through food as the general population, according to FDA's estimates.[100]

In addition, according to FDA's database on inactive ingredients in approved drugs,[101] the maximum daily exposure (MDE) of FD&C Red No. 3 from chewable tablets can be 1 mg/day (this is the only form where an MDE is given). Some oral suspensions can contain 1 mg of FD&C Red No. 3 per 5 ml dose according to the FDA database and some capsules can contain over 11 mg per unit dose. For FD&C Red No. 3 aluminum lake, chewable tablets can contain 4.25 mg per unit dose.

In 2021, the California Office of Environmental Health Hazard Assessment (OEHHA) published a health hazard assessment of potential neurobehavioral effects of synthetic food dyes in children (hereafter, the 2021 OEHHA report).[102] The 2021 OEHHA report estimated intakes of certified color additives, including FD&C Red No. 3 intake, for pregnant women, women of childbearing years (18-49), and children of five different age groups, using a similar methodology as FDA.[103]

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[98] Prior to terminating the provisional uses of FD&C Red No. 3, FDA estimated that exposure from dietary ingestion was 9 mg/day both for young children (ages 2-5, equivalent to 600 ug/kg of body weight/day) and other age groups (ages 2 plus, equivalent to 150 ug/kg of body weight/day); that consumers may be exposed to an additional 1 to 3 mg FD&C Red No. 3 per day from ingested drugs or dietary supplements (17-50 ug/kg of body weight per day, derived from a 60 kg body weight); and that in patients consuming drug syrups, the combined short-term exposure to FD&C Red No. 3 may increase to almost 2-fold the levels estimated for chronic exposure, to approximately 20 mg/day, or 1300 ug/kg of body weight/day for children 2-5. These estimates are provided in a FDA Memorandum dated August 11, 1989, from David G. Hattan, Ph.D., Deputy Director, Division of Toxicological Review and Evaluation, HFF-152, to Ronald Lorentzen, Ph.D., Assistant to Director for Carcinogenicity Assessment, HFF-100, "Evaluation of Data Concerning Possible Mechanism(s) Mediating Rat Thyroid Tumorigenesis by FD&C Red No. 3," which cited Food and Color Additives Review Section memorandum, December 11, 1986 to Division of Food and Color Additives. In 2011 FDA estimated that the daily intake of FD&C Red No. 3 in the U.S. population from all food sources was 0.61 mg per person per day, and 6.1 mg per person per day for high consumers, using the amount batch certified, and assuming that 73 percent of color additives certified are used for human food in the United States.

[99] Doell et al. Op. Cit. (Table 6) states that 0.7 mg per day is equivalent to 0.01 mg per kg of body weight, and that 3.2 mg/day is equivalent to 0.07 mg per kg of body weight for mean intake under the high-exposure scenario, and 0.05 mg per kg of body weight for the 90th percentile intake under the high-exposure scenario.

[100] Doell et al. Op. Cit. (Table 7) states that mean intake under a lower-exposure scenario is 0.02 mg per kg of body weight, and that the 90th percentile intake under a high exposure scenario is 0.1 mg per kg of body weight (compare to values in previous footnote).

[101] U.S. Food and Drug Administration. Inactive Ingredients in Approved Drug Products Search. Database Last Updated October 19, 2022 (Data Through October 1, 2022). https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm.

[102] California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Children's Environmental Health Center. Health Effects Assessment: Potential Neurobehavioral Effects of Synthetic Food Dyes in Children. April 2021. https://oehha.ca.gov/risk-assessment/report/health-effects-assessment-potential-neurobehavioral-effects-synthetic-food.

[103] Both FDA and OEHHA used NHANES food consumption data, but OEHHA used more recent data (2015-2016) than FDA (which used 2007-2010 NHANES data). OEHHA produced estimates that covered different populations (i.e., pregnant women, women of childbearing years (18-49)) and more age groups of children (0-<2 years, 2-<5 years, 5-<9 years, 9-<16 years, 16-18 years), than FDA (FDA used the US population over 2, children 2-5, and males 13-18). OEHHA reported mean, median, 75th and 95th percentile consumption, whereas FDA reported mean and 90th percentile. Both assessments used FDA measurements of FD&C color additives, as reported in Doell et al.

The 2016 FDA and 2021 OEHHA report estimates are comparable for similar groups under similar scenarios.[104]

However, the highest exposures on a body weight basis to Red No. 3, according to the OEHHA 2021 report, were for children under two, an age range that FDA did not consider. This is significant given that early life is a period of potentially increased susceptibility to carcinogens.[105] The estimates for single day exposures in the OEHHA 2021 report for this youngest age group reached 7.90 mg/kg of body weight under the high-exposure scenario and 4.83 mg/kg of body weight under the typical exposure scenario at the 95th percentile, which exceeded FDA's acceptable daily intakes (ADIs).[106]

Exposure to FD&C Red No.3 is much lower in other countries where the color additive is limited to certain foods. For example, in the European Union, erythrosine is exclusively authorized for use in cocktail and candied cherries, and Bigarreaux cherries.[107] Other countries, including Australia and New Zealand, as well as the Codex Alimentarius Commission, also limit the use of

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2016, although the OEHHA 2021 report also incorporated updated food dye concentration measurements in certain critical foods from UC Davis. FDA used 2 day and 10-14 day food consumption data and three exposure scenarios (low, average, high) whereas OEHHA calculated exposure over 1 and 2 days, and two exposure scenarios (typical and high). For many FD&C color additives and food categories, the FDA and UC Davis measurements were similar. However, the maximum FD&C Red No. 3 content that UC Davis reported in frostings and icings was lower than what FDA reported in 2016.

[104] For example, for children 2-5 using 2-day food consumption data, and an average or typical exposure scenario, FDA's mean estimate is 0.1 mg/kg of body weight and OEHHA's is 0.07 mg/kg of body weight; for a high exposure scenario, FDA's mean estimate is 0.2 mg/kg of body weight and OEHHA's is 0.17 mg/kg of body weight.

[105] U.S. Environmental Protection Agency. Supplemental Guidance for Assessing Susceptibility from Early-life Exposure to Carcinogens. EPA/630/R-03/003F, 2005, Washington, DC. Available at: https://www.epa.gov/risk/supplemental-guidance-assessing-susceptibility-early-life-exposure-carcinogens; World Health Organization. Summary of Principles for Evaluating Health Risks in Children Associated with Exposure to Chemicals. 2011. Available at https://apps.who.int/iris/bitstream/handle/10665/44533/9789241501170_eng.pdf; Hines RN et al. Approaches for Assessing Risks to Sensitive Populations: Lessons Learned from Evaluating Risks in the Pediatric Population. Toxicol Sci 133(1):4-26, 2010. https://doi.org/10.1093/toxsci/kfp217; California Environmental Protection Agency. In Utero and Early Life Susceptibility to Carcinogens: The Derivation of Age-at-exposure Sensitivity Measures. May 2009. Available at https://oehha.ca.gov/media/downloads/crnr/appendixjearly.pdf.

[106] California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Children's Environmental Health Center. Health Effects Assessment: Potential Neurobehavioral Effects of Synthetic Food Dyes in Children. April 2021. https://oehha.ca.gov/risk-assessment/report/health-effects-assessment-potential-neurobehavioral-effects-synthetic-food.

[107] European Food Safety Authority (EFSA). Scientific Opinion on the Re-evaluation of Erythrosine (E 127) as a Food Additive. *EFSA Journal 9*(1): 1854, 2011, citing European Parliament and Council Directive 94/36/EC of 30 June 1994. https://doi.org/10.2903/j.efsa.2011.1854.

erythrosine to certain foods and establish maximum levels,[108] and in Korea, the use of most synthetic colors is prohibited in certain foods preferred by children and teenagers.[109]

However, in the United States, use of FD&C Red No. 3 in foods is unrestricted; it can be used in any food and there are no numerical limits established.

None of FDA's 2016 exposure estimates for FD&C Red No. 3 exceed FDA's ADI.[110] However, this ADI is not appropriate or sufficient. It was established prior to FD&C Red No. 3's approval in 1969, before information was available that FD&C Red No. 3 is a carcinogen,[111] and it is widely recognized by authorities, including FDA, that it is inappropriate to establish an ADI for carcinogens.[112] Furthermore, even setting aside the fact that FD&C Red No. 3 is carcinogenic, the ADI is inadequate for non-cancer effects. The OEHHA 2021 report identified animal studies showing effects at doses lower than the no observed adverse effect level (NOAEL) used by FDA to set its now outdated ADI.[113]

V. Request for Fee Waiver

Pursuant to 21 C.F.R 70.19(q), petitioners request a waiver of the color additive petition fees and deposit requirements. The petitioners are non-profit organizations and individuals who submit this petition because it is in the public interest to protect public health by reducing carcinogenic

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[108] In Australia and New Zealand, erythrosine is limited to use in preserved cherries (maximum 200 mg/kg) and icing and frosting (maximum 2 mg/kg). [Source: Australia New Zealand Food Standards Code, Schedule 15, Substances That May Be Used As Food Additives. https://www.legislation.gov.au/Details/F2021C00607.] In the Codex Alimentarius General Standard for Food Additives, erythrosine is limited to use in six foods. Limits range from 30 mg/kg to 200 mg/kg. [Source: FAO/WHO Food Standards, Codex Alimentarius. General Standard for Food Additives (GSFA) Online. Erythrosine (127). https://www.fao.org/gsfaonline/additives/details.html?id=87.

[109] Ha M-S. et al. Exposure Assessment of Synthetic Colours Approved in Korea. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2013;30(4):643-53. https://doi.org/10.1080/19440049.2013.768358.

[110] The ADI is 2.5 mg/kg-bw/day or 75 mg/day for a 30 kg child. Food and Drug Administration. Background Document for the Food Advisory Committee: Certified Color Additives in Food and Possible Association with Attention Deficit Hyperactivity Disorder in Children. March 30-31, 2011.

[111] As noted by OEHHA in its 2021 report (p. 255), FDA's ADI was established using a chronic rat study conducted by FDA in the 1950s, based on "distended cecum."

[112] U.S. Food and Drug Administration. Toxicological Principles for the Safety Assessment of Food Ingredients: Redbook 2000. July 2000, Revised July 2007. Chapter II. Agency Review of Toxicology Information Submitted in Support of the Safety Assessment of Food Ingredients (available in 1993 Draft "Redbook II"). This guidance states "*For non-cancer endpoints* [emphasis added], the NOEL is divided by a safety factor to obtain an estimate of the maximum ADI of the additive for humans." It has a separate section on carcinogenic risk assessment, which FDA uses primarily for estimating the risk from carcinogenic contaminants and for setting priorities. It states, "In general, FDA and CFSAN follow the National Research Council guidelines for risk assessment, described in *Risk Assessment in the Federal Government: Managing the Process*." This 1983 report further clarifies the different approaches to risk assessment for carcinogenic vs. non-carcinogenic substances, for example stating that, "In all cases *except that of carcinogens* [emphasis added], establishment of acceptable intakes was accomplished by applying safety factors to experimentally derived no-observed-effect exposures . . . This approach continues to be used for noncarcinogenic food additives …." National Research Council. Risk Assessment in the Federal Government: Managing the Process. National Academy Press, 1983, p. 53.

[113] California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Children's Environmental Health Center. Health Effects Assessment: Potential Neurobehavioral Effects of Synthetic Food Dyes in Children. April 2021, page 255. https://oehha.ca.gov/risk-assessment/report/health-effects-assessment-potential-neurobehavioral-effects-synthetic-food.

exposures, including to FD&C Red No. 3. They have no financial interests in FD&C Red No. 3 or any of the alternatives that may benefit from removing this color additive from the market. Waiver of the fee under these circumstances promotes the public interest by removing a financial barrier that would otherwise serve as a deterrent to such efforts.

VI. Conclusion: The FDA Must Revoke the Permanent Listing of FD&C Red No. 3 for Use in Food, Dietary Supplements, and Ingested Drugs, and Should Do So Quickly.

In light of the evidence, the FDA cannot continue to permit the use of FD&C Red No. 3 in any product it regulates. The FDA has acknowledged that FD&C Red No. 3 causes tumors in rats since 1982.[114] In 1990, the FDA determined that there is a cancer risk posed by FD&C Red No. 3 and, citing the Delaney Clause, terminated the provisional uses of FD&C Red No. 3. Illogically, the FDA continues to allow manufacturers to put this color additive into foods and drugs that are directly ingested and represent a far greater source of exposure than had the uses that the agency banned more than a quarter-century ago. FD&C Red No. 3 is unsafe, and its listing for use in food, dietary supplements, and ingested drugs violates the Delaney Clause.

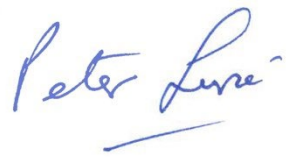
The FDA must therefore remove approval of FD&C Red No. 3 by amending the permanent list of color additives approved for use in food, including dietary supplements, 21 C.F.R. § 74.303.

We further urge the FDA to revoke approval of FD&C Red No. 3 for use in drugs at 21 C.F.R. § 74.1303. Per 21 U.S.C. § 351(a), ingested drugs that contain an unsafe color additive are adulterated, and therefore cannot remain FDA-approved.[115]

This petition contains no confidential information and should be included in the docket for any regulatory action so that the public can assess the information.

If you have questions or comments, please contact Jensen Jose, our agent on this petition, at jjose@cspinet.org, and copy Thomas Galligan (tgalligan@cspinet.org) and Lisa Lefferts (llefferts@earthlink.net) on all responses.

Sincerely,



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[114] Color Additives Notice, pp. 3524, 3537.
[115] 21 U.S.C. § 351(a).

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Appendices: Responses to Information Required by 21 C.F.R. § 71.1

Per 21 C.F.R. § 71.1, we provide responses to the information requested for color additive petitions specified under the several lettered headings, submitted on separate pages and suitably identified.

Appendix A: Name and Pertinent Information Concerning the Color Additive

As explained by FDA in 1990, "FD&C Red No. 3, a bluish red color of the xanthene class, is identified in Chemical Abstracts as the disodium salt of 3′,6′-dihydroxy-2′,4′,5,7′-tetraiodospiro[isobenzofuran-1(3H),9′-[9H]xanthen]-3-one (CAS Reg. No. 16423-68-0). FDA and industry communications have established the common name "fluorescein" as a means of identifying derivatives of that chemical moiety. Therefore, FDA identifies this color additive as principally the disodium salt of 2′,4′,5′,7′-tetraiodofluorescein (CAS Reg. No. 16423-68-0) with smaller amounts of the disodium salts of 2′,4′,5′-triiodofluorescein (CAS Reg. No. 56254-06-9) and 2′,4′,7′-triiodofluorescein (CAS Reg. No. 83498-90-2). The designation "FD&C Red No. 3" is permitted only for those batches of the color additive that the agency has certified to be in compliance with § 74.303 (21 CFR § 74.303). Uncertified material is commonly called erythrosine or other names, including Colour Index (C.I.) Acid Red 51; C.I. No. 45430; and C.I. Food Red 14."[116]

FDA regulations[117] provide information regarding the chemical identity and composition, properties, and specifications of FD&C Red No. 3:

"Sec. 74.303 FD&C Red No. 3.

(a) *Identity.* (1) The color additive FD&C Red No. 3 is principally the monohydrate of 9 (*o*-carboxyphenyl)-6-hydroxy - 2,4,5,7-tetraiodo-3H-xanthen-3-one, disodium salt, with smaller amounts of lower iodinated fluoresceins.

(2) Color additive mixtures for food use made with FD&C Red No. 3 may contain only those diluents that are suitable and that are listed in part 73 of this chapter as safe for use in color additive mixtures for coloring foods.

(b) *Specifications.* FD&C Red No. 3 shall conform to the following specifications and shall be free from impurities other than those named to the extent that such other impurities may be avoided by good manufacturing practice:

Volatile matter (at 135 deg. C.) and chlorides and sulfates (calculated as the sodium salts), total not more than 13 percent.

Water-insoluble matter, not more than 0.2 percent.

Unhalogenated intermediates, total not more than 0.1 percent.

Sodium iodide, not more than 0.4 percent.

[116] Termination of Provisional Listings of FD&C Red No. 3 for Use in Cosmetics and Externally Applied Drugs and of Lakes of FD&C Red No. 3 for All Uses, 55 Fed. Reg. 3516 (February 1, 1990).
[117] 21 C.F.R § 74.303.

Triiodoresorcinol, not more than 0.2 percent.

2(2',4'-Dihydroxy-3', 5'-diiodobenzoyl) benzoic acid, not more than 0.2 percent.

Monoiodofluoresceins not more than 1.0 percent.

Other lower iodinated fluoresceins, not more than 9.0 percent.

Lead (as Pb), not more than 10 parts per million.

Arsenic (as As), not more than 3 parts per million.

Total color, not less than 87.0 percent.

(c) *Uses and restrictions.* FD&C Red No. 3 may be safely used for coloring foods generally (including dietary supplements) in amounts consistent with good manufacturing practice except that it may not be used to color foods for which standards of identity have been promulgated under section 401 of the act unless added color is authorized by such standards."

In FDA's Substances Added to Food (formerly EAFUS),[118] the following other names are listed:

- FD&C RED NO. 3
- ERYTHROSINE
- DISODIUM 2',4',5',7'-TETRAIODOFLUORESCEIN
- ERYTHROSINE BS
- SODIUM ERYTHROSIN
- C.I. ACID RED 51
- D&C RED NO. 3
- EBS
- SPIRO(ISOBENZOFURAN-1(3H),9'-(9H)XANTHEN)-3-ONE, 3',6'-DIHYDROXY-2',4',5',7'-TETRAIODO-, DISODIUM SALT
- FLUORESCEIN, 2',4',5',7'-TETRAIODO-, DISODIUM SALT
- C.I. FOOD RED 14
- C.I. 45430
- ERYTHROSINE B
- DISODIUM 9-(O-CARBOXYPHENYL)-6-HYDROXY-2,4,5,7-TETRAIODO-3H-XANTHEN-3-ONE MONOHYDRATE
- DISODIUM 3',6'-DIHYDROXY-2',4',5',7'-TETRAIODOSPIRO(ISOBENZOFURAN-1(3H),9'-(9H)XANTHEN)-3-ONE
- ACID RED 51
- FOOD RED 14

[118] U.S. Food and Drug Administration. Substances Added to Food (formerly EAFUS). https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus.

- ERYTHROSINE SODIUM
- FOOD RED NO. 3

Appendix B: The Amount of the Color Additive Proposed for Use

None. FD&C Red No. 3 presents a cancer risk and therefore is not a permissible color additive.

Appendix C: Methods

We are requesting FDA to remove its approval for the remaining uses of FD&C Red No. 3 as a color additive, effectively banning its use. Currently, FD&C Red No. 3 is batch certified by FDA using analytical chemistry methods developed for this purpose by the FDA. Certification analytical methods are available from FDA. Methods for the detection of FD&C Red No. 3 in foods have also been developed by FDA and are described in an article published by FDA scientists.[119]

[119] Doell et al. Op. Cit.

Appendix D: Full Reports of Investigation Made with Respect to the Safety of the Color Additive

Part 1: Pivotal Studies/Evaluations Relied on by FDA to Conclude FD&C Red No. 3 is Carcinogenic

The descriptions of these studies are primarily taken from FDA's description in its Denial of Petition for Listing of FD&C Red No. 3 for Use in Cosmetics and Externally Applied Drugs (55 Fed. Reg. 3520-01 (Feb 1, 1990)).

Certified Color Manufacturers Association, International Research and Development Corporation (IRDC) Study No. 410-002, August 31, 1981; CAP No. 96.[120]

> According to FDA, the dosage levels of FD&C Red No. 3 were 0, 0, 0.1, 0.5, and 1.0 % of the diet. Mean thyroid weight was higher in females in the 0.5% and 1% dose levels compared with controls. CCMA contended that there were no significant results for neoplastic lesions. However, FDA's microscopic examination revealed statistically significant, higher incidences of male rats with combined thyroid follicular cell adenomas and carcinomas in 0.1%, 0.5%, and 1% dose groups, compared with the combined control animals ($p = 0.016, 0.0007, 0.029$, respectively).[121]

Certified Color Manufacturers Association, International Research and Development Corporation (IRDC) Study No. 410-011, August 2, 1982; CAP No. 96[122]

> After Study No. 410-002 (above) had begun, FDA concluded that the results of the pre-1976 studies on FD&C Red No. 3 and the multigeneration reproduction study then underway showed that the animals could tolerate a higher dose level. The agency, therefore, requested this additional chronic feeding study in rats using the 4% dose level. Thyroid gland enlargement (as determined by increased weight) occurred in the male rats in the 4% treated group. FDA disagreed with CCMA's reporting of results. FDA's review found 14/68 or 20.6% follicular cell adenomas in the 4% group compared with 1/68 or 1.5% in the controls, and carcinomas in 5/68 or 7.4% of the 4% group compared with 1/68 or 1.5% in the controls. The agency's analysis demonstrated a very large statistically significant increase ($p < 0.0007$) in the incidence of combined adenomas and carcinomas: 18/68 (26.5%) in the 4% group compared with 2/68 (2.9%) in controls. The incidence of thyroid follicular cell hyperplasia in the treated animals was also higher than that in rats in the concurrent control group. The agency also confirmed that there were a few more rats with parafollicular cell (C-cell) tumors in the 4% treated group compared with the control group. Given the variability in the spontaneous occurrence of C-cell

[120] As cited in FDA Memorandum from David G. Hattan, Ph.D., Deputy Director, Division of Toxicological Review and Evaluation, HFF-152 to Ronald Lorentzen, PhD, Assistant to Director for Carcinogenicity Assessment, HFF-100, Evaluation of data concerning possible mechanism(s) mediating rat thyroid tumorigenesis by FD&C Red No. 3, August 11, 1989.

[121] Color Additives Notice p. 3524.

[122] FDA Memorandum from David G. Hattan, Ph.D., FDA Deputy Director, Division of Toxicological Review and Evaluation, HFF-152 to Ronald Lorentzen, PhD, Assistant to Director for Carcinogenicity Assessment, HFF-100, Evaluation of data concerning possible mechanism(s) mediating rat thyroid tumorigenesis by FD&C Red No. 3, August 11, 1989.

lesions in the rat, however, FDA declined to attribute the C-cell lesions in the rat study to the administration of FD&C Red No. 3.[123]

National Toxicology Program Board of Scientific Counselors, Technical Reports Review Subcommittee. Report. December 27, 1983.

At FDA's request, an NTP Subcommittee conducted a peer review of the IRDC Study Nos. 410-002 and 410-011 data. Based upon its review, the Subcommittee concluded that there is "convincing evidence of carcinogenicity" of FD&C Red No. 3 in male rats. The NTP Subcommittee also considered the combining of carcinomas and adenomas to be appropriate. On the basis of the existing evidence, the Subcommittee concluded that no determination could be made as to the mechanism (primary or secondary) of carcinogenic effects for FD&C Red No. 3 in the thyroid of male rats. It agreed that new data presented at the meeting by consultants for the Certified Color Manufacturers Association did not change its conclusions. It recommended additional studies, including more definitive studies on the genotoxic potential, not only in microbial systems but also in mammalian cells; further clarification of apparent metabolic effects of the color as evidenced by increased food consumption, decreased body weight and alterations in levels of thyroid hormones (triiodothyronine (T3) and thyroxine (T4)) and thyroid stimulating hormone (TSH, also called thyrotropin), as well as determination of a no effect level for inhibition of T4 conversion to T3; and studies on the pharmacokinetics of the color in male rats encompassing gastro-intestinal absorption, biotransformation, tissue binding and storage, and excretion.[124] Additional studies were submitted by the petitioners and are described in Part 2 of this Appendix.

Part 2: Other Relevant Studies/Evaluations Considered by FDA

The descriptions of the unpublished studies are primarily taken from FDA's description in its Denial of Petition for Listing of FD&C Red No. 3 for Use in Cosmetics and Externally Applied Drugs (55 Fed. Reg. 3520-01 (Feb 1, 1990)).

Borzelleca, J.F, CC Capen, Hallagan JB. Lifetime toxicity/carcinogenicity study of FD&C Red No. 3 (erythrosine) in rats. Food and Chemical Toxicology 25(10): 723-33, 1987. https://doi.org/10.1016/0278-6915(87)90226-2.

This is the published version of the IRDC studies in Charles River CD-1 rats exposed in utero to FD&C Red No. 3, listed above under Part 1 (pivotal studies). The published version differed from the unpublished version, according to FDA: "Significantly, Borzelleca, Capen, and Hallagan did not report that any of the treated rats sacrificed at 1 year showed evidence of thyroid follicular cell hyperplasia."[125]

Borzelleca, JF, Hallagan JB. Lifetime toxicity/carcinogenicity study of FD&C Red No. 3 (erythrosine) in mice. Food and Chemical Toxicology 25(10): 735-37, 1987. https://doi.org/10.1016/0278-6915(87)90227-4.

[123] Color Additives Notice, p. 3524, 3525
[124] Color Additives Notice, p. 3524.
[125] Color Additives Notice, p. 3534.

This is the published version of IRDC Study No. 410-005 in Charles River CD-1 mice, below.

Certified Color Manufacturers Association, International Research and Development Corporation (IRDC) Study No. 410-005, submitted August 31, 1981; CAP No. 96.

Tested 60 animals/sex/dose group at five different dose levels (including controls). FDA concluded that long-term exposure of Charles River CD(R)-1 mice to FD&C Red No. 3 did not produce a carcinogenic response.[126]

Certified Color Manufacturers' Association. Study of the hormonal effects of FD&C Red No. 3 in rats. Bio/dynamics, 1989.

A 60-day study in male rats designed to provide evidence that FD&C Red No. 3 causes hormonal effects resulting in increased stimulation of the thyroid by TSH, and if evident, determine the threshold for these effects. Animals were fed 0.0, 0.25, or 4% FD&C Red No. 3. FDA stated that the proponents used inappropriate methods of statistical analysis to evaluate hormonal results and reanalyzed the results, concluding that both dose groups resulted in a statistically significant increase of TSH, T4, and reverse triiodothyronine (rT3; an isomer of T3 levels compared to control values throughout most of the study, and a statistically significant decrease in serum T3 as compared with controls at day 30 for the 0.25% dose and throughout the study for the 4% dose. These effects were dose related. FDA concluded that the morphological results were paradoxical, and thus inconclusive, since effects during the first 30 days (e.g., decreased follicle size) were reversed (e.g., increased follicle size) in the second 30-day period.[127]

Certified Color Manufacturers' Association. Final report on the absorption, distribution, metabolism, and excretion of FD&C Red No. 3 in rats, Hazleton Laboratories, 1989.

This study examined the tissue distribution and urinary and fecal excretion of 14 C-labeled erythrosine and 125 I-labeled erythrosine after oral administration to rats. The rats received the 14 C- or 125 I-labeled erythrosine by gavage after consuming diets containing 0%, 0.5%, or 4% FD&C Red No. 3 in the diet. The excretion patterns and magnitude of the radioactive residues in the liver, kidney, and blood were not dependent on sex, radiolabel, or the amount of FD&C Red No. 3 in the diet. The results suggest that the thyroid was saturated with iodide prior to the administration of the radioactive material, since the 125 I residues in the thyroid gland in the high dose group were not significantly higher than those in the low dose group, The Agency and the proponents agreed that the amount absorbed by the gastrointestinal system is limited, with most excreted unchanged in the feces; and of the limited amount absorbed, some was deiodinated, with deiodinated products excreted primarily in the urine.[128]

Certified Color Manufacturers' Association. Hazleton Laboratories. 7-month study in rats (unpublished). Project No. 6145-101. 1984 (initial report) and 1988 (final report).

The study was designed to determine the influence of 7 months of continuous exposure to FD&C Red No. 3 on thyroid function and whether changes in thyroid physiology and

[126] Color Additives Notice, p. 3524, 3525.
[127] Color Additives Notice, p. 3524, 3530, 3532, 3537-38.
[128] Color Additives Notice, p. 3533-3534.

morphology induced by FD&C Red No. 3 could be reversed by administration of T3, consistent with the secondary mechanism hypothesis. (Under this hypothesis, T3 administration should result in decreased serum levels of T4, rT3, and TSH, and return the follicular cells to a normal state (unhypertrophied)). Rats were dosed with 0.0, 0.25, 0.5, 1.0, 2.0, and 4.0% of FD&C Red No. 3 in the diet and circulating thyroid hormone and TSH levels and urinary iodine levels were measured after administration of FD&C Red No. 3. During the last month, 5 animals per sex from each of the six dose groups (each of which contained 15 animals/sex) were injected with T3. At the end of the study, the thyroids of all animals were examined by electron microscopy. Additionally, deiodination studies with homogenates of the liver and pituitary were performed to test the hypothesis that FD&C Red No 3 inhibits the conversion of T4 to T3 in these tissues. Male rats in the 4% dose group had decreased mean body weights, a greater food consumption, greater excretion of total iodine, and greater mean thyroid weights. The male rats showed an increase in serum T4, a decrease in serum T3, and an increase in serum rT3, compared with controls. Also, the mean serum TSH values were higher in treated animals than in control animals, although the difference was not statistically significant for the entire study; there is no evidence of sustained, statistically significant differences in TSH levels between the treated and control animals throughout the course of the study. FDA reviewed the electron micrographs and was unable to confirm that FD&C Red No 3 resulted in cellular hypertrophy, although FDA found that quantitative measures of the cells supported an interpretation of cellular hypertrophy, provided that the tissue sampling was unbiased, which FDA was unable to ascertain. There was no evidence of any further progressive proliferative changes, such as hyperplasia. FDA agreed that the follicular cell hypertrophy regressed upon administration of T3 in the subgroup of rats injected with T3, but not to a normal state (additional lysosomal bodies remained in the follicular cells at the end of the study). While FDA agreed that the liver homogenate data support a dose-dependent inhibition of the formation of T3 from T4, FDA considered that the study provides only limited evidence that FD&C Red No. 3 inhibits the conversion of T4 to T3, since the pituitary results (showing a lack of inhibition of T4 metabolism) contradicted the hypothesis.[129] Overall, the agency concluded that the study provides only limited evidence that FD&C Red No. 3 inhibits the conversion of T4 to T3, and does not establish the hormonal changes necessary to support the secondary hypothesis.[130]

Certified Color Manufacturers' Association. 3-week study in rats (unpublished). Witorsch, 1984. The purpose was to determine whether dietary FD&C Red No. 3, sodium iodide, or fluorescein disrupted the normal thyroid-pituitary feedback relationship by producing a pituitary gland that was hyperresponsive to thyrotropin-releasing hormone (TRH). Animals were dosed with 0.0, 0.5, 1.0, and 4% (2464 mg/kg/day) of FD&C Red No. 3 in the diet, sodium iodide at 100 mg/kg/day, or fluorescein at 1000 mg/kg/day for 3 weeks. The researchers measured TSH, T3, T4, and T3 resin uptake before and after an intravenous bolus of TRH. FDA concluded that the study does not provide acceptable evidence of increased TSH secretion. There was no difference in the proportion of increase in TSH between animals treated with FD&C Red No. 3 and the control animals

[129] Color Additives Notice, p. 3533.
[130] Color Additives Notice, p. 3531-3533, 3538.

after both groups were injected with TRH. A key part of the secondary mechanism hypothesis—that TSH levels are chronically higher than normal during the portion of the life of the rat necessary to produce thyroid follicular neoplasms—was not demonstrated. In addition, the study reported an increase in T3, rather than the predicted decrease.[131]

Primate Research Institute, 1983. An endocrine evaluation of the thyroidal effects of FD&C Red #3. Submitted for Certified Color Manufacturers Association, Inc. Final Report, Study No. Cm-70r.

Conducted on behalf of the proponents, this 27-week study in rats was provided to support their initial contention that the thyroid tumors observed in chronic rat studies were a response mediated by exposure to excess iodide supplied by FD&C Red No. 3. FDA determined that the data from this study did not support the "iodide-mediated" hypothesis. Proponents also argued that morphological data from this study supported their hypothesis of a secondary mechanism, asserting that there was evidence of thyroid gland activation as indicated by follicular cell hypertrophy in treated animals. However, FDA evaluated electron micrographs of follicular cells from the thyroids of control and treated animals and concluded there were no conspicuous or consistent treatment related differences in the ultrastructural appearance of follicular cells, other than an increased concentration of lysosomes. Although there was evidence of significantly elevated levels of TSH, these were only in one set of analyses (the "in-life" phase, and not the "serial" serum sampling).

Ruiz, M, Ingbar SH. Effect of erythrosine (2',4',5',7'-tetraiodofluorescein) on the metabolism of thyroxine in rat liver study. Endocrinology 110(5):1613-7, 1982. https://doi.org/10.1210/endo-110-5-1613.

This study was designed to determine whether FD&C Red No. 3 causes a dose-related inhibition of T4 metabolism in rats. Male rats were administered erythrosine by intraperitoneal injection. Only the liver homogenates from treated rats (not those from untreated rats) showed a dose-dependent reduction in the conversion of T4 to T3 and I. FDA concluded that this result offered some limited support for the postulate that FD&C Red No. 3 inhibits the peripheral conversion of T4 to T3, but that it was not definitive since the color additive was administered intraperitoneally, which results in a substantially larger systemic exposure than would oral administration, and since it does not provide evidence regarding effects on T4 metabolism with continuous prolonged exposure.[132]

U.S. Food and Drug Administration, Color Additive Scientific Review Panel, Final Report, 1986.[133]

This review panel was composed of scientific experts from the U.S. Public Health Service that had been convened to review whether valid quantitative risk assessments could be performed for six provisionally listed color additives, including FD&C Red No. 3, and whether the available information supported the data analyses and the risk

[131] Color Additives Notice, p. 3532, 3538.
[132] Color Additives Notice, p. 3533.
[133] 51 FR 7856-03, 1986. Color Additives: D&C Red No. 8, 9, 19, and 37; D&C Orange No. 17; and FD&C Red No. 3; Report Availability.

assessments that were before the agency. Due to the complexity presented by the FD&C Red No. 3 data, the FDA Commissioner convened a new panel to consider the data that appeared to suggest that FD&C Red No. 3 acts as a secondary carcinogen (see below).[134]

U.S. Food and Drug Administration, FD&C Red No. 3 Peer Review Panel, Final Report, 1987.[135]

> The Panel was unable to come to any conclusion concerning the exact mechanism by which FD&C Red No. 3 induced thyroid tumors in rats. It stated that it is more likely to be the result of an indirect (secondary) mechanism and suggested additional studies that could be conducted to investigate further the mechanism of action of FD&C Red No. 3.[136]

Part 3: Relevant Studies/Evaluations Since FDA's 1990 Conclusion that FD&C Red No. 3 is Carcinogenic

We used the following approach to identify and evaluate all studies and evaluations relevant to the carcinogenicity of FD&C Red No. 3 that were published since FDA reached its conclusion in 1990:

- Identify and evaluate scientific opinions by the European Food Safety Authority (EFSA) on erythrosine since 1990, namely the 2011 EFSA "Scientific Opinion on the re-evaluation of Erythrosine (E 127) as a food additive" as well as all references it cited that were published in or after 1990 and were related to carcinogenicity, genotoxicity, or thyroidal effects.[137] We excluded references on reproductive toxicity, neurobehavioral toxicity/behavioral effects, serotonin release, allergenicity, hypersensitivity, teratogenicity, aluminum, intake estimates, analytical methods, impurities, and interactions with other additives.

- Identify and evaluate scientific opinions by the WHO JECFA on erythrosine since 1990, namely those published in 1991 and 2019, as well as all references cited in the 2019 (Eighty-sixth) report relevant to the carcinogenicity, genotoxicity, or thyroidal effects of erythrosine and published in or after 1990, excluding references on reproductive and neurobehavioral toxicity and references not specifically related to Red No. 3 or erythrosine.[138]

- Identify and evaluate cancer monographs on erythrosine by the International Agency for Research on Cancer (IARC) since 1990. (None identified either before or after 1990).

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[134] Color Additives Notice, p. 3522.
[135] 52 FR 29728-02, 1987. FD&C Red No. 3: Availability of final report of FD&C Red No. 3 Peer Review Panel.
[136] Color Additives Notice, p. 3522.
[137] European Food Safety Authority (EFSA). Scientific Opinion on the Re-evaluation of Erythrosine (E 127) as a Food Additive. *EFSA Journal 9*(1): 1854, 2011. https://doi.org/10.2903/j.efsa.2011.1854.
[138] Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants. Eighty-sixth report. WHO Technical Report Series 1014, 2019, p. 27. https://apps.who.int/iris/bitstream/handle/10665/279832/9789241210232-eng.pdf.

- Identify and evaluate other relevant documents published by authorities in the United States. We identified two relevant documents available on the California's Office of Environmental Health Hazard Assessment website and another relevant document on thyroid cancer risk assessment from the U.S. Environmental Protection Agency. There are no U.S. National Toxicology Program carcinogenicity studies on FD&C Red No. 3.

- Finally, we searched PubMed and Web of Science for publications published from 1/1/1990 and through 9/1/2022 using the PubMed MeSH Keyword for erythrosine in PubMed, which includes the following terms[139] (used for Web of Science):

  - Erythrosin B
  - F D and C #3
  - 2',4',5',7'-Tetraiodofluorescein
  - FD and C Red No. 3
  - FDC Red No. 3
  - Erythrosine B
  - Erythrosin

  Specifically, we applied the following cancer terms: cancer OR carcinogenesis OR carcinogenic OR tumor OR tumour OR neoplasia OR adenoma OR carcinoma.[140]

  In addition, we applied the following genotoxicity terms: mutagen* OR mutation OR "genetic toxicity" OR genotoxic OR "gene toxicity" OR "DNA damage" OR "DNA adducts" OR Ames OR "comet assay" OR clastogen*.[141]

  Lastly, we applied the following thyroid relevant terms: thyroid OR T4 OR TBG OR thyroxine.[142]

  We excluded results that were not relevant to the ability of FD&C Red 3 to cause or promote cancer, tumors, genotoxic effects, or effects on the thyroid.

Here are our summaries of the resulting 40 relevant studies/evaluations published between 1/1/1990 and 9/1/2022:

[139] National Library of Medicine. National Center for Biotechnology Information, MeSH (Medical Subject Headings) in the NLM controlled vocabulary thesaurus used for indexing articles for PubMed. Search for Erythrosine. https://www.ncbi.nlm.nih.gov/mesh/68004923.
[140] The PubMed search is (erythrosine AND (1990/1/1:2022/9/1[pdat])) AND (cancer OR carcinogenesis OR carcinogenic OR tumor OR tumour OR neoplasia OR adenoma OR carcinoma).
[141] The PubMed search is (erythrosine AND (1990/1/1:2022/9/1[pdat])) AND (mutagen* OR mutation OR "genetic toxicity" OR genotoxic OR "gene toxicity" OR "DNA damage" OR "DNA adducts" OR Ames OR "comet assay" OR clastogen*)
[142] The PubMed search is (erythrosine AND (1990/1/1:2022/9/1[pdat])) AND (thyroid OR T4 OR TBG OR thyroxine)

Anwar, F, Singh R, Mushtaq G et al. Cancer initiating properties of erythrosine supplements with sub necrotic dose of diethyl nitrosamine: potential effects on biochemical parameters of liver, Vitamin C and E. Mol Cell Toxicol 11:357-366, 2015. https://doi.org/10.1007/s13273-015-0036-0.

This study examined how oral exposure to erythrosine affected a number of biochemical parameters and histopathology of the liver in rats given a single exposure to the carcinogen n-diethyl nitrosamine (DENA) by intraperitoneal injection. DENA causes liver cancer (hepatocellular carcinoma). Compared with rats exposed only to DENA, erythrosine plus DENA caused statistically significant alterations in many biochemical parameters examined. Histopathological examination of liver tissue showed a marked effect of DENA plus erythrosine exposure on liver structure. The authors concluded that erythrosine was a promoter of hepatocellular carcinoma.

Aziz, AHA, Shouman SA, Attia AS, Saad SF. A Study on the Reproductive Toxicity of Erythrosine in Male Mice. Pharm Res 35(5):457-462, 1997. https://doi.org/10.1006/phrs.1997.0158.

Although this study is on reproductive toxicity, we include it here since erythrosine was shown to increase sperm head abnormalities in male albino mice, and the authors state about 90% of agents that test positive on this test are established carcinogens or mutagens. Specifically, the incidence of sperm with abnormal head significantly increased ($p < 0.01$) by about 57% and 65% after 5 daily administrations by gavage of 680 and 1360 mg/kg (equivalent to 10 and 20% of its LD-50 (lethal dose 50%)), respectively, compared to control animals given distilled water. No increase was seen in animals dosed with 340 mg/kg. The authors discuss this result in the context of other studies showing erythrosine affecting DNA in somatic cells and promoting thyroid tumors in rats.

California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (OEHHA), Final Prioritized Candidate Chemicals Under Consideration for Carcinogenicity Evaluation: Fifty-four chemicals within Batch #3, 1999, p. 77. https://oehha.ca.gov/media/downloads/crnr/fbatch3.pdf

This document presents OEHHA's preliminary review of the carcinogenicity and exposure data, as part of its continuous prioritization process. It has not conducted an in-depth review. The document notes FDA's 1990 decision and lists and briefly describes animal bioassays and other relevant data. It concludes that the color additive has not been placed on the candidate list, meaning, it has not been designated of High Carcinogenicity Concern. All chemicals not assigned a final "high" level of carcinogenic concern are assigned to Category II. The document states, "Action is not anticipated on Category II chemicals until all high priority chemicals on the Candidate List with known or potential exposure have been brought before the Committees. At that point, with Committee and public input, OEHHA will refine the existing process in order to determine which of the Category II prioritized chemicals should be brought forward for consideration by the CIC [Carcinogen Identification Committee]." In reaching this conclusion, the document notes the statistically significant increase in the incidence of benign thyroid tumors in rats in one study, and no increased tumor incidence in rats in a "similar" feeding study by Hansen et al. 1973. It also noted that the color additive was

generally negative in gene mutation tests but did increase micronuclei and chromosomal aberrations *in vitro*. It noted there was a "high" level of concern over the extent of exposure.

California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Children's Environmental Health Center. Health Effects Assessment: Potential Neurobehavioral Effects of Synthetic Food Dyes in Children. April 2021. https://oehha.ca.gov/risk-assessment/report/health-effects-assessment-potential-neurobehavioral-effects-synthetic-food.

This report systematically reviews human, animal, and mechanistic evidence on potential neurobehavioral effects of certified color additives. It also includes an exposure assessment that updates and expands FDA's assessment by Doell et al. 2016 to include more age groupings, as previously described. The highest 95th percentile single-day dose estimates based on the typical and high-exposure scenarios were reported for FD&C Red No. 3 in children under 2 years old. OEHHA noted (p. 208) that, "These high values appear to be outliers compared to other values; however, we reviewed all source data and code and these results derive correctly from the underlying information." Children's mean FD&C Red No. 3 intake estimates based on a single serving of frozen desserts and frostings and icings sometimes exceeded the JECFA ADI (0.1 mg/kg/day) for some brands. The report finds that the FDA ADI for FD&C Red No. 3 would be considerably lower if it was based on the results of more modern studies that observed neurobehavioral effects.

Capen, C.C. Correlation of mechanistic data and histopathology in the evaluation of selected toxic endpoints of the endocrine system. Toxicol Lett 102-103:405-9, 1998. https://doi.org/10.1016/S0378-4274(98)00244-6.

A section of this article focuses on the effect of FD&C Red No. 3 on thyroid structure and function. Capen concluded that a primary action of FD&C Red No. 3 on the thyroid is unlikely due to (a) failure of the color to accumulate in the thyroid after feeding FD&C Red No. 3 to rats at 0.5 or 4.0% for a week, (b) "negative" genotoxicity and mutagenicity assays (though it should be noted that the article made no mention of studies finding genotoxicity or FDA's different view of the evidence), (c) lack of oncogenic response in mice and gerbils, (d) lack of tumors at lower doses (1% or less) and (e) lack of tumor increases in other organs. The only other mechanistic study described was a short-term study that Capen had conducted which, as FDA stated in 1990, does not establish that TSH levels remain elevated for the duration necessary to produce thyroid tumors. Overall, this evaluation does not negate the fact that FD&C Red No. 3 is carcinogenic in rats, nor does it establish the mechanism by which the color additive causes tumors in rats, as FDA determined in 1990.

Capen, C.C. Mechanisms of chemical injury of thyroid gland. Prog Clin Biol Res 387:173-91, 1994. https://pubmed.ncbi.nlm.nih.gov/7526405/.

Describes different ways chemicals may affect the thyroid gland. The author asserts that FD&C Red No. 3 inhibits 5'-monodeiodinase, which converts T4 in peripheral sites (e.g., liver and kidney) to T3, and that this inhibition lowers circulating T3 levels, which results

in a compensatory increased secretion of TSH, thyroid follicular cell hypertrophy and hyperplasia, and increased incidence of follicular cell tumors in chronic studies of rats.

Capen, C.C. Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicol Path 25(1):39-48, 1997. https://doi.org/10.1177/019262339702500109.
Repeats the same information on erythrosine as Capen (1994).

Capen, C.C. Pathophysiology of chemical injury of the thyroid gland. Toxicol Lett 64-65:381-388, 1992. https://doi.org/10.1016/0378-4274(92)90211-2.
Repeats the same information on erythrosine as Capen (1994).

Chequer, F.M., Venâncio V.P., Bianchi M.L., Antunes L.M. Genotoxic and mutagenic effects of erythrosine B, a xanthene food dye, on HepG2 cells. Food Chem Tox 50(10):3447-3451, 2012. https://doi.org/10.1016/j.fct.2012.07.042.
This study aimed to analyze the genotoxicity of erythrosine using the alkaline comet assay and mutagenicity using the cytokinesis block micronucleus cytome (CBMN-Cyt) assay in HepG2 cells. These cells were chosen because they express phase I and phase II biotransformation enzymes and thereby can mimic in vivo metabolism of xenobiotics, which is important should in vivo biotransformation lead to formation of mutagenic metabolites of erythrosine. The cells were treated with seven concentrations (0.1-70.0 μg/mL) of the dye, and the results showed genotoxicity (comet assay) at the two highest concentrations and mutagenicity (CBMN-Cyt assay) at six concentrations.

Chequer, F.M., Venâncio V.P., Almeida M.R. et al. Erythrosine B and quinoline yellow dyes regulate DNA repair gene expression in human HepG2 cells. Toxicol Ind Health 33(10):765-774. 2017. https://doi.org/10.1177/0748233717715186.
Following up on Chequer et al. (2012) this study investigated the molecular basis underlying the genotoxicity of erythrosine in HepG2 cells. The authors conclude that erythrosine "significantly decreased the expression of two genes (FEN1 and REV1) related to DNA base repair."

Dees, C,. Askari M., Garrett S. et al. Estrogenic and DNA-damaging activity of Red No. 3 in human breast cancer cells. Environ Health Perspect 105(Suppl 3):625-632, 1997. https://doi.org/10.1289/ehp.97105s3625.
In this study, FD&C Red No. 3 increased the growth of estrogen receptor-positive human breast carcinoma cells in vitro, but not that of estrogen receptor-negative human breast carcinoma cells, and bound the estrogen receptor in these cells (i.e., it exhibited estrogenic properties). Effects were seen at environmentally relevant levels. The authors conclude that "[c]onsumption of Red No. 3, which has estrogen-like growth stimulatory properties and may be genotoxic, could be a significant risk factor in human breast carcinogenesis."

Devi, C.P.A., Raghavan L., Vivekanandhi J., Jayaraman K. In vivo effects of Erythrosine on mouse chromosomes. Toxicol Int 11: 63-67, 2004.
We were only able to obtain an abstract for this study. EFSA also states that only an abstract is available providing limited details. EFSA states, "Swiss albino mice were

exposed to Erythrosine by oral gavage for 30 days (controls 15 mice; control reversals five mice, low dose (62.5 mg/kg bw/day) 10 mice, intermediate dose (125 mg/kg bw/day) 15 mice, intermediate reversals five mice and high dose (250 mg/kg bw/day) 10 mice. A significant dose dependent decrease in cell proliferation was observed. The percentage of chromosomal aberrations was significantly decreased in the intermediate and the high dose group (Devi et al. 2004). Only an abstract was available providing limited details."

European Food Safety Authority (EFSA). Scientific Opinion on the re-evaluation of erythrosine (E 127) as a food additive. EFSA Journal 9(1): 1854, 2011. https://doi.org/10.2903/j.efsa.2011.1854.

EFSA's Scientific Panel on Food Additives and Nutrient Sources agreed with the previous review by the EU Scientific Committee for Food (SCF) in 1989, that reviewed the same evidence that FDA did prior to FDA's 1990 determination that FD&C Red No. 3 is a carcinogen. The EFSA Panel stated that, "the weight-of-evidence still showed that the tumorigenic effects of Erythrosine in the thyroid gland of rats are secondary to its effects on thyroid function and not related to any genotoxic activity," and "may be considered of limited relevance to humans."

In 1990, as documented in its denial of the petition for listing FD&C Red No. 3 for use in cosmetics and externally applied drugs (55 FR 3520-01), FDA fully considered the hypothesis that FD&C Red No. 3 causes thyroid cancer through a secondary mechanism and carefully evaluated the mutagenicity data. Ultimately, FDA considered the evidence to be too mixed to conclude that FD&C Red No. 3 is not mutagenic, and that the data did not establish that the carcinogenic effect of FD&C Red No. 3 is due to a secondary mechanism. It considered that the evidence was sufficient to "firmly establish" that FD&C Red No. 3 causes thyroid cancer in male rats.

The EFSA Panel set an ADI based on Gardner et al. 1987, a study of 30 healthy young men lasting 14 days, which it identifies as "the critical study," and "a pivotal clinical study." This is the same study JECFA used in 1990. That study gave one of three doses (20, 60, or 200 mg/day) to ten men each, and measured several markers of thyroid function, including serum T4, T3, reverse T3 (rT3), and TSH, T3-charcoal uptake, serum PBI (protein-bound iodine), total serum iodine, and total urinary iodine excretion, on days 1, 8, and 15 and TRH on days 1 and 15. At the highest dose, mean basal serum TSH and mean peak TSH increment after TRH significantly increased. Significant dose-related increases in serum total iodide and PBI concentrations occurred in all three groups and significant dose-related increases in urinary iodide excretion occurred in the 60 and 200 mg/day dose groups. The authors suggested that the increase in TSH secretion was related to the increased serum iodide rather than a direct effect of erythrosine on thyroid hormone secretion or peripheral metabolism. Dosing with 60 mg/day did not produce significant effects on any of these metrics. Assuming a body weight of 60 kg and applying a 10-fold safety factor to allow for the small number of subjects used and the study's relatively short duration, the Panel derived an ADI of 0-0.1 mg/kg bw/day. It estimated intake for adults on average as 0.0031 mg/kg bw/d and 0.01 mg/kg bw/d at the 95th percentile, and consequently below the ADI. Note that these intake estimates for the

European population are considerably lower than those that FDA developed for the U.S. population (e.g., 0.01 – 0.05mg/kg bw/day).

FDA fully considered Gardner et al. and concluded, "the submitted evidence cannot be used to establish the 60 mg/day dose of FD&C Red No. 3 as a NOEL because the study design did not provide sufficient statistical power to establish a NOEL."[143]

EFSA notes that various studies of erythrosine would not be in full compliance with current regulatory protocols, and that certain aspects of Gardner et al. have been questioned, including the statistical analysis, failure to correct for significant differences between groups in basal and maximal TRH-stimulated TSH concentrations on day 1, and lack of a control group. EFSA did not appear to explicitly consider, or at least express any concern about, the study's low statistical power.

Importantly, the Panel stated that since the JECFA and SCF evaluations, no new data are available on chronic toxicity/carcinogenicity. Thus, there are no long-term studies that contradict the FDA conclusion that FD&C Red No. 3 causes cancer in rats.

EFSA disagrees with FDA's conclusion and favors that of the published version of the industry study, Borzelleca et al. EFSA characterizes FDA's review as "not accessible," not "verified," and producing "slight discrepancies in the diagnoses of adenomas/carcinomas" compared with the published version of the study by Borzelleca et al.[144] In fact, FDA's own microscopic examination revealed relatively large—not slight—differences in the diagnoses of adenomas/carcinomas compared with those of industry researchers.[145] Critically, EFSA fails to note that FDA's statistical evaluations (as documented in its 1990 denial (55 Fed. Reg. 3524-3525) showed that FD&C Red No. 3 produced statistically significant increases in follicular cell adenomas plus carcinomas at all doses tested ($p = 0.016, 0.0007, 0.03$, respectively in the first rat study using doses of 0.1, 0.5, and 1%).

EFSA's Panel contends that rats are more sensitive than humans to effects on the thyroid, including thyroid cancer. It states that humans possess a high affinity binding protein ,TBG, that binds T4 (and T3 to a lesser degree), and that rodents lack this protein. Species with TBG have lower percentages of unbound active T4 (and T3) than species without TBG (e.g., the rat, according to EFSA). As a result, the Panel argues, T4 is cleared and excreted faster in rats, and rats must produce more T4 to compensate, compared to humans. The Panel stated that the accelerated production of thyroid hormone in the rat is driven by serum TSH levels that are about 6- to 60-fold higher than in humans. EFSA's Panel hypothesized that increases in TSH levels above basal levels in rats, "could more readily move the gland toward increased growth and potential

[143] Color Additives Notice, page 3535-3536.
[144] European Food Safety Authority (EFSA). Scientific Opinion on the Re-evaluation of Erythrosine (E 127) as a Food Additive. *EFSA Journal 9*(1): 1854, 2011. https://doi.org/10.2903/j.efsa.2011.1854. at 19.
[145] Color Additives Notice, *supra* note 7, at 3524; FDA Memorandum from David G. Hattan, Ph.D., Deputy Director, Division of Toxicological Review and Evaluation, HFF-152 to Ronald Lorentzen, PhD, Assistant to Director for Carcinogenicity Assessment, HFF-100, Evaluation of data concerning possible mechanism(s) mediating rat thyroid tumorigenesis by FD&C Red No. 3, August 11, 1989, at 25-26.

neoplastic change than in humans." It noted that the male rat has higher circulating TSH levels than females and is more sensitive to follicular cell hyperplasia and neoplasia. In humans, there is no sex difference in thyroid hormone levels, but females more frequently develop thyroid cancer.

EFSA concedes that rodents represent a conservative indicator of potential risk for thyroid cancer in humans, but argues that the relevance of experimental conditions in rodent cancer studies must be considered, relative to anticipated human exposures "i.e., dose, frequency, and time," and that chemically induced effects that are produced by short-term disruption in thyroid-pituitary functioning appear to be reversible when the stimulus is removed.

As discussed previously, this argument is flawed, since it is based on the incorrect assumption that rats lack a major high affinity thyroid hormone binding protein, with properties similar to those of human TBG. Although this was believed to be true in 1985, more recent scientific evidence (cited previously in section III.b.ii. of this petition) demonstrates this assumption to be incorrect.

In short, the EFSA evaluation, like that of the proponents of FD&C Red No. 3, does not conclusively establish that FD&C Red No. 3 induces thyroid cancer through a secondary mechanism. In regard to this argument from proponents of FD&C Red No. 3, FDA stated:

> The proponents of FD&C Red No. 3 have submitted the results of a number of studies to support the secondary mechanism hypothesis for the thyroid carcinogenesis of FD&C Red No. 3. However, this evidence does not sustain the proponents' hypothesis. Specifically, the proponents' evidence does not establish: (1) That TSH levels remain elevated for the duration of administration of the color additive necessary to produce thyroid tumors; (2) the full sequence of expected morphological events in response to prolonged elevation of TSH levels; (3) that these changes would ultimately result in thyroid neoplasms; and (4) that FD&C Red No. 3 is not genotoxic. Indeed, the available data do not sufficiently rule out the possibility of a direct-acting mechanism. In particular, the evidence from the short-term studies is not inconsistent with an alternative hypothesis that FD&C Red No. 3 operates through a mechanism whereby the thyroid gland is initially hyperstimulated by TSH, then returns by compensation to a normal hormonal state, and, independent of these effects, is the site of primary carcinogenesis. Accordingly, although the secondary mechanism hypothesis is scientifically plausible, the agency concludes that the existing data do not support a finding that FD&C Red No. 3 acts through the hypothesized secondary mechanism to produce thyroid carcinogenesis.[146]

Food Standards Australia New Zealand. Final assessment report – Application A603 – Red 3 Erythrosine in food colouring preparations, May 2010. https://www.foodstandards.gov.au/code/applications/pages/applicationa603red3e4006.aspx.

[146] Color Additives Notice, p. 3540.

(EFSA cited the Initial Assessment Report from 2008, also available at the above link).

The final assessment report states, "The weight of evidence indicates that erythrosine is not carcinogenic, however, benign thyroid tumours have been observed at very high doses (>2500 mg/kg bw/day) in a minority of long-term feeding studies in rats. It is most likely that the occurrence of these tumours was secondary to the compound's hormonal effects and is not relevant to humans based on well-recognised interspecies differences in thyroid physiology."

Ganesan, L., Margolles-Clark E., Song Y., Buchwald P. The food colorant erythrosine is a promiscuous protein-protein interaction inhibitor. Biochem Pharm 81(6):810-818, 2011. https://doi.org/10.1016/j.bcp.2010.12.020.

Erythrosine was the only color additive of all those certified for use in the United States that inhibited important receptor-ligand type protein-protein interactions in the tumor necrosis factor (TNF) superfamily. These interactions are important in immune system functioning and inhibition of tumorigenesis. Erythrosine was found to be a non-specific promiscuous and relatively potent inhibitor of such protein-protein interactions, inhibiting many such interactions within the TNF superfamily at low concentrations (approximately 2-20 mg/L).

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Hagiwara, M., Watanabe E., Barrett E.W., Tsutsui T. Assessment of genotoxicity of 14 chemical agents used in dental practice: Ability to induce chromosome aberrations in Syrian hamster embryo cells. Mutation Research 603:111-120, 2006. https://doi.org/10.1016/j.mrgentox.2005.08.011.

In this study, erythrosine B induced chromosome aberrations in Syrian hamster embryo cells when treated in the presence of exogenous metabolic activation. It did not induce chromosome aberrations in the absence of metabolic activation. The percentage of cells with aberrant metaphases as well as with polyploidy or endoreduplication was enhanced by erythrosine B (330 μM) in the presence of exogenous metabolic activation.

Hill, R.N., Crisp T.M., Hurley P.M. et al. Risk assessment of thyroid follicular cell tumors. Environ Health Perspect 106(8):447-57, 1998. https://doi.org/10.1289/ehp.98106447.

These authors served on the technical panel for the 1998 U.S. EPA document on "Assessment of thyroid follicular cell tumors" and the content is very similar. See U.S. EPA document.

International Programme on Chemical Safety. Part 1: IPCS framework for analyzing the relevance of a cancer mode of action for humans and case-studies. Harmonization Project Document No. 4. World Health Organization, 2007. https://www.who.int/publications/i/item/9789241563499.

This document includes a case-study on thiazopyr and thyroid disruption for analyzing the relevance of a cancer mode of action for humans. Thiazopyr, like FD&C Red No. 3, also increases the incidence of male rat thyroid follicular cell tumors. The authors used Bradford Hill criteria and concluded that there is sufficient experimental evidence to establish a thyroid disruption mode of action for thiazopyr-induced thyroid follicular cell tumors in rats, and that thiazopyr does not pose a carcinogenic hazard for humans. They identified key events in thiazopyr's mode of carcinogenic action. They noted that rats

tend to be more sensitive to thyroid carcinogenesis than mice, and that male rats are frequently found to be more sensitive than female rats with respect to the proportion of chemicals that induce thyroid tumors. They assert that no genetic toxicity has been demonstrated for thiazopyr in four different types of assays, suggesting that genotoxicity is not a prerequisite characteristic of substances that cause thyroid tumors.

Jennings, A.S., Schwartz S.L., Balter N.J. et al. Effects of oral erythrosine (2',4',5',7'tetraiodofluorescein) on the pituitary-thyroid axis in rats. Toxicol Appl Pharmacol 103:549-556, 1990. https://doi.org/10.1016/0041-008x(90)90327-q.

In this study, rats were fed erythrosine (0.5, 1.0, or 4.0%), sodium iodide (0.16%), or fluorescein (1.6%) in the diet for three weeks, and then shipped to another laboratory to measure TRH. In a second group of experiments, rats received intraperitoneal saline or 50 mg/kg/day erythrosine for 2 days. Twenty four hours after the second injection, their livers were perfused to study the effect of erythrosine on the metabolism of T4 and rT3. The authors suggested that treatment with erythrosine increased the TSH responsiveness of the pituitary to TRH by altering thyrotrophic cell conversion of T4 to T3, and that chronic ingestion of erythrosine may promote thyroid tumor formation in rats via chronic stimulation of the thyroid by TSH. However, this short-term study does not establish that TSH levels would continue to remain elevated for the duration necessary to produce thyroid tumors, or that the changes observed would ultimately result in thyroid tumors. As FDA noted in 1990 (55 Fed. Reg. 3540), the thyroid gland may be initially hyperstimulated by TSH, then return by compensation to a normal hormonal state. Also, limited conclusions about the effect of FD&C Red No. 3 in the diet can be drawn from 2-day intraperitoneal injections.

Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Thirty-seventh report. WHO Technical Report Series 806, 1991, p. 19. http://apps.who.int/iris/bitstream/handle/10665/40288/WHO_TRS_806.pdf.

The Committee concluded erythrosine is not genotoxic and that the occurrence of thyroid tumors in rats was "most likely" secondary to hormonal effects, and set an ADI of 0-0.1 mg/kg of body weight, based on the no observed effect level of 60 mg/person/day (equivalent to 1 mg/kg of bodyweight per day) and a safety factor of 10, based on Gardner et al. 1987, as described previously (under EFSA).

Joint FAO/WHO Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. Eighty-sixth report. WHO Technical Report Series 1014, 2019, p. 27. https://apps.who.int/iris/bitstream/handle/10665/279832/9789241210232-eng.pdf.

This report briefly summarizes previous reviews of erythrosine by the Committee dating back to 1965. It noted that a toxicological dossier that included new studies on genotoxicity and other endpoints was submitted, and that a comprehensive literature search conducted in PubMed retrieved three additional relevant studies. JECFA did not discuss or cite the new studies on genotoxicity, simply stating that, "the overall weight of evidence indicates that erythrosine is not genotoxic." The Committee also considered studies previously evaluated. It noted that no additional metabolic or kinetic studies had become available since the Committee's previous evaluation. It noted that two long-term feeding studies with erythrosine found an increase in the incidence of thyroid follicular cell adenomas in male rats, and that another study from 1988 indicated that erythrosine

promoted the development of thyroid follicular tumors in partially thyroidectomized rats, but not in non-thyroidectomized rats, and stated that, "the rat is not considered a suitable model for potential effects on the thyroid in humans." It further stated that a large number of in vitro and in vivo genotoxicity tests have been conducted on erythrosine, and that the Committee was still of the view that the overall weight of evidence indicates that erythrosine is not genotoxic. It again used the 14-day study of 30 healthy male volunteers to estimate the NOAEL (no observed adverse effect level) of 1 mg/kg of body weight per day and applied a 10-fold factor to derive an ADI of 0.1 mg/kg body weight. In its review of dietary exposure information, it noted that mean dietary estimates derived from maximum use levels exceeded the ADI for toddlers and reached the ADI for children, adolescents, adults, and elderly adults. The estimate based on maximum use levels for high percentile toddlers and adults exceeded the ADI by four-fold and two-fold, respectively. Estimates using "analytical levels" produced lower estimates that did not exceed the ADI, although the high percentile estimate for children came close (0.09 mg per kg body weight). It further noted that in addition, exposure through pharmaceuticals was previously estimated to occur at up to approximately 0.1 mg/kg bw per day in specific populations, generally over a short period of time. Surprisingly, the Committee considered that such exposure should not be taken into account in the assessment of long-term exposure in a healthy population to erythrosine as a food additive. It reasoned the exposure for high-exposure children was unlikely to occur every day over a lifetime and that therefore dietary exposures do not present a safety concern. It concluded there are no concerns with respect to genotoxicity and that new data that have become available since its previous evaluation do not give reason to revise the ADI of 0.1 mg/kg of body weight.

Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of certain food additives and contaminants. Thirty-seventh report. WHO Food Additive Series 28, 1991. https://inchem.org/documents/jecfa/jecmono/v28je12.htm.

This is the toxicological monograph prepared for the thirty-seventh meeting described above, by Dr. J.C. Larsen of the National Food Agency of Denmark. It provides more detail on specific studies than does the Technical Report Series from 1991 cited above but reaches the same conclusions, i.e., that erythrosine is not genotoxic, and that the occurrence of thyroid tumors in rats was "most likely" secondary to hormonal effects. Although it cited the 1990 Federal Register notice, "Termination of provisional listing of FD&C Red No. 3 for use in cosmetics and externally applied drugs and of lakes of FD&C Red No. 3 for all uses," clearly it was not persuaded by the views expressed there.

Kanno, J., Matsuoka C., Furuta K. et al. Tumor promoting effect of goitrogens on the rat thyroid. Toxicol Pathol 18(2):239-46, 1990. https://doi.org/10.1177/019262339001800202.

The authors discuss that FD&C Red No. 3 and Rose Bengal B (a synthetic food coloring used in Japan) were reported to induce thyroid neoplasia in rodent bioassays and that FD&C Red No. 3 reportedly interfered with the peripheral deiodination of T4 to T3 and increased the secretion of TSH from the pituitary. However, they note that some investigators reported that the goiters induced by the two color additives were morphologically quite different from those induced by the TSH-increasing goitrogens. Therefore, they compared the effects of Rose Bengal B with various known goitrogens (thiourea, phenobarbital sodium, potassium thiocyanate) on the rat, administering each to

DHPN-initiated and non-initiated F344 male rats in drinking water for 25 weeks. In their experiment, the goiters induced by Rose Bengal B could be morphologically distinguished from those induced by typical TSH-mediated goitrogens. They proposed that thyroid tumor promoters be classified into two groups mainly by their effect on thyroid morphology, i.e., iodine deficiency type promoters such as thiourea, phenobarbital and potassium thiocyanate, and iodine excess type promoters such as Rose Bengal B. (Rose Bengal B, also called tetrachlorotetraiodofluorescein potassium salt, is chemically very similar to FD&C Red No. 3, tetraiodofluorescein sodium salt.)

Kapadia, G.J., Tokuda H., Sridhar R. et al. Cancer chemopreventive activity of synthetic colorants used in foods, pharmaceuticals and cosmetic preparations. Cancer Lett 129:87-95. 1998. https://doi.org/10.1016/S0304-3835(98)00087-1.

Erythrosine and several other color additives were tested in vitro and in vivo for antitumor promoting potential. Erythrosine was found to be a potent inhibitor of skin tumor promotion in mice treated with the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) and the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA).

Kawaguchi, S., Sasaki Y.F., Tsuda S. Evaluation of in vivo genotoxicity of twelve synthetic tar dyes permitted in Japan using mouse Comet assay. Abstracts/Mutat Res 483 (Suppl. 1): S170, 2001.

We have not been able to obtain this article/abstract; it appears that it may have been published as part of a larger study under Sasaki et al. 2002 (see below). It is described by EFSA (2011). EFSA said, "Groups of four mice were dosed once orally with Erythrosine, and eight organs (glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow) were analysed in the Comet assay 3 hours and 24 hours after exposure. Erythrosine induced dose-related DNA damage in the glandular stomach, colon and urinary bladder after oral administration of 100 mg/kg bw and 2000 mg/kg bw and in the lung at 2000 mg/kg bw in the groups that were sacrificed 3 hours after exposure. In the groups that were sacrificed 24 hours after exposure, no DNA damage was evident (Kawaguchi et al. 2001, Sasaki et al. 2002). The negative result in the bone marrow was consistent with the negative results in bone marrow chromosomal aberration studies."

Mekkawy, H.A., Massoud A.A., El-Zawahry A.M. Mutagenic effects of the food colour Erythrosine in rats. Problems of Forensic Sciences [Z Zagadnien Nauk Sadowych] 43, 184-191, 2000. http://www.forensicscience.pl/pfs/43_mekkawy.pdf.

In this study, male rats (20 animals/dose) were fed erythrosine (0, 0.08, or 0.4 g/kg diet) for 30 days. The researchers measured chromosomal aberrations of rat bone marrow, nucleic acids, and total protein concentrations of rat liver and brain in 10 animals/group. The study found that erythrosine induced chromosomal aberrations at both doses. The high dose group had higher incidence of aberrations of all types measured, including diploidy, centric fusion, breaks, gaps, centromeric attenuation, deletions, ring-shaped, stickiness, and end-to-end. The mitotic index was increased at the lower dose and decreased at the higher dose. The nucleic acids and total protein concentrations were significantly increased at both doses. EFSA said: "There was neither a dose-response nor consistency in the findings and therefore no conclusions can be drawn from this study."

Merinas-Amo, R,. Martínez-Jurado M., Jurado-Guüeto S. et al. Biological effects of food coloring in in vivo and in vitro model systems. Foods 8(5):176, 2019. https://doi.org/10.3390/foods8050176.

Researchers tested erythrosine and other food colorings in two strains of *Drosophila melanogaster* at a range of concentrations intended to be higher than, lower than, and equivalent to the ADI for humans. According to the researchers, the *Drosophila* animal model is known to have more than 75% of human disease homologous genes related to different human degenerative illnesses, allergic diseases, and other conditions. They also used human leukemia (HL-60) cells to assess whether these colorings inhibited growth of the tumor cells, caused DNA damage, or affected DNA methylation status. Survival of *Drosophila* treated with erythrosine was significantly lower than controls at all concentrations except one (equivalent to the ADI), and longevity was significantly decreased at all concentrations except the lowest. Treatment with hydrogen peroxide (mutagenic in *Drosophila*) and erythrosine resulted in a synergistic adverse effect on survival at the lowest and highest concentrations tested. Erythrosine increased tumor cell growth except at the highest concentration but did not damage DNA or modify DNA methylation status.

Metwaly, A., Aboul-Enein A., Abd-Allah A., Hanafy E. Do synthetic food additives possess higher genotoxic effect than natural ones? Biosci Res 15(4):3329-3336, 2018. https://www.scopus.com/record/display.uri?eid=2-s2.0-85063182244&origin=inward&txGid=c5bb37d6d86c9cbd7d647edb2337f606.

Several additives, including erythrosine, were evaluated for genotoxicity using the alkaline comet assay in rat lymphocyte cells. Erythrosine was genotoxic in this system at the highest dose tested (100 ug/ml), causing DNA strand breaks of lymphocyte cells.

Miyachi, T, Tsutsui T. Ability of 13 chemical agents used in dental practice to induce sister-chromatid exchanges in Syrian hamster embryo cells. Odontology 9: 24-29, 2005. https://doi.org/10.1007/s10266-005-0055-8.

In this study, erythrosine did not induce chromosome aberrations in Syrian hamster embryo cells in the absence of metabolic activation. This is consistent with Hagiwara et al., in which aberrations were only observed in the presence of metabolic activation and not in the absence of metabolic activation.

Mpountoukas, P., Pantazaki A., Kostareli E. et al. Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. Food Chem Toxicol 48(10):2934-44, 2010. https://doi.org/10.1016/j.fct.2010.07.030.

Erythrosine at 8, 4, and 2 mM showed high cytotoxicity and cytostaticity in human peripheral blood cells in vitro. Results from spectroscopic titration studies, DNA electrophoretic mobility experiments, and PCR amplification of DNA fragments showed the capacity for DNA binding. This study was not reviewed by EFSA.

Poulsen, E.[147] Case study: erythrosine. Food Addit Contam 10(3):315-23, 1993. https://doi.org/10.1080/02652039309374154.

This article discussed whether it is appropriate to establish an ADI for erythrosine. It concluded that it is appropriate and that the NOEL in human studies is the most appropriate basis for the ADI. It recommends further human studies to elucidate variability of human pharmacokinetics of erythrosine.

Sarıkaya, R., Selvi M., Erkoç F. Evaluation of potential genotoxicity of five food dyes using the somatic mutation and recombination test. Chemosphere 88(8):974-9, 2012. https://doi.org/10.1016/j.chemosphere.2012.03.032.

Erythrosine was one of five food dyes evaluated for genotoxicity in the Somatic Mutation and Recombination Test (SMART) of Drosophila melanogaster in this study. The results for erythrosine were inconclusive. This study was not reviewed by EFSA.

Sasaki, Y.F., Kawaguchi S, Kamaya A et al. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res 519(1–2): 103-119, 2002. https://doi.org/10.1016/S1383-5718(02)00128-6.

The authors treated groups of four male ddY mice once orally with erythrosine, among other additives, at up to $0.5\times LD_{50}$ or the limit dose (2000 mg/kg) and performed the comet assay on the glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow 3 and 24 h after treatment. Of all the additives, color additives were the most genotoxic. Erythrosine (as well as six other color additives) induced dose-related DNA damage in the glandular stomach, colon, and/or urinary bladder. The lowest dose that induced statistically significant DNA damage in the glandular stomach and colon was 100 mg/kg. All seven color additives induced DNA damage in the gastrointestinal organs at a low dose (10 or 100 mg/kg).

Satoh, K., Nonaka E., Ishikawa F. et al. In Vitro Screening Assay for Detecting Aromatase Activity Using Rat Ovarian Microsomes and Estrone ELISA. Biol Pharm Bull 31(3):357-362, 2008. https://doi.org/10.1248/bpb.31.357.

Aromatase is a key enzyme that catalyzes the conversion of androgens to estrogens and plays an important role in maintaining a homeostatic balance between androgen and estrogen. In this study 45 chemicals were tested using rat ovarian microsomes, and erythrosine was found to be the second strongest inhibitor of aromatase activity of the chemicals tested, after the structurally related rose bengal. Erythrosine inhibited aromatase about 50x that of 4-hydroxy-androstendione and 1/100x that of fadrozole, two known aromatase inhibitors. Although not directly relevant to carcinogenicity or genotoxicity, we included this study since it may have relevance to the sexually dimorphic tumor response observed in rats treated with erythrosine.

Shimizu, R. Iodotyrosine deiodinase, a novel target of environmental halogenated chemicals for disruption of the thyroid hormone system in mammals. Biol Pharm Bull 37(9):1430-1434, 2014. https://doi.org/10.1248/bpb.b14-00240.

[147] Another article by Poulsen published in 1991, "Evaluation of Substances Consumed as Technical Ingredients (Food Additives) (Food Addit Contam 1991;8(2):125-33) is not included since the abstract indicates that it discusses JECFA and SCF evaluations (including for erythrosine) which are directly covered elsewhere in this petition. We were unable to obtain the full article.

This review discusses iodotyrosine deiodinase (IYD), an enzyme that salvages iodide from iodinated byproducts of thyroid hormone production. Iodide is used to synthesize thyroid hormones in the body. The authors speculate that IYD, which is inhibited by erythrosine, may play a role in the induction of thyroid tumors by erythrosine.

Shimizu, R., Yamaguchi M., Uramaru N. et al. Structure-activity relationships of 44 halogenated compounds for iodotyrosine deiodinase-inhibitory activity. Toxicol 314(1):22-29, 2013. https://doi.org/10.1016/j.tox.2013.08.017.

Erythrosine was the second most potent inhibitor of IYD of 44 halogenated compounds studied using microsomes of human embryonic kidney (HEK-293) T cells.

TemaNord. Food Additives in Europe 2000; Status of safety assessments on food additives presently permitted in the EU. Nordic Council of Ministers; 560:92-100, 2002. Available: https://www.google.com/books/edition/Food_Additives_in_Europe_2000/Fvm-sqd90-oC?hl=en&gbpv=0.

This document recommended in 2002 that a re-evaluation of erythrosine was not necessary given the present state of knowledge combined with very low potential exposure (its use in Europe is restricted to certain forms of processed cherries (cocktail, candied, and Bigarreaux cherries in syrup and in cocktails) and the amount used is limited (200 mg/kg except 150 mg/kg for Bigarreaux cherries)). The ADI of 0.1 mg/kg bw can be reached by consuming 30 g (about one ounce) of such cherries. However, the calculated intake by adults and the whole population is reported as 0% of the ADI in the EU, and one member state reports 0% intake in young children. The report reviewed the findings of JECFA in 1990 and the European Scientific Committee for Food in 1987 and 1990. It acknowledged that there has been, "an intense debate about the tumourigenic effects of erythrosine in the thyroid gland," concluding that the weight of evidence shows that the tumorigenic effects are secondary to effects on thyroid and pituitary functions and not related to genotoxic activity. This conclusion is at odds with FDA's (55 Fed. Reg. 3520-01).

U.S. Environmental Protection Agency Risk Assessment Forum. Assessment of thyroid follicular cell tumors. EPA/630/R-97/002, March 1998. https://www.epa.gov/osa/assessment-thyroid-follicular-cell-tumors.

Describes EPA procedures for evaluating thyroid follicular cell tumors in experimental animals and the data needed to make judgments regarding anticipated human risk. The document was peer reviewed in 1988 and 1996 by independent reviewers, including several FDA staff and the EPA Science Advisory Board.

The document requires considerable data to move from the presumption that chemicals that produce rodent thyroid follicular cell tumors may pose a carcinogenic hazard for humans. These required data are not available for FD&C Red No. 3. Only when no mutagenic effects are present and antithyroid effects are established is a margin of exposure methodology used. When experimental data needed to understand the cause of thyroid tumors is lacking and the mode of action is unknown, a linear dose-response procedure should be assumed. The data required to demonstrate antithyroid activity, includes data showing the reversibility of changes in thyroid cell morphology and number

and in thyroid-pituitary hormones upon cessation of chemical dosing, are not available for FD&C Red No. 3. Given conflicting data on the mutagenicity of FD&C Red No. 3, and a lack of required data demonstrating antithyroid activity, a margin of exposure approach cannot be adopted for FD&C Red No. 3, and a linear dose-response procedure should be assumed.

The document also notes that hormone levels may return to normal over time because of homeostatic compensatory increases in thyroid activity and mass. As noted by FDA in its 1990 decision, there are no data on thyroid hormone changes beyond 7 months. These data are still lacking. Thus, the data do not demonstrate that FD&C Red No. 3 results in long-term hormonal changes necessary to support the hypothesis that the tumors are secondary to hormonal changes.

Yamaguchi, F., Tsutsui T. Cell-transforming activity of fourteen chemical agents used in dental practice in Syrian hamster embryo cells. J Pharmacol Sci 93(4):497-500, 2003. https://doi.org/10.1254/jphs.93.497.

Erythrosine B did not induce morphological transformation in the Syrian hamster embryo cell transformation assay system.

Zijno, A., Marcon F., Leopardi P. et al. An assessment of the in vivo clastogenicity of erythrosine. Food Chem Toxicol. 32(2):159-63, 1994. https://doi.org/10.1016/0278-6915(94)90178-3.

Male B6C3F1 mice were treated by intraperitoneal injection at doses of 0, 50, 100 and 200 mg/kg of erythrosine twice, 24 hours apart. A positive control (mitomycin C) was used. Signs of toxicity were observed at the highest dose of erythrosine. There were no statistically significant differences between controls and animals treated with erythrosine in sister chromatid exchange frequencies in peripheral blood lymphocytes, frequencies of micronuclei in bone marrow polychromatic erythrocytes (PCEs) or frequencies of micronuclei in peripheral blood reticulocytes (PBRs). Statistically significant differences were seen between negative and positive controls. The authors concluded that erythrosine is inactive as a clastogen in mouse blood and marrow cells.

Appendix E: Data/Information on Probable Exposure to FD&C Red No. 3

We are requesting FDA to remove the remaining approvals for the use of FD&C Red No. 3 as a color additive, effectively banning its use.

Prior to terminating the provisional uses of FD&C Red No. 3, FDA stated:

> "The current exposure to FD&C Red No. 3 and its lakes (based on Food and Color Additive Review Section memorandum, December 11, 1986 …) from dietary ingestion is estimated to be 9.0 mg/day for young children (ages 2-5) and for other age groups (ages 5 plus.) On a weight basis, however, the exposure is estimated to be 600 ug/kg for children (ages 2-5) and 150 ug/kg for other groups (ages 2 plus).
>
> Consumers may also be exposed to FD&C Red No. 3 from ingested drugs or dietary supplements (17-50 ug/kg derived from 60 kg body weight). In patients consuming drug syrups, the combined short term exposure to FD&C Red No. 3 may increase to almost 2-fold the levels estimated for chronic exposures, to approximately 20 mg/day. Thus, children in the 2-5 years category could receive short term exposures of 1300 ug/kg/day."[148]

According to its most recent exposure assessment, which did not include exposures received through drug syrups or other medications, FDA estimated that 84% of consumers in the US population aged 2 years and older consume FD&C Red No. 3, based on 10-14-day food consumption data and that mean exposures range from 0.7-3.2 mg/person/day.[149] FDA estimated that 87% of children aged 2-5 years consume FD&C Red No. 3 and that mean exposures range from 0.3 – 3.1 mg/person/day for this age group.[150] The agency estimated that 85% of teenage boys aged 13-18 years consume FD&C Red No. 3 and that mean exposures range from 0.7 – 2.3 mg/person/day.[151] The highest consumption estimated was 2.7 mg/day for teenage boys (a 90th percentile, high exposure scenario). On a body weight basis, the highest consumption estimated was for children ages 2-5 years: 0.1 mg/kg of body weight (a 90th percentile, high exposure scenario), which is equivalent to the European and JECFA ADI.

A more recent assessment conducted by California's OEHHA in 2021, which used a comparable methodology to FDA's but more recent data and additional age groupings, determined that the highest exposures on a body weight basis to FD&C Red No. 3 were for children under 2, an age

[148] FDA Memorandum dated August 11, 1989, from David G. Hattan, Ph.D., Deputy Director, Division of Toxicological Review and Evaluation, HFF-152, to Ronald Lorentzen, Ph.D., Assistant to Director for Carcinogenicity Assessment, HFF-100, "Evaluation of Data Concerning Possible Mechanism(s) Mediating Rat Thyroid Tumorigenesis by FD&C Red No. 3," which cited Food and Color Additives Review Section memorandum, December 11, 1986 to Division of Food and Color Additives.
[149] Equivalent to 0.01-0.07 mg/kg of body weight per day. The lower estimate is the mean intake under a lower exposure scenario, and the higher estimate is the mean intake under a high exposure scenario, both using 10-14 day food consumption data. From Doell et al. op. cit.
[150] 0.02-0.2 mg/kg of body weight per day. From Doell et al. op. cit.
[151] 0.01-0.04 mg/kg of body weight per day. From Doell et al. op. cit.

range that FDA did not consider. Single day exposures for children under 2 reached 7.90 mg/kg of body weight under the high-exposure scenario and 4.83 mg/kg of body weight under the typical exposure scenario at the 95th percentile. Averaging consumption over two days, and reporting mean exposures, children under two were exposed to 0.47 mg/kg of body weight under a high-exposure scenario, much higher than the highest consumption FDA estimated on a body weight basis.

A study examining the prevalence of artificial colors found that FD&C Red No. 3 was present in 11.1% of candies, 3.3% of toaster pastries, 2.6% of fruit-flavored snacks, and 2.6% of packaged cakes marketed to children in a sampled grocery store.[152]

In 2021, FDA certified 215,780.42 pounds of FD&C Red No. 3.[153] According to FDA, FD&C Red No. 3 is in baby foods, breakfast cereal, cakes and cupcakes, chewing gum, cookies, decoration/chips for baking, dried fruit, frostings and icings, frozen breakfast foods, hard candy, ice cream/frozen yogurt/sherbet, ice cream cones, ice pops/frozen fruit bars, meal replacement drinks and bars, soft candy/gummies, and toaster pastries.[154] In its 2016 exposure study, FDA reported particularly large amounts of FD&C Red No. 3 in products such as some frostings and icings, cones used for ice cream, and meal replacement drinks.[155] A nutrition supplement baby food, PediaSure Shake Strawberry, contains 2 mg per serving of FD&C Red No. 3; two servings per day are recommended.[156] Thus, it is easy to see how consumers, especially children, could consume large amounts of FD&C Red No. 3.

Manufacturers also use FD&C Red No. 3 in dietary supplements and oral drugs.[157] We obtained 2,555 results after running a search[158] on its use as an inactive ingredient in human drugs

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[152] Batada A, Jacobson MF. Prevalence of Artificial Food Colors in Grocery Store Products Marketed to Children. *Clin Pediatr*.55(12):1113-9, 2016. https://doi.org/10.1177/0009922816651621.

[153] F.D.A., Color Certification Reports for January 1 2021 through December 31 2021 (second, third, and fourth quarters of fiscal year 2021 through first quarter fiscal year 2022), https://www.fda.gov/industry/color-certification/color-certification-reports.

[154] Doell DL, Folmer DE, Lee HS, *et. al*. Exposure Estimate for FD&C Colour Additives for the US Population. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2016 May; 33(5):782-797. https://doi.org/10.1080/19440049.2016.1179536.

[155] Doell et al. Op. cit. For example, Wilton Gel Food Colors with Magenta, Orange, Teal, Purple contained 20 mg/serving, Betty Crocker Cupcake Icing Rose Red contained 9.7 mg/serving, Giant Rainbow Ice Cream Cups contained 4.6 mg/serving, and Special K Protein Shake Dark Chocolate contained 8 mg/serving. The milligrams-per-serving data were calculated based on FDA's data on ppm of Red 3 contained in the products (see Supplemental Material at https://www.tandfonline.com/doi/suppl/10.1080/19440049.2016.1179536?scroll=top), and products' serving sizes listed on company websites.

[156] According to Doell et al 2016 (Supplemental tables), the average Red 3 content of PediaSure strawberry shake is 8.6 mg/kg. Serving size is 8 fluid ounces (see https://pediasure.com/nutrition-drinks-for-kids/compare-pediasure-nutrition-facts); 8 fluid ounces is approximately 0.2366 kg; 8.6 mg/kg x 0.2366 kg is 2.0 mg. The Pediasure website states "Kids must consume 2 full servings of PediaSure per day for optimal results." See https://pediasure.com/what-is-pediasure.

[157] Drugs.com. FD&C Red No. 3 Excipient. Top Medications with this Excipient. https://www.drugs.com/inactive/fd-c-red-no-3-247.html

[158] The search producing 2555 results was obtained using the term RED 3. Running the search different ways produced different results. For example, searching on "red no. 3" and limiting the results to human drugs produced 1029 results. Searching on "red 3" produced 4 results. Searching by FD&C RED NO. 3 produced 0 results. Searching by UNII code PN2ZH5LOQY and limiting to human drugs produced 1023 results. Searching by UNII

(including both prescription and over the counter drugs) in DailyMed, a database sponsored by the National Library of Medicine which contains labeling submitted to FDA by companies.[159] According to FDA's database on inactive ingredients in approved drugs,[160] the maximum daily exposure (MDE) from chewable tablets can be 1 mg/day (this is the only form where an MDE is given). Some oral suspensions can contain 1 mg of FD&C Red No. 3 per 5 ml dose according to the FDA database.

code 8TL7LH93FM retrieved 3 results. Results were not verified to confirm that FD&C Red No. 3 was actually listed as an inactive ingredient in all products. Searches run on September 13, 2022.

[159] National Library of Medicine. DailyMed. [Note: Search does not display on all browsers]. DailyMed - Search Results for INACTIVE_INGREDIENT:(RED 3) (nih.gov).

[160] U.S. Food and Drug Administration. Inactive Ingredients in Approved Drug Products Search. Database Last Updated July 18, 2022 (Data Through July 1, 2022). https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm.

Appendix F: Proposed Tolerances and Other Limitations on the Use of the Color Additives, If Required

The petition requests that FD&C Red No. 3 be removed from the permanent list of color additives approved for use in food and dietary supplements, 21 C.F.R. § 74.303, and for use in ingested drugs, 21 C.F.R. § 74.1303.

Appendix G: If Exemption from Batch Certification is Requested

We are requesting FDA to remove its remaining approvals for the use of FD&C Red No. 3 as a color additive. No exemption from batch certification is requested and batch certification will not be required if our petition is granted.

Appendix H: Proposed Changes to the Original Regulations

Title 21 of the Code of Federal Regulations

§74.303 FD&C Red No. 3.

(a) *Identity.* (1) The color additive FD&C Red No. 3 is principally the monohydrate of 9 (*o*- carboxyphenyl)-6-hydroxy - 2,4,5,7-tetraiodo-3H-xanthen-3-one, disodium salt, with smaller amounts of lower imdinated fluoresceins.

(2) Color additive mixtures for food use made with FD&C Red No. 3 may contain only those diluents that are suitable and that are listed in part 73 of this chapter as safe for use in color additive mixtures for coloring foods.

(b) *Specifications.* FD&C Red No. 3 shall conform to the following specifications and shall be free from impurities other than those named to the extent that such other impurities may be avoided by good manufacturing practice:

Volatile matter (at 135 °C.) and chlorides and sulfates (calculated as the sodium salts), total not more than 13 percent.

Water-insoluble matter, not more than 0.2 percent.

Unhalogenated intermediates, total not more than 0.1 percent.

Sodium iodide, not more than 0.4 percent.

Triiodoresorcinol, not more than 0.2 percent.

2(2′,4′-Dihydroxy-3′, 5′-diiodobenzoyl) benzoic acid, not more than 0.2 percent.

Monoiodofluoresceins not more than 1.0 percent.

Other lower iodinated fluoresceins, not more than 9.0 percent.

Lead (as Pb), not more than 10 parts per million.

Arsenic (as As), not more than 3 parts per million.

Total color, not less than 87.0 percent.

(c) *Uses and restrictions.* FD&C Red No. 3 may be safely used for coloring foods generally (including dietary supplements) in amounts consistent with good manufacturing practice except that it may not be used to color foods for which standards of identity have been promulgated under section 401 of the act unless added color is authorized by such standards.

(d) *Labeling*. The label of the color additive and any mixtures prepared therefrom intended solely or in part for coloring purposes shall conform to the requirements of §70.25 of this chapter.

(e) *Certification.* All batches of FD&C Red No. 3 shall be certified in accordance with regulations in part 80 of this chapter.

§74.1303 FD&C Red No. 3.

(a) *Identity and specifications*. (1) The color additive FD&C Red No. 3 shall conform in identity and specifications to the requirements of §74.303(a)(1) and (b).

(2) Color additive mixtures for ingested drug used made with FD&C Red No. 3 may contain only those diluents that are suitable and that are listed in part 73 of this chapter as safe for use in color additive mixtures for coloring ingested drugs.

(b) *Uses and restrictions*. FD&C Red No. 3 may be safely used for coloring ingested drugs in amounts consistent with good manufacturing practice.

(c) *Labeling*. The label of the color additive and any mixtures prepared therefrom intended solely or in part for coloring purposes shall conform to the requirements of §70.25 of this chapter.

(d) *Certification*. All batches of FD&C Red No. 3 shall be certified in accordance with regulations in part 80 of this chapter.

Appendix I: Request for Fee Waiver

Pursuant to 21 C.F.R 70.19(q), petitioners request a waiver of the color additive petition fees and deposit requirements. The petitioners are non-profit organizations and individuals who submit this petition because it is in the public interest to protect public health. See Section V of the petition.

Appendix J: Environmental review component.

An environmental assessment is not required because the proposed action for foods because it is categorically excluded pursuant to 21 C.F.R. § 25.32(m) as an "action to prohibit or otherwise restrict or reduce the use of a substance in food, food packaging, or cosmetics." For food and drugs, this action complies with the categorical exclusion criteria pursuant to 40 C.F.R. § 1508.4. Because the proposed action will not "significantly affect the quality of the human environment,"[161] no extraordinary circumstances as defined at 21 C.F.R. § 25.21 exist for the action requested in this petition which would require the submission of an Environmental Assessment.

A food or drug manufacturer may determine that the FD&C Red No. 3 is not essential and choose not to replace it. Other than a change in the color of some foods and drugs, we could identify no extraordinary circumstances that would result from this removal without replacement.[162]

A manufacturer would likely turn to 21 C.F.R §§ 73.1-99.501 to identify alternatives should the manufacturer determine that another color additive was needed to replace FD&C Red No. 3. CSPI does not condone the use of certified color additives in food,[163] although a manufacturer can consider those for foods or drugs. Color additives exempt from certification can be employed, and some can create a similar color in food, including beet powder, grape skin extract, vegetable juice (e.g., from radish, red cabbage, black/purple carrot, purple sweet potato), and fruit juice (e.g., from elderberries).[164] While many color additives were approved by FDA before the National Environmental Policy Act of 1969 was adopted and have not been reassessed by the agency for their current risk, we did not identify a potential for serious harm to the environment or protected species from the marginal increase in production or use of these alternatives.

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[161] 21 C.F.R. § 25.21

[162] Note that the petitioner is not required under section 171.130(b) to provide information that only the manufacturer would have, *In re Natural Resources Defense Council*, 645 F.3rd 400, 407 (DC Cir 2011).

[163] See CSPI. *Petition to Ban the Use of Yellow 5 and Other Food Dyes, in the Interim to Require a Warning on Foods Containing these Dyes, to Correct the Information the Food and Drug Administration Gives to Consumers On the Impact of These Dyes on the Behavior of Some Children, and to Require Neurotoxicity Testing of New Food Additives and Food Colors*. Available https://cspinet.org/resource/cspi-petition-fda-re-food-dyes. Accessed April 20, 2020.

[164] Natural Alternatives for Synthetic, FD&C Colors. Natural Products Insider, Sep. 24, 2013. https://www.naturalproductsinsider.com/ingredients/natural-alternatives-synthetic-fdc-colors; Food Standards Agency. Guidelines on approaches to the replacement of tartrazine, allura red, ponceau 4R, quinoline yellow, sunset yellow and carmoisine in food and beverages. http://www.reading.ac.uk/foodlaw/pdf/uk-11026-removing-colours-guidance.pdf.