Comments submitted via regulations.gov.

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Re: Docket No. FDA–2023–N–0437: Filing of Color Additive Petition From Center for Science in the Public Interest, et al.; Request To Revoke Color Additive Listing for Use of FD&C Red No. 3 in Food and Ingested Drugs**.**

The undersigned organizations appreciate the opportunity to provide comments on the Food and Drug Administration's ("FDA" or "Agency") notice of Petition for rulemaking, "Filing of Color Additive Petition From Center for Science in the Public Interest, et al.; Request To Revoke Color Additive Listing for Use of FD&C Red No. 3 in Food and Ingested Drugs," 88 Fed. Reg. 10,256 (Feb. 17, 2023). Our trade associations represent various parts of the supply chain, from farmers and agricultural processors, to packaged goods, and retail.

We appreciate FDA's ongoing efforts to ensure food and food additives are safe, including its efforts in conducting a thorough evidence-based scientific review of FD&C Red No. 3 ("Red No 3") under the rigid standard of the Delany Clause. The Delany Clause prohibits the listing of a color additive if FDA makes a finding that the additive "induce[s] cancer when ingested by man or animal,"[1] and we understand that this limits FDA's discretion to determine the safety of Red No 3, regardless of the probability, or risk, of cancer associated with exposure. Although FDA found Red No 3 to be carcinogenic in 1990 and denied listing it as a color in cosmetics[2] ("FDA 1990 Denial"), the science and research on carcinogenesis has evolved over the last 33 years providing a clear basis that Red No 3 is not genotoxic. As set forth below, studies and expert evaluations provide that Red No 3 is non-genotoxic, operates as a secondary mechanism of carcinogenesis, and the findings on Red No 3 in male rats is not relevant to humans. We respectfully request that FDA conduct a thorough scientific review of studies and expert evaluations discussed below as well as other studies not referenced herein on secondary

[1] 21 U.S.C. § 379e(b)(5)(B).
[2] FDA, 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, 3542 (Feb. 1, 1990).

mechanism of rat thyroid carcinogenesis to determine the safe use of Red No 3 in food and dietary supplements and make a determination to maintain Red No 3 in the permanent list of color additives.

I. Executive Summary

FDA's 1990 Denial and finding that Red No 3 is an animal carcinogen was based upon the Agency's inability to determine genotoxicity and industry's inability to show Red No 3 operates as a secondary mechanism. At that time, FDA concluded that "unresolved issues concerning the genotoxicity of Red No 3 remain"[3], and the agency had insufficient evidence to show that Red No 3 operates through a secondary mechanism of carcinogenesis.[4] In cancer risk assessments, as FDA recognizes today, non-genotoxic substances are "not directly DNA reactive but operating through a secondary mechanism," and are "assumed to have a threshold of exposure level below which tumor development is not anticipated and the risk of cancer is negligible."[5] As set forth below, Red No 3 is well-established to be non-genotoxic, and this inherent property is justification for further consideration of the science of Red No 3's secondary mechanism of carcinogenesis.

Mechanistic studies examining rat thyroid carcinogenesis have been published on a wide range of chemical compounds, including Red No 3 prior to and since the 1990 FDA delisting of Red No 3. For example, in a 1987 Color Additives Review Panel, convened by FDA to consider evidence of Red No 3 as a secondary carcinogen, concluded "there is no reason to suspect that this toxicity [from results of an International Research and Development Corporation study] results from direct interaction [Red No 3] with the DNA" and that there is "no evidence for a direct mechanism for [Red No 3]."[6] In other words, in 1987, expert evaluations concluded that Red No 3 was non-genotoxic.

Between 1988 and 1998, more than 600 papers on thyroid function, regulation, carcinogenesis, and epidemiology have appeared in the literature.[7] Additional support that Red No 3 is non-genotoxic developed during this time as the fields of toxicology and carcinogenesis advanced to better interpret the results on secondary mechanism of carcinogenesis in animals including rodents and to adequately determine the applicability of these findings to humans. Expert evaluations by the Joint FAO/WHO Expert Committee on Food Additives ("JECFA"), European Food Safety Authority ("EFSA"), and other scientific bodies concluded that **Erythrosine/Red No 3 did not show any genotoxic activity and is a non-genotoxic compound** based on in vivo and

[3] FDA, 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. 352, 3526 (Feb. 1, 1990).
[4] *Id.* at 3529.
[5] FDA, Final Rule, Partial Denial of Petition, "Food Additive Regulations; Synthetic Flavoring Agents and Adjuvants," 83 Fed. Reg. 50.490 (Oct. 18, 2018).
[6] FDA, Color Additives Review Panel, 52 Fed. Reg. 29,728 (Aug. 11, 1987).
[7] Hard, GC., Recent developments in the investigation of thyroid regulation and thyroid Carcinogenesis. Env. Health Perspective, 106, 427 (1998).

in vitro mutagenicity studies.[8,9,10,11] Further, as discussed below, studies and expert scientific evaluations have concluded that chemically induced tumors in rodent test animals via a secondary mechanism of carcinogenesis allows the observed tumors to be considered of limited or no relevance to humans.

Expert evaluations by JECFA and EFSA and several studies on Red No 3 lead to the following conclusions:

- Red No 3 is shown to be carcinogenic in only one rodent study;
- Red No 3 is carcinogenic in one species, the rat;
- Red No 3 is only carcinogenic in one sex, the male;
- Red No 3 is only carcinogenic at one dose, the 4% highest dose;
- Tumorigenic effects of Erythrosine/Red No 3 are secondary to its effects on thyroid function and not related to any genotoxic activity;
- Studies on Red No 3 and general studies on mechanism of thyroid carcinogenesis support the secondary mechanism for thyroid carcinogenicity;
- A threshold for Red No 3 is supportable based on secondary mechanistic studies; and
- A safe human intake level for Red No 3 can be demonstrated.

Expert evaluations by the International Agency for Research on Cancer ("IARC")[12] and several studies demonstrating chemically induced thyroid carcinogenesis through secondary mechanism which is also applicable to Red No 3 lead to the following conclusions:

- The male rat is not considered a suitable model for potential effects on the thyroid in humans; and
- Thyroid follicular tumors in male rats are secondary to hormonal effects and have species-specific sensitivity.

Similar to the basis of FDA's 1990 Denial, the current petition ("Petition") by the Center for Science in Public Interest ("CSPI"), et al., does not point to any studies or evaluations that conclude that Red No 3 is genotoxic or acts directly upon the thyroid to produce cancer. The petition positions Red No 3 as neither genotoxic, nor non-genotoxic, and without strong evidence concludes "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."[13] As we describe herein, it is well established that Red No 3 is **non-genotoxic**, and this inherent

[8] JECFA, Evaluation of Certain Food Additives and Contaminants, Twenty-ninth Report, WHO Technical Report Series, No. 733 (1986).
[9] JECFA, Evaluation of Certain Food Additives and Contaminants, Thirty-seventh Report, WHO Technical Report Series 806, 19 (1991), *available at* http://apps.who.int/iris/bitstre am/handle/10665/40288/WHO_TRS_806.pdf.
[10] JECFA, Evaluation of Certain Food Additives and Contaminants, Eighty-sixth Report, WHO Technical Report Series 1014, 32 (2019), *available at* https://apps.who.int/iris/bitstream/handle/10665/279832/9789241210232-eng.pdf.
[11] EFSA, Scientific Opinion on the Re-evaluation of Erythrosine (E 127) as a Food Additive, *EFSA Journal 9*(1), 1854 (2011), *available at* https://doi.org/10.2903/j.efsa.2011.1854.
[12] IARC, *Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis,* IARC Scientific Publications, No. 147, 230 (1999).
[13] Petition at 5.

property is justification for further consideration of the science of Red No 3's **secondary mechanism of carcinogenesis**. Further, studies have concluded that the rat is not considered a suitable model for potential effects on the thyroid in humans.

II. Background: FDA 1990 Decision; CSPI Petition; Non-Genotoxicity and Secondary Mechanism

The Center for Science in Public Interest's ("CSPI") Petition ("Petition") includes a number of arguments, which largely center around many of the arguments made in 1990 that formed the basis of FDA's 1990 Denial, to support the idea that Red No 3 "induce[s] cancer in man or animal. . ."[14] The Petition includes a detailed analysis of the regulatory history and toxicology findings on Red No 3 dating back to studies published in the 1980's by the color manufacturers.

FDA's 1990 Denial was due to the agency's determination that Red No 3 was an animal carcinogen based on a key adverse finding of tumors in the male rat thyroid, which were tested orally and only at the highest dose (4%). Red No 3 was found not to be carcinogenic in the female rat or in male or female mice tested orally (3% highest dose tested) in studies led by the color manufacturers.[15,16]

The FDA concluded that no determination could be made regarding the biochemical "mechanism" (primary or secondary) of the observed carcinogenic effects in the male rat thyroid gland. The Agency disagreed with the industry that Red No 3 was carcinogenic at the highest dose, due not to a direct primary mechanism (such as genotoxicity) but to a secondary mechanism of action related to the compound's toxic effects in the thyroid. The Agency also disagreed with the industry's position that there was a threshold intake level of Red No 3 below which there would be no thyroid tumors produced in humans, even though all lower doses in the rat oral study showed no carcinogenic effects. Further, the FDA did not consider the mechanistic studies as convincing evidence, provided by the industry, since the publication of the rat and mouse 2-year bioassay studies.

Industry positions cited in FDA's 1990 Denial are in stark contrast to those of the Agency in 1990 and current Petition. Industry explained the secondary mechanism of carcinogenesis hypothesis and the proof of the safety of Red No 3, which FDA summarized as follows:

> "[Industry] agreed with the Agency that [Red No 3] caused follicular cell neoplasms in the thyroid glands of male rats fed [Red No 3] at a dose level of 4 percent. However, in their May 1988 submission, [industry] contend that 'there is no evidence that FD&C Red No 3

[14] Petition at 2.
[15] Borzelleca, JF., et al., Lifetime toxicity/carcinogenicity study of FD&C Red No. 3 (erythrosine) in rats, 25(10) Food Chem. Tox. 723-733 (1987).
[16] Borzelleca, JF. and Halligan JB., Lifetime toxicity/carcinogenicity study of FD&C Red No. 3 (Erythrosine) in mice, Food Chem. Toxicol, 25(10):735-737 (1987).

> acts through a direct (primary) mechanism to induce rat thyroid follicular cell tumors; FD&C Red No. 3 is not genotoxic, and it does not accumulate in the rat thyroid after ingestion.' Further, the proponents contend that there is a threshold level below which the hormone imbalance will not occur, and that FD&C Red No 3 may be safely used in products at or below that level."[17]

In 1990, shortly after the FDA's termination of "provisional listings" of Red No 3, the Certified Color Manufacturers Association ("CCMA") submitted a Citizen Petition ("1990 CCMA Petition") to FDA requesting that the issue be referred to a color additive advisory committee for review.[18] The CCMA specifically asked that the advisory committee make recommendations whether the evidence demonstrates that Red No 3 causes thyroid follicular tumors by a secondary rather than a direct mechanism. The 1990 CCMA Petition laid out in detail the scientific arguments and many referenced studies supporting the secondary mechanism for the tumor findings in the male rat:

> "In summary, findings from the four described studies strongly support the conclusion that the thyroid follicular cell adenomas noted in 4.0% male rats in the high-dose study resulted from the TSH-mediated secondary mechanism of rat thyroid oncogenesis."[19]

The crux of the Agency's conclusion that Red No 3 is an animal carcinogen as basis for FDA's 1990 Denial[20] was (1) the inability to determine if Red No 3 was genotoxic or (2) determine whether it operates through a secondary mechanism. At that time, FDA concluded that "unresolved issues concerning the genotoxicity of Red 3 remain"[21] and the agency had insufficient evidence to show that Red No 3 operates as a secondary mechanism carcinogen.[22] Today, however, FDA recognizes that in a cancer risk assessment, non-genotoxic substances are "not directly DNA reactive but operating through a secondary mechanism," and are "assumed to have a threshold of exposure level below which tumor development is not anticipated and the risk of cancer is negligible."[23] On the other hand, if the chemical is genotoxic (i.e., directly DNA reactive), it is assumed that there is risk of cancer at any level of exposure.

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[17] FDA, 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, 3542, 3537 (Feb. 1, 1990).

[18] CCMA, Citizen Petition to FDA, Re: FD&C Red No. 3, Docket FDA-1990-P-0322 (Mar. 5, 1990).

[19] *Id.* at 19.

[20] FDA, 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, 3542 (Feb. 1, 1990).

[21] *Id* at 3526.

[22] *Id.* at 3529.

[23] FDA, Final Rule, Partial Denial of Petition, "Food Additive Regulations; Synthetic Flavoring Agents and Adjuvants," 83 Fed. Reg. 50.490 (Oct. 18, 2018).

Our comments present a scientific basis regarding non-genotoxicity of Red No 3, as examined, evaluated, and reported in scientific studies and in expert evaluations conducted by scientific bodies specific to Red No 3 and on the secondary mechanism of carcinogenesis.

III. Red No 3 is Well-established to be Non-genotoxic

The determination of a chemical's non-genotoxicity is critical in allowing experts in chemical carcinogenesis to justify the deeper physiological/biochemical/toxicological studies needed to demonstrate that a chemical shown to be carcinogenic in animals may be operating by a secondary mechanism of carcinogenesis.

As mentioned above, CSPI's Petition does not show or conclude that Red No 3 is genotoxic, much how FDA conceded in 1990 that "based on the available data, the agency is unable to conclude that the color additive is not genotoxic."[24] Despite the agencies inability to draw a conclusion, prior to 1990 and since then, studies and expert evaluations conclude that Red No 3 is non-genotoxic.

Research Leading Up to 1990

Evidence prior to 1990 was highlighted in CCMA's Petition,[25] which included the following conclusion by Lin and Brusick (1986):

> "Based on the weight of evidence from a large body of well-conducted studies with negative findings . . . we conclude that [Red No 3] is ***not mutagenic and that the limited tumorigenic activity identified in male rats [in the IRDC study] is not believed to be the result of genotoxic initiation***."[26]

This conclusion was supported by the 1987 Color Additive Review Panel and the Panel's final report concluded, "there is no reason to suspect that this toxicity [i.e., the results of the IRDC study] results from direct interaction [Red No 3] with the DNA" and that there is "no evidence for a direct mechanism for [Red No 3]."[27] In addition, the CCMA addressed misinterpretation of other published genotoxicity studies, demonstrating that Red No 3 is not genotoxic:

> "The presence of a few, isolated positive assays does not establish genotoxicity when the weight of the evidence is to the contrary…A balanced, weight-of-the-evidence analysis of the genotoxicity data on

[24] 55 Fed. Reg. 3526.
[25] CCMA, Citizen Petition to FDA, Re: FD&C Red No. 3, Docket FDA-1990-P-0322 (Mar. 5, 1990).
[26] Lin, GH. and Brusick, DJ., Mutagenicity studies on FD&C Red No. 3, Mutagenesis, 1(4) 253-259 (1986) (emphasis added).
[27] FDA, Color Additives Review Panel, 52 Fed. Reg. 29,728 (Aug. 11, 1987).

> FD&C Red No. 3, which is the proper way to resolve the issue, can lead only to the conclusion that the color additive is **not genotoxic.**"[28]

Many of the relevant studies between 1990 and 2019 on genotoxicity of Red No 3 have been addressed by EFSA and JECFA, and both concluded that Red 3 is a non-genotoxic compound.

European Food Safety Authority Evaluation and Conclusion

In 2011, EFSA published a comprehensive Scientific Opinion on Erythrosine (E 127) as a Food Additive and based on weight-of-evidence approach, concluded, that Red No 3 is not genotoxic:

> "Although some older and more recent in vitro studies showed positive results for the genotoxicity of Erythrosine, there are three negative in vivo genotoxicity studies (mammalian micronucleus, sister chromatid exchange and Comet assay). The weight-of-evidence from the available studies supports the **conclusion that Erythrosine is neither genotoxic nor clastogenic in vivo**.[29]
>
> . . .
>
> 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**."[30]

The EFSA panel concurred with EU Scientific Committee for Food ("SCF"), JECFA, and the TemaNord evaluations "concluded, based on in vivo and in vitro mutagenicity studies available at that time, that **Erythrosine did not show any genotoxic activity.**"[31] Then, when assessing results of new mutagenicity studies, including oral in vivo activity which were negative, the EFSA panel "considered the weight-of-evidence still showed that the **tumorigenic effects of Erythrosine are secondary to its effects on thyroid function and not related to any genotoxic activity**."[32]

Joint FAO/WHO Expert Committee on Food Additives Evaluation and Conclusion

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[28] CCMA, Citizen Petition to FDA, Re: FD&C Red No. 3, Docket FDA-1990-P-0322 at 22 (Mar. 5, 1990) (emphasis added).

[29] *EFSA Journal 9*(1), 1854 at 3 (2011) (emphasis added), *available at* https://doi.org/10.2903/j.efsa.2011.1854.

[30] *Id.* at 4 (emphasis added).

[31] *Id.* at 3 (emphasis added), citing TemaNord, Food Additives in Europe; Status of Safety Assessments on Food Additives Presently Permitted in the EU. Nordic Council of Ministers; 560:92-100 (2002); SCF, Reports of the Scientific Committee for Food, Commission of the European Communities, Twenty-first Series, 11-12 (1989); JECFA, Evaluation of Certain Food Additives and Contaminants, Twenty-ninth Report, WHO Technical Report Series, No. 733 (1986); JECFA, Evaluation of Certain Food Additives and Contaminants, Thirty-seventh Report, WHO Technical Report Series 806, 19 (1991).

[32] *Id.* at 3.

In 2019, JECFA reviewed a toxicological dossier that included new studies on genotoxicity, reproductive and developmental toxicity, neurological effects, and hypersensitivity. JECFA conducted a weight-of-evidence approach evaluating the genotoxicity database for Red No 3 and firmly concluded, like EFSA, that Red No 3 was non-genotoxic:

> "A large number of in vitro and in vivo genotoxicity tests have been conducted on erythrosine. The Committee confirmed that the overall weight of evidence indicates that **erythrosine is not genotoxic**."[33]

The CSPI Petition provides brief reviews of the above conclusions of TemaNord, EFSA, and JECFA. The Petition posits without any explanation that TemaNord's conclusion is "at odds with FDA's"[34] 1990 conclusions, but did not challenge the JECFA 1991 conclusion that Red No 3 is not genotoxic.[35] It also challenges the 2011 EFSA evaluation because, in 1990, "FDA considered the evidence" and could not conclude that Red No 3 "was not mutagenic." Although the Petition focuses on the fact that the EFSA panel did not review new data on chronic toxicity/carcinogenicity, the panel did review new studies conducted since FDA's 1990 Denial. This argument also ignores that JECFA confirmed EFSA's findings in 2019 and assessed "evidence newly available."[36] CSPI's defense against other studies concluding Red No 3 is non-genotoxic is largely premised on "FDA's [1990] view of the evidence."[37]

CSPI then moves its arguments away from genotoxicity to discuss how secondary mechanism is not established, spending much time on an analysis based on U.S. Environmental Protection Agency policy or guidance on risk assessments for chemicals that may produce thyroid follicular cell tumors in experimental animals ("EPA Guidance").[38] However, the EPA Guidance is based on the assumption that chemicals act by affecting DNA directly to cause mutations, i.e., genotoxic chemicals.[39] If the chemical is genotoxic, then the guidance would not require an evaluation of the mode of action.

As demonstrated above, it is well-established that Red No 3 is not genotoxic, which warrants evaluation of whether it operates by a secondary mechanism of action.

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[33] JECFA, Evaluation of Certain Food Additives and Contaminants, Eighty-sixth Report, WHO Technical Report Series 1014 at 29 (2019) (emphasis added).
[34] Petition at 51.
[35] Petition at 47.
[36] JECFA, Evaluation of Certain Food Additives and Contaminants, Eighty-sixth Report, WHO Technical Report Series 1014 at 32 (2019).
[37] E.g., Petition at 40 (Discussion of Capen, C.C., Correlation of Mechanistic Data and Histopathology in The Evaluation of Selected Toxic Endpoints of the Endocrine System, Tox. Lett., 102-103, 405-9 (1998), *available at* doi.org/10.1016/S0378-4274(98)00244-6.
[38] U.S. Environmental Protection Agency, Risk Assessment Forum, Assessment of Thyroid Follicular Cell Tumors, EPA/630/R-97/002 (Mar. 1998), *available at*: www.epa.gov/osa/assessment-thyroid-follicular-cell-tumors.
[39] *Id.* at 1.

IV. Scientific Expert Evaluations and Studies Specific to Red No 3's Secondary Mechanism of Carcinogenesis

The CSPI Petition argues that Red No 3 is an animal carcinogen with little consideration or analysis for the secondary mechanism hypothesis. Although the Petition cites and summarizes studies on secondary mechanism, it (1) does not believe a secondary mechanism has been established, and (2) even if it has, FDA still must remove Red No 3 from the list of colors:

> "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."[40]

On the second argument, assuming FDA finds that Red No 3 is non-genotoxic, and acts a secondary mechanism, CSPI incorrectly asserts the requirements of the Delaney Clause, Congressional intent, and FDA's position. The CSPI correctly quotes FDA's comment that 'the Delaney Clause does not differentiate between non-genotoxic and genotoxic carcinogens.'[41] However, the agency recognizes in that same final rule, as it did in FDA's 1990 Denial, the distinction between primary and secondary mechanisms as it relates to "inducing cancer" under the Delaney Clause.[42] As Congress intended, FDA recognizes that it has "discretion and judgment in deciding whether a substance has been shown to cause cancer."[43] To that end, FDA has recognized a secondary mechanism does not induce cancer within the Delaney Clause.[44] More specific to Red No 3, FDA left open the possibility of a secondary mechanism of action:

> "FDA believes that such a secondary mechanism hypothesis has merit from a scientific perspective [then asserting the data in 1990 was inadequate to evidence a secondary mechanism]."[45]

If FDA finds that a chemical is shown to be carcinogenic by a secondary mechanism of action, the Delaney Clause would not apply because secondary mechanisms of action do not induce cancer. That said, FDA would still be subject to the "General Safety Clause" which requires FDA to review data to establish the color is safe under the conditions of use specified in the regulations, will be safe . . ."[46] Under the General Safety Clause, FDA would look to review data

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[40] Petition at 17.
[41] Petition at 12, *citing* 83 Fed. Reg. at 50500.
[42] *Id*. (non-genotoxic substances are "not directly DNA reactive but operating through a secondary mechanism," and are "assumed to have a threshold of exposure level below which tumor development is not anticipated and the risk of cancer is negligible").
[43] Color Additives, Hearings before the House Comm. on Interstate and Foreign Commerce, 86th Cong., 2d Sess. 12 (1960).
[44] 38 Fed. Reg. 10458 (April 27, 1973); 51 Fed. Reg. 41,765, 41,773 (Nov. 19, 1986); 55 Fed. Reg. 3526; 83 Fed. Reg. at 50500.
[45] 55 Fed. Reg. at 3529.
[46] 21 U.S.C. § 376(b)(4).

to establish whether there may be concentrations by which a color additive is or is not carcinogenic.

Based on the foregoing, FDA is obligated to reconsider the available evidence to determine whether a secondary mechanism of action applies to Red No 3. We next address separately expert evaluations and several studies demonstrating secondary mechanism of action for Red No 3 specifically, and secondary mechanism for rat thyroid carcinogenesis which also applies to Red No 3.

European Food Safety Authority Evaluation and Conclusion

In 2011, EFSA published a comprehensive Scientific Opinion on Erythrosine (E 127) As a Food Additive, which indicated and as mentioned in the Petition, that there is no new data available on chronic toxicity/carcinogenicity since the prior JECFA (1991) and SCF (1989) evaluations. The EFSA panel considered the weight-of-evidence which still showed that the tumorigenic effects of Erythrosine are "**secondary to its effects on thyroid function and not related to any genotoxic activity.**"[47] Although two studies showed an oncogenic effect in the thyroid gland of rats, the panel concluded, "the weight of evidence is that these tumours are elicited by a **non-genotoxic mechanism . . . rodent thyroid tumors may be considered of limited relevance to humans**."[48]

Concerning permitted uses in foods, the EFSA Panel noted that Erythrosine is exclusively authorized for use in cocktail and candied cherries, and Bigarreaux cherries. In addition, in 2011 levels of use intake estimates were below the Acceptable Daily Intake ("ADI") for both children and adults. Taking all the toxicology, human clinical, thyroid physiology, and exposure database into consideration, the EFSA panel on Food Additives and Nutrient Sources Added to Foods Panel concluded that "the present database does not provide a basis to revise the ADI of 0.1 mg/kg bw/day."[49]

Joint FAO/WHO Expert Committee on Food Additives Evaluation and Conclusion

Since 1965, JECFA has evaluated the safety of Erythrosine numerous times, most recently in 2019. The 2019 panel comprised of prominent scientists/toxicologists, including from FDA and Europe, with decades of experience. A toxicological dossier that included new studies on genotoxicity, reproductive and developmental toxicity, neurological effects, and hypersensitivity was reviewed. The new studies included several long-term oral toxicity studies that showed no compound-related increases in tumor incidences in mice, rats, and gerbils. The Committee also evaluated the 1987 lifetime toxicity/carcinogenicity study of Red No 3 in rats

[47] *EFSA Journal 9*(1), 1854 at 32 (2011) (emphasis added).
[48] *Id.* at 4.
[49] *Id*. at 5.

which reported an increased incidence of thyroid follicular cell adenomas at the highest dose tested. The Committee concluded the following on the findings of the 1987 study:

> "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. Another study indicated that erythrosine promoted the development of thyroid follicular tumours in partially thyroidectomized rats, but not in non-thyroidectomized rats . . . The present Committee noted that **the rat is not considered a suitable model for potential effects on the thyroid in humans.** . . ."[50]

As summarized in the report the Committee concluded that:

> "the thyroid tumours in male rats previously reported in long-term toxicity studies were **secondary to thyroid hormone changes and species-specific sensitivity.**"[51]

After determining the non-genotoxicity of Red No 3, its effect via secondary mechanism, and lack of applicability of the rat model for effects on the human thyroid, JECFA evaluated data to ascertain safe intake levels. It determined that human data is more appropriate to establish ADI levels, and "**dietary exposures to erythrosine for all age groups do not present a safety concern**":[52]

> "The evidence newly available at this meeting indicates that there are no concerns with respect to genotoxicity and reproductive and developmental toxicity of erythrosine. The previously established ADI of 0–0.1 mg/kg bw is based on a NOAEL of 60 mg per person per day (equivalent to 1 mg/kg bw per day for a 60 kg person) identified in a human study, with a default uncertainty factor of 10. . . . minimal effects on thyroid function were observed at 200 mg per person per day (equivalent to 3.3 mg/kg bw per day). Effects in experimental animals were observed at doses at least 60-fold higher than the NOAEL in this human study; **these effects supported the use of the human data as the basis for the ADI**."[53]

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[50] JECFA, Evaluation of Certain Food Additives and Contaminants, Eighty-sixth Report, WHO Technical Report Series 1014 at 29 (2019) (emphasis added), *referencing* Borzelleca, J.F, et al., Lifetime Toxicity/Carcinogenicity Study of FD&C Red No. 3 (erythrosine) in Rats, Food and Chemical Toxicology 25(10), 723 (1987), *available at* https://doi.org/10.1016/0278-6915(87)90226-2.

[51] *Id.* at 28.

[52] *Id.* at 32.

[53] JECFA, Evaluation of Certain Food Additives and Contaminants, Eighty-sixth Report, WHO Technical Report Series 1014 at 29 (2019) (emphasis added), *referencing* Borzelleca, JF., et al., Lifetime Toxicity/Carcinogenicity Study of

Studies Specifically Addressing Red No 3's Secondary Mechanism of Rat Thyroid Carcinogenesis

A number of studies, such as Shimizu et al. (2013), confirm Red No 3's secondary mechanism of carcinogenesis hypothesis. The authors investigated the possible influence of 44 halogenated compounds (including three food colors) on thyroid hormone metabolism via inhibition of iodotyrosine deiodinase (IYD) activity using microsomes of HEK-293 T cells expressing recombinant human IYD. Among them, 25 halogenated phenolic compounds inhibited IYD activity at the concentration of 1×10^{-4}M or 6×10^{-4}M. Rose bengal was the most potent inhibitor, followed by erythrosine B (Red No 3). These results suggested that halogenated compounds may disturb thyroid hormone homeostasis via inhibition of IYD, and that the structural requirements for IYD-inhibitory activity include halogen atom and hydroxyl group substitution on a phenyl ring.[54]

Studies [55,56] published in the late 1980's and 1990's by Capen, an expert on thyroid cancer mechanisms, concluded Red No 3 to be an example of a well-characterized compound that results in perturbations of thyroid function in rodents which is associated with an increased incidence of benign thyroid tumors[57] as shown in long term studies. Mechanistic studies on Red No 3 report the following:

> "[suggest] that a **primary (direct) action of [Red No 3] on the thyroid is unlikely** due to: (a) failure of the color (^{14}C-labeled) to accumulate in the gland; (b) negative genotoxicity and mutagenicity assays; (c) lack of an oncogenic response in mice and gerbils; (d) a failure to result in thyroid tumor development at dietary concentrations of 1% or less in male and female rats; and (e) a lack of increased tumor development in other organs."[58]

Further, Capen reported that "the color is poorly absorbed from the gastrointestinal tract with < 5% of the dose in rats and < 1% in humans absorbed following oral exposure." [59]

FD&C Red No. 3 (erythrosine) in Rats, Food and Chemical Toxicology 25(10), 723 at 32 (1987), *available at* https://doi.org/10.1016/0278-6915(87)90226-2.

[54] Shimizu, R., Yamaguchia, M., Uramarub, N., Kurokic, H., et al., Structure–activity relationships of 44 halogenated compounds for iodotyrosine deiodinase-inhibitory activity. Toxicol. 314:22-29 (2013).

[55] Capen, CC., Hormonal imbalances and mechanisms of chemical injury of the thyroid gland, *cited by* Jones, TC., et al., Endocrine System. Series II. Monographs on the Pathology of Laboratory Animals. International Life Sciences Institute Series, Springer-Verlag, Berlin, Heidelberg, New York, 217-238 (1996).

[56] Capen, CC., Mechanistic data and risk assessment of selected toxic endpoints of the thyroid gland. Toxicol. Pathol., 25:39-48 (1997).

[57] Capen, CC., Mechanistic considerations for thyroid gland neoplasia with FD and C Red No. 3 (erythrosine). In: The Toxicology Forum. Proceedings of the 1989 Annual Winter Meeting, Washington, D.C., 113-130 (1989).

[58] Capen, CC., Mechanistic data and risk assessment of selected toxic endpoints of the thyroid gland. Toxicol. Pathol., 25:39-48 (1997).

[59] Capen, CC., Mechanistic considerations for thyroid gland neoplasia with FD and C Red No. 3 (erythrosine). In: The Toxicology Forum. Proceedings of the 1989 Annual Winter Meeting, Washington, D.C., 113-130 (1989).

In another 60-day study, Capen (1995)[60] examined the effects of Red No 3 on thyroid hormone (TSH, T3, T4 levels) and direct morphometric changes (diameter of thyroid follicles, area of follicular colloid, height of follicular cells) in male rats fed either 4% (high dose) or 0.25% (low dose) Red No 3 compared to controls. Capen described a several-fold elevation in serum TSH levels that may be related, in part, to exposure of the thyroid to a high iodine content (58% of molecular weight of Red No 3 is iodine) and interference with the receptor-mediated response to TSH.

Capen and many other thyroid experts believe that such mechanistic data can aid in the interpretation of animal toxicology studies and help to clarify their significance in determining human relevance of observed tumors.

V. Scientific Expert Evaluation and Studies Generally Addressing the Secondary Mechanism of Rat Thyroid Carcinogenesis Applicable to Red No 3

A 1997 IARC Working Group examined the scientific basis for possible species differences in mechanisms by which thyroid follicular-cell tumors in mice and rats, renal tubule-cell tumors in male rats, and urinary bladder tumors in rats may be produced. The workshop also addressed the predictive value of these tumors for the identification of carcinogenic hazards to humans by evaluating the hypotheses underlying the proposed species-specific mechanisms for each of the above-mentioned tumor types. The experts then provided recommendations on how the mechanistic data could be used in the overall evaluation of carcinogenicity in humans.

The Working Group addressed the possibility that an agent causes cancer in animals through a mechanism that does not operate in humans, and therefore the mechanism must be considered when trying to determine human relevance. The Working Group evaluated many studies on carcinogenicity in organs, including thyroid follicular-cell tumours associated with imbalances in thyroid stimulating hormone levels resulting from persistent hyperplasia in specific cell types from which neoplasms arise. The Working Group published a Consensus Report,[61] which was agreed by all participants. Key findings in the IARC Consensus Report include the following:

- Confirms: "A number of **nongenotoxic rodent thyroid carcinogens act through an indirect mechanism** involving a sustained increase in TSH levels."[62]
- FDA's 1990 Denial noted that tumors were only present in studies on male rats, as opposed to studies involving mice, and other species. IARC report provides an explanation on the species-specific effects of thyroid carcinogens "**[m]utagenic thyroid carcinogens are more potent in rats than in mice.** Chemicals causing thyroid tumours

[60] Capen, CC., Toxic responses of the endocrine system. In: Klaassen, CD., (Ed), Cassarett and Doull's Toxicology: The Basic Science of Poisons, 5th ed. McGraw Hill, New York, 617-640 (1995).
[61] IARC, *Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis,* IARC Scientific Publications, No. 147, 230 (1999).
[62] *Id.* at 26.

through an indirect mechanism via sustained elevation of TSI-I levels are presumably much more potent in rats than in humans due to the large differences in thyroid physiology between these two species."[63]

- Confirms: "Pathology of thyroid follicular proliferative lesions. The majority of proliferative lesions found in both man and animals are benign."[64]
- Confirms: "Virtually all compounds which induce thyroid follicular tumours in animals in the long term have been shown to interfere with thyroid hormone homeostasis in the short term."[65]
- Concludes: "In conclusion, we consider that there is effectively no risk of thyroid carcinogenesis in man from limited exposure to low doses of a compound that has been shown to produce thyroid tumours in rodents only when administered for long periods of time at high doses, providing that the substance has been shown not to be mutagenic, and that it has been shown to interfere with thyroid hormone homeostasis by a defined mechanism."[66]

Studies Generally Addressing the Secondary Mechanism of Rat Thyroid Carcinogenesis

The European Society of Toxicologic Pathology organized an expert workshop in May 2018 to address considerations related to thyroid follicular cell hypertrophy and/or hyperplasia (FCHH), which is a common finding in nonclinical toxicity studies that can have important implications for risk assessment of pharmaceuticals, food and color additives, and environmental chemicals. The workshop was not intended to provide comprehensive coverage of thyroid gland biology, but rather to focus specifically on information relevant to interpreting FCHH in non-clinical toxicity studies. Red No 3 was not specifically addressed in the workshop, but findings can be applied to observations in thyroid hormone disruption by Red No 3. Results from the workshop[67] include the following key observations associated with thyroid gland carcinogenesis that are relevant to Red No 3 toxicity studies (the chronic rat bioassays and subsequent mechanistic studies):

> "There are inherent differences in the incidence, tumor types, and clinical history of thyroid cancer in mammals. Thyroid follicular tumors are particularly common in rats, depending on the strain, which suggests that rats may have a genetic predisposition for thyroid tumors. In rats, thyroid tumors are more common in males compared to females, and follicular adenoma is the most common subtype. As described above, the rat also has important differences in TH production, metabolism, and excretion (TH

[63] *Id.* at 27.
[64] *Id.* at 45.
[65] *Id.* at 47.
[66] *Id.* at 55.
[67] Huisinga, M., Bertrand, L., Chamanza, R., Damiani, I., et al., Adversity considerations for thyroid follicular cell hypertrophy and hyperplasia in nonclinical toxicity studies: Results From the 6th ESTP International Expert Workshop. Toxicol. Pathol. 48:920-938 (2020).

> economy) compared to other mammalian species and humans. Rats, particularly males, have high secretory capacity, rapid metabolism, and excretion of TH. Perturbation of TH economy results in rapid (as little as 1 week) morphologic changes in the rat thyroid gland that includes FCHH . . . Follicular cell adenomas and carcinomas of the thyroid gland in rats are usually preceded by FCHH, which can be induced by chemicals associated with disruption of TH economy. The FC tumors in rats can also be promoted by mitogenic stimulation of focal thyroid hyperplasia."[68]

A comprehensive review by Bartsch et al. (2018), including senior author Dr. Helmut Greim, examined numerous studies on the human relevance of follicular thyroid tumors in rodents caused by non-genotoxic substances. The authors conclusions are concisely summarized as follows:

> "Chronic stimulation of the thyroid gland of rodents by TSH leads to thyroid follicular hyperplasia and subsequently to thyroid follicular adenomas and carcinomas . . . the function and regulation of the thyroid gland are described and the types of thyroid tumors and the causes of their development in humans and animals are examined. Based on these data and the evidence that so far, except radiation, no chemical is known to increase the incidence of thyroid tumors in humans, it is concluded that rodent thyroid tumors resulting from continuous stimulation of the thyroid gland by increased TSH levels are **not relevant to humans.** Consequently, compounds that induce such tumors **do not warrant classification as carcinogenic.**"[69]

While the authors did not specifically address Red No 3, important toxicologic, pathologic, hormonal, and mechanistic observations that have been published on Red No 3 for decades are included in this paper's conclusion:

> "In conclusion: rats develop thyroid tumors resulting from constant stimulation of the thyroid gland and the continuous increase of TSH levels. In humans, as indicated by unchanged T3, T4 and TSH levels no disturbance of the thyroid homeostasis even after long-term high doses of drugs that enhance elimination of thyroid hormones is observed. Consequently, **non-genotoxic substances that only cause thyroid adenomas/carcinomas in rats,** which can be attributed to a disturbance in thyroid function such as the induction of phase II enzymes e.g., UGTs, are considered of **no relevance to humans and do not warrant classification as carcinogenic.** This also applies to tumors induced by substances

[68] Huisinga, M., Bertrand, L., Chamanza, R., Damiani, I., et al., Adversity considerations for thyroid follicular cell hypertrophy and hyperplasia in nonclinical toxicity studies: Results From the 6th ESTP International Expert Workshop. Toxicol. Pathol. 48:920-938 (2020).

[69] Bartsch, R., Brinkmann, B., Jahnke, G., Laube, B., Lohmann, R., et al., Human relevance of follicular thyroid tumors in rodents caused by nongenotoxic substances, Regul. Toxicol. Pharmacol. 98:199-208 (2018) (emphasis added).

> that **impair thyroid hormone synthesis** or release such as impaired iodine uptake, inhibition of iodine peroxidase, of thyreoglobulin synthesis, of deiodinases or of hormone release from the thyroid follicles when there is evidence for increased thyroid stimulation by increased TSH levels."[70]

A landmark paper entitled "Chemical Carcinogenesis" by Cohen and Arnold (2011)[71] addressed DNA-reactive versus non-DNA-reactive carcinogens, distinguishing between the former and those chemicals which increase cancer risk by increasing cell proliferation, which has been a breakthrough in delineating overall mechanisms. According to the authors, improvements in the assessment of modes of action involved in animal and in vitro models have led to more rational approaches to assessing relevance to humans. Based on extensive investigations over many decades, there have also been many chemicals identified that increase the risk of cancer in animal models but are not DNA reactive, and in each of these instances, the carcinogenic effect is because of an increase in cell proliferation. We believe that this has been quite adequately demonstrated for Red No 3, just as the author has definitively proven for sodium saccharin.

Sodium saccharin was shown in the 1970's to increase bladder cancer in rats in lifetime studies. The effect was greater in male rats than female rats, and mice and monkeys were shown to be unaffected. Cohen[72,73,74] showed that sodium saccharin in the rat led to pronounced alterations in the composition of the various normal urinary constituents, leading to the formation of calcium phosphate–containing precipitate.[75] This precipitate was cytotoxic and led to regenerative proliferation and ultimately to the development of a low incidence of tumors, and the formation of this precipitate occurred more readily in male rats than in female rats. Cohen's years of in-depth studies of this secondary mechanism of action for sodium saccharin in the male rat bladder led to its global removal from all lists of carcinogens.

In the section on "Non–DNA-Reactive Carcinogens," Cohen points out that in animal models, hormones and various treatments that affect the endocrine system frequently led to an increased risk of tumors in the target population. This includes tumors of the rat thyroid, the rat testicular Leydig cells, and the endocrine cells of the stomach. However, the authors also stressed that the only endocrine-related tumors in animal models that appear to be pertinent to humans are those associated with estrogen.

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[70] Bartsch, R., Brinkmann, B., Jahnke, G., Laube, B., Lohmann, R., et al., Human relevance of follicular thyroid tumors in rodents caused by nongenotoxic substances. Regul. Toxicol. Pharmacol. 98:199-208 (2018) (emphasis added).

[71] Cohen, SM. and Arnold, LL., Chemical carcinogenesis, Toxicol. Sci., 120 (Suppl. 1) (2011).

[72] Cohen, SM., Human relevance of animal carcinogenicity studies. Regul. Toxicol. Pharmacol. 21:75-80 (1995).

[73] Cohen, SM., Garland, EM., Cano, M, St John MK., et al., Effects of sodium ascorbate, sodium saccharin and ammonium chloride on the male rat urinary bladder. Carcinogenesis 16:2743-2750 (1995).

[74] Cohen, SM., Calcium phosphate-containing urinary precipitate in rat urinary bladder carcinogenesis. IARC Sci. Publ. 147:175-189 (1999).

[75] Cohen, SM. and Arnold LL., Chemical carcinogenesis, Toxicol. Sci., 120, Suppl. 1 (2011).

In contrast, Cohen noted that there are numerous chemicals that have been identified in rats that produce a direct mitogenic effect such as increasing thyroid-stimulating hormone by one means or another. The authors cited several publications supporting this concept and several of these studies are discussed elsewhere in this document. Although this review paper did not mention Red No 3 specifically, the following conclusions on this important area of thyroid chemical carcinogenesis research over many decades are relevant to Red No 3:

> "This leads to a direct mitogenic stimulus of the rat thyroid follicular cells and ultimately the development of benign and malignant tumors. Although humans have a similar feedback mechanism involving circulating thyroid hormones and TSH, the quantitative aspects are quite different. Humans have a circulating thyroid-binding globulin so that thyroid hormone is readily available if a stimulus occurs that leads to a decrease in circulating thyroid. In contrast, the rat does not have the circulating, readily available thyroid hormone, so its response is to increase TSH to stimulate the thyroid to produce more hormone by the follicular cells. Furthermore, the response to a hypothyroid stimulus in rats is to produce TSH, stimulating proliferation of follicular cells, leading to tumors. In contrast, hypothyroidism in humans leads to an increase in TSH, but this does not lead to an increase in follicular cell proliferation. It has been concluded that **this mode of action in rats is not relevant to humans**, based predominantly on a quantitative assessment of the process but also involving some qualitative issues. Again, the tumors arise from a process that leads to cell proliferation, and this process occurs early in the overall carcinogenicity of these chemicals. Epidemiologic investigations have not shown increased thyroid cancer associated with hypothyroidism, nor has it been shown to be related to chemical exposure, only radiation. Many chemicals have been shown to be toxic to the thyroid in animal models and in humans, but not thyroid carcinogens in humans."[76]

A comprehensive review by Gordon Hard[77] of the American Health Foundation covered new mechanistic information from 1988-1998 relevant to normal and abnormal thyroid growth and function. Hard noted that recent studies have shown that thyroid regulation occurs via a complex interactive network mediated through several different messenger systems, with increased TSH levels activating the signal transduction pathways to stimulate growth and

[76] Cohen, SM. and Arnold LL., Chemical carcinogenesis, Toxicol. Sci., 120, Suppl. 1 at S86 (2011) (emphasis added).
[77] Hard, GC., Recent developments in the investigation of thyroid regulation and thyroid Carcinogenesis, Environ. Health Perspect. 106:427-436 (1998).

differentiation of the follicular cell. The important role of TSH in thyroid growth and function helps to explain how perturbations in the thyroid-pituitary axis may influence the development of thyroid neoplasia in rodents (including rat) treated with chemicals, thus supporting the concept that chronic stimulation of the thyroid induced by goitrogenic compounds can result in thyroid tumors. Based on the literature review, Hard concluded that some comparative physiologic and mechanistic data highlight certain critical differences between rodents and humans that could confer an increased vulnerability of rodents to chronic hypersecretion of TSH (which has been routinely observed in male rat for Red No 3 at high doses). Furthermore, he concluded that newer studies provided further support that chemically induced thyroid neoplasia are linked to disruptions in the thyroid-pituitary axis, and that a practical threshold for the effects of such a chemical-causing thyroid tumors in rodents would be expected. Such conclusions have also been reached by more recent studies and reviews by authoritative bodies.

A series of paper published by McClain[78,79,80] in the first five years after the FDA delisted Red No 3 for certain uses, summarized mechanistic considerations for the relevance of animal data on thyroid tumors to human risk assessment. McClain described two basic mechanisms where chemicals produce thyroid gland neoplasia in rodent models: (1) involving chemicals that exert a direct carcinogenic effect in the thyroid gland; and (2) involving chemicals which, through a variety of mechanisms, disrupt thyroid function (especially hormone imbalance) and produce thyroid gland neoplasia secondary to hormone imbalance. McClain traced the historical development of the secondary mechanism hypothesis back to Kennedy and Purves (1941), who found thyroid adenomas in rats fed a diet containing brassica seeds, a naturally occurring goitrogen. McClain further described the wide acceptance of this consistent mechanism to explain the pathogenesis of thyroid tumors induced in rats treated with anti-thyroid drugs[81,82] and these observations are very relevant to what occurred in the Red No 3 rat study:

> "Anti-thyroid drugs initially produce a hormonal imbalance by interfering with thyroid hormone production. As a result, a sustained increase in the synthesis and secretion of TSH occurs via the negative feedback system of the pituitary gland to stimulate thyroid function. Increased TSH stimulation produces a variety of

[78] McClain, RM., The significance of hepatic microsomal enzyme induction and altered thyroid function in rats: Implications for thyroid gland neoplasia, Toxicol. Pathol. 17:294-306 (1989).
[79] McClain, RM., Thyroid gland neoplasia: non-genotoxic mechanisms, Toxicol. Lett. 64-65:397-408 (1992).
[80] McClain, RM., Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment, Mutat. Res., 333:131-142 (1995).
[81] *Id*. at 132 , citing Furth, J., Pituitary cybernetics and neoplasia. Harvey Lectures, Academic Press, New York/London, 47 (1968).
[82] McClain, RM., Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment, Mutat. Res. 333:131, 132 (1995), citing Furth, J., A meeting of ways in cancer research: Thoughts on the evolution and nature on neoplasms, Cancer Res., 19:241 (1959).

> morphological and functional changes in the follicular cell including follicular cell hypertrophy, hyperplasia, and ultimately neoplasia. The sustained excessive level of TSH is considered to be the pathogenic factor responsible for thyroid tumor production."[83]

McClain further described important species differences in thyroid gland physiology between rodents and humans which may account for a marked species difference in the inherent susceptibility for neoplasia secondary to hormone imbalance, pointing to high TSH levels (as seen in the Red No 3 study in male rat) that are acknowledged in rodents to be a determining factor for the development of thyroid cancer. McClain stressed that the rodent exhibits an increase in thyroid gland neoplasia due to even mild to moderate increases in TSH, and that this chronic stimulation of the thyroid gland by TSH in the rodent leads to the well-recognized progression of follicular cell hypertrophy, hyperplasia and eventually neoplasia.

McClain concluded that it is important to consider mechanism in the evaluation of potential cancer risks, as follows:

> ". . .there would be little if any risk for apparently nongenotoxic chemicals that act secondary to hormone imbalance at exposure levels that do not disrupt thyroid function. Further, the degree of thyroid dysfunction produced by a chemical would present a major toxicological problem before such exposure would increase the risk for neoplasia for humans."[84]

In the study by Swenberg et al. (1992), the section *"Role of carcinogens and goitrogens in the pathogenesis of thyroid gland neoplasia in rodents"* discussed the secondary mechanism of action in the rat thyroid tumors. The authors pointed out that "[a] basic understanding of the mechanism of chemical induction of thyroid neoplasia was obtained during experimentation in the 1940s and 1950s"[85] demonstrating the adverse impact of thyroid hormone imbalance. Further, the authors stated that excessive secretion of even endogenous TSH alone, in the absence of any chemical treatment, produces a high incidence of thyroid tumors as had been clearly established by several experiments during the 1950s where rats were fed diets deficient in iodine.

The authors concluded, in agreement with many other scientists (including many whose conclusions appear more recently and elsewhere in our comments), that:

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[83] McClain, RM., Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment, Mutat. Res. 333:131-142 at 132 (1995).
[84] *Id.* at 131.
[85] Swenberg JA., et al., Species-specific mechanisms of carcinogenesis, in *IARC Scientific Publication* 116:477, (1992).

> "Because of marked species differences in thyroid gland physiology and apparent susceptibility to hypothyroidism, the rodent is an inappropriate model from which to extrapolate cancer risk to man for chemicals that operate secondary to hormone imbalance. . . the rodent model is likely to be conservative and to provide overestimates of risks for species with different thyroid gland function because of the strong promoting effect of high levels of TSH."[86]

VI. CONCLUSION

As outlined in the comments, there is strong scientific evidence demonstrating that Red No 3 is:

- Non-genotoxic;
- Thyroid carcinogenesis observed in the male rat only is due to a secondary mechanism and not a direct primary mechanism; and
- More importantly, the carcinogenicity observed in the thyroids of male rats is species-specific, at the very highest dose tested only, and concluded by authoritative scientific bodies and many world-renowned toxicologists over many decades that the findings of thyroid carcinogenesis in male rats is not relevant to humans.

We respectfully request that FDA conduct a thorough scientific review of studies and expert evaluations referenced as well as those not referenced herein on secondary mechanism of rat thyroid carcinogenesis to determine the safety and use of Red No 3 in food and dietary supplements and decide to maintain Red No 3 in the permanent list of color additives.

Sincerely,

American Bakers Association
Consumer Brands Association
National Confectioners Association

[86] Swenberg, JA., et al., Species-specific mechanisms of carcinogenesis, in *IARC Scientific Publication* 116:477 at 491, (1992).