55 FR 3520-01
NOTICES
DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration
[Docket Nos. 76C-0044 and 76N-0366]
RIN 0905-AB60

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

Thursday, February 1, 1990

AGENCY: Food and Drug Administration.

ACTION: Notice; order denying petition.

SUMMARY: The Food and Drug Administration (FDA) is denying the color additive petition (CAP 9C0096) that requests the "permanent" listing of FD&C Red No. 3 as a color additive for use in cosmetics, including lipsticks and other ingested cosmetics, and externally applied drugs. The agency is taking this action because it has concluded that the proponents of FD&C Red. No. 3, principally the Cosmetic, Toiletry and Fragrance Association, Inc., and the Certified Color Manufacturers' Association, have not established that the use of this color additive in cosmetics, including lipsticks and other ingested cosmetics, and externally applied drugs is safe within the meaning of section 706 of the Federal Food, Drug, and Cosmetic Act. FD&C Red No. 3 causes a carcinogenic response in rats. Published elsewhere in this issue of the Federal Register is a document announcing the termination of the provisional listing of FD&C Red No. 3 for use in all cosmetics and externally applied drugs, and for all uses of lakes of FD&C Red No. 3 in food, drugs, and cosmetics.
DATES: Written objections and requests for a hearing by March 5, 1990.

ADDRESSES: Written objections to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:Catherine J. Bailey, Center for Food Safety and Applied Nutrition (HFF-334), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-472-5690.

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I. Introduction

In 1960, the Federal Food, Drug, and Cosmetic Act (the act) was amended by the Color Additive Amendments of 1960 (Title II, Pub. L. 86-618, 74 Stat. 404-407) (the amendments). As amended, the act provides that a color additive is deemed unsafe for use in or on food, drugs, cosmetics, certain medical devices, or the human body unless FDA has issued a regulation permanently listing that color additive for its intended uses (section 706(a) (21 U.S.C. 376(a))). FDA will issue such a regulation only if the agency has been presented with data that establish with reasonable certainty that no harm will result from the use of the color additive. The burden of presenting such data is on the person seeking approval of the use of the additive (21 U.S.C. 376(b); 45 FR 6252 at 6254 and 6255 (January 25, 1980)). Thus, since 1960, the proponents of all color additives, including FD&C Red No. 3, have had the legal obligation to establish, with sound scientific data, the safety of those color additives. As shown below, consistent with the D.C. Circuit Court finding that the Delaney Clause established an "extraordinarily rigid" standard (Public Citizen v. Young, 831 F.2d 1108, 1122 (D.C. Cir. 1987), the proponents of FD&C Red No. 3 have not met their burden in that FD&C Red No. 3 has been found to cause a carcinogenic response in rats.

In Certified Color Mfrs. Ass'n v. Mathews, 543 F.2d 284 (D.C. Cir. 1976), the United States Court of Appeals for the District of Columbia Circuit explained the purpose of the amendments:

The Color Additive Amendments of 1960 reflect a Congressional and administrative response to the need in contemporary

society for a scientifically and administratively sound basis for determining the safety of artificial color additives, widely used for coloring food, drugs, and cosmetics. The Amendments reflect a general unwillingness to allow widespread use of such products in the absence of scientific information on the effect of these products on the human body. The previously used system had some glaring deficiencies, and the 1960 Amendments were designed to overcome them [footnotes omitted].* * *

543 F.2d at 286-87.

In section 203(b) of the transitional provisions of the amendments, Congress provided for the provisional listing of the color additives in use at the time of the amendments, pending completion of the scientific investigations necessary to determine the safety of these additives. In 1960, numerous color additives were "provisionally" listed. Over the years, those color additives were gradually ***3521** removed from the provisional list either by permanent listing or by removal from the market. At this time, the only straight color additive remaining on the provisional list is FD&C Red No. 3 for use in externally applied drugs and cosmetics.

II. Background and Regulatory History

The color additive FD&C Red No. 3 has been in use for many years. It was first listed for use in food as "erythrosin" on July 13, 1907 (Food Inspection Decision 76, U.S. Department of Agriculture) and listed for food, drug, and cosmetic use as "FD&C Red No. 3" on May 9, 1939 (4 FR 1922). Because FD&C Red No. 3 and its lakes were in use at the time of the 1960 amendments, both FD&C Red No. 3 and its lakes were provisionally listed for food, drug, and cosmetic use (25 FR 9759; October 12, 1960).

On March 27, 1968, the Certified Color Industry Committee (now the Certified Color Manufacturers' Association (CCMA)) submitted a petition (CAP 8C0067) to FDA requesting the permanent listing of FD&C Red No. 3 for use in food, dietary supplements, and ingested drugs. FDA announced the filing of CCMA's petition in the Federal Register of July 2, 1968 (33 FR 9627). Subsequently, FD&C Red No. 3 was permanently listed for use in food and in ingested drugs under 21 CFR 8.242 and 8.4102 (34 FR 7446; May 8, 1969). These regulations were later codified at 21 CFR 74.303 and 74.1303.

Thereafter, on September 5, 1969, the Toilet Goods Association, Inc. (now the Cosmetic, Toiletry and Fragrance Association, Inc. (CTFA)), submitted a color additive petition (CAP 9C0096), requesting permanent listing of FD&C Red No. 3 for coloring cosmetics, including lipsticks, and externally applied drugs. FDA published a notice of filing of the petition in the Federal Register of August 6, 1973 (38 FR 21199).

Subsequently, in a letter dated May 14, 1974, CTFA requested that its petition be amended to include listing FD&C Red No. 3 in cosmetics for eye-area use. FDA published an amended filing notice for the petition in the Federal Register of March 5, 1976 (41 FR 9584), to include the listing of FD&C Red No. 3 for eye-area use and all types of cosmetics that are subject to ingestion. FDA notified the petitioner by letter dated May 14, 1976, of the need for data to support the use of FD&C Red No. 3 in cosmetics intended for use in the area of the eye. In a letter dated October 24, 1978, FDA advised the petitioner to consider withdrawing the portion of the petition that sought approval of the use of FD&C Red No. 3 in cosmetics intended for use in the area of the eye because it appeared that the required data from studies to support eye-area use of the color additive would not be readily available. Since that time, the petitioner has not submitted the required data on eye-area use. Therefore, FDA considers the portion of the petition relating to the listing of FD&C Red No. 3 for eye-area use to be withdrawn without prejudice in accordance with the provisions of 21 CFR 71.6(c).

FD&C Red No. 3 has remained provisionally listed for use in cosmetics and externally applied drugs under 21 CFR 81.1(a) since submission of CTFA's petition. The provisional listing of FD&C Red No. 3 currently has a closing date of January 29, 1990. Published elsewhere in this issue of the Federal Register is a document announcing the termination of this provisional listing. Specifications for certification of FD&C Red No. 3 for all uses are listed under 21 CFR 73.303.

Although it is not the petitioner for the permanent listing of the cosmetic and externally-applied drug uses, CCMA has submitted to the agency much of the data relevant to the safety of FD&C Red No. 3 because of the organization's overall interest in the status of the color additive. Thus, CCMA and CTFA will hereafter be referred to collectively as the proponents of FD&C Red No. 3. In determining whether to grant or deny this petition, FDA has considered all of the data submitted to the agency that is relevant to the safety of the color additive, regardless of who submitted it.

In the Federal Register of February 4, 1977 (42 FR 6992), FDA published revised provisional listing regulations which required new chronic toxicity studies on 31 color additives, including FD&C Red No. 3, as a condition of their continued provisional listing. FDA required the new chronic toxicity studies because previously submitted studies were deficient in several respects. FDA described these deficiencies in the Federal Register of September 23, 1976 (41 FR 41860 at 41863):

1. Many of the studies were conducted using groups of animals, i.e., control and those fed the color additive, that are too small to permit conclusions to be drawn today on the chronic toxicity or carcinogenic potential of the color. The small number of animals used does not, in and of itself, cause this result, but when considered together with the other deficiencies in this listing, does do so. By and large, the studies used 25 animals in each group; today FDA recommends using at least 50 animals per group.

2. In a number of the studies, the number of animals surviving to a meaningful age was inadequate to permit conclusions to be drawn today on the chronic toxicity or carcinogenic potential of the color additives tested.

3. In a number of the studies, an insufficient number of animals was reviewed histologically.

4. In a number of the studies, an insufficient number of tissues was examined in those animals selected for pathology.

5. In a number of the studies, lesions or tumors detected under gross examination were not examined microscopically.

In the February 4, 1977, final rule, FDA postponed the closing date for the provisional listing of FD&C Red No. 3 until January 31, 1981, to allow for completion of the new chronic toxicity studies. The authority of the Commissioner of Food and Drugs to grant this extension of the FD&C Red No. 3 closing date was judicially sustained. Health Research Group v. Califano, No. 77-293 (D.D.C. September 23, 1977).

Due to unforeseen difficulties in completing the new chronic toxicity studies, FDA postponed the closing date for the provisionally listed uses of FD&C Red No. 3 and its lakes to October 2, 1983, by final rule published in the Federal Register of March 27, 1981 (46 FR 18954). Once again, the Commissioner's authority to postpone the closing date for the provisionally listed uses of FD&C Red No. 3 was challenged and sustained. McIlwain v. Hayes, 690 F.2d 1041 (D.C. Cir. 1982). The October 2, 1983, closing date was subsequently postponed to December 2, 1983, in the Federal Register of October 4, 1983 (48 FR 45237). Thereafter, FDA postponed the closing date for the provisionally listed uses of FD&C Red No. 3 and its lakes for brief periods to provide the agency additional time to complete its review and consider the scientific and legal aspects of the petitioned uses of the color additive. Each of these extensions was annnounced in the Federal Register (48 FR 53694, November 29, 1983; 49 FR 4202, February 3, 1984; 49 FR 13344, April 4, 1984; 49 FR 23039, June 4, 1984; 49 FR 30926, August 2, 1984; 49 FR 38935, October 2, 1984; 49 FR 47228, December 3, 1984; 50 FR 4642, February 1, 1985; 50 FR 13018, April 2, 1985).

As part of the agency's review of the FD&C Red No. 3 toxicity data, and at FDA's request, the National Toxicology Program Board of Scientific Counselors, Technical Reports Review Subcommittee (NTP Subcommittee) convened a public meeting for the purpose of providing peer review of the data from the chronic bioassay of FD&C Red No. 3 in Charles River CD-1 rats conducted by the International Research and Development Corporation (IRDC) ***3522** for CCMA. (This study and FDA's evaluation of it are discussed in detail below.) Notice of this meeting was published in the Federal Register of October 11, 1983 (48 FR 46104). At the meeting, the NTP Subcommittee heard presentations by FDA pathologists and toxicologists and by scientists and consultants representing CCMA. CCMA also asked the NTP Subcommittee to consider new data at the meeting. The new data included a study designed to determine if the effects observed were due to an iodine excess from the sodium iodide constituent of FD&C Red No. 3. After discussion and deliberation, the NTP Subcommittee issued its report on December 27, 1983. As discussed in detail below in section IV, the NTP Subcommittee concluded that chronic bioassay of FD&C Red No. 3 in Charles River CD-1 rats provided convincing evidence that FD&C Red No. 3 is an animal carcinogen.

On June 3, 1985, FDA announced that, as a result of the agency's review and consideration of all of the data and other information available on FD&C Red No. 3, it had become clear that the use of FD&C Red No. 3 raised significant policy and scientific questions that could not be immediately resolved. Consequently, the agency postponed the closing date for FD&C Red No. 3 and its lakes to September 3, 1985 (50 FR 23294; June 3, 1985). In a proposed rule published in the Federal Register of June 26, 1985 (50 FR 26377), FDA explained the significant scientific and policy questions presented by the data from the chronic feeding studies of FD&C Red No. 3. In particular, the agency discussed the proponents' secondary mechanism hypothesis (discussed below in section IV) as it relates to the increased incidence of thyroid follicular cell carcinomas, adenomas, and hyperplasia in male rats that were fed the color additive at the 4.0-percent level. The agency also discussed the possibility that a chronic study might be useful in resolving questions related to the secondary mechanism and the agency's willingness at that time to extend the provisional listings for FD&C Red No. 3 to permit such a study.

Finally, in the June 26, 1985, proposed rule, FDA proposed to extend the closing date for FD&C Red No. 3 and its lakes to September 3, 1986, to allow the agency to receive and evaluate the report of a review panel composed of scientific experts from the U.S. Public Health Service (the 1986 Panel) that had been convened to review two issues concerning the risk

assessments for five provisionally listed color additives, including FD&C Red No. 3. The issues were: (1) Whether valid quantitative risk assessments could be performed for those color additives and (2) whether the available information supported the data analyses and the risk assessments that were performed and were before the agency. (The availability of the report of the 1986 Panel was subsequently announced in the Federal Register of March 6, 1986 (51 FR 7856).) In a final rule published in the Federal Register of September 4, 1985 (50 FR 35783), the agency responded to public comments on the June 26, 1985, proposal and postponed the closing date for FD&C Red No. 3 and its lakes to September 3, 1986.

Due to the complexity presented by the FD&C Red No. 3 data, the Commissioner of Food and Drugs, on June 16, 1986, convened a new Color Additives Review Panel (the 1987 Panel) to consider the data that appeared to suggest that FD&C Red No. 3 acts as a secondary carcinogen. The Commissioner requested that the 1987 Panel consider whether the data demonstrated that a secondary mechanism of action exists for FD&C Red No. 3; if not, what further studies would resolve the issue; and what human health concerns would be posed by continued use of the color additive until these questions were resolved. The closing date for the provisionally listed uses of FD&C Red No. 3 and its lakes was again extended to November 3, 1986, to allow the 1987 Panel to complete its work and make its report to the Commissioner, and for the agency to evaluate the 1987 Panel's report and to develop appropriate Federal Register documents (51 FR 31323; September 3, 1986). This November 3, 1986, closing date was further extended to November 3, 1987, to allow the 1987 Panel additional time to complete its report and for FDA to review the report and publish its proposed action based upon the 1987 Panel's recommendations (51 FR 39856; November 3, 1986). (The 1987 Panel submitted its report to FDA in July 1987; the availability of the 1987 Panel's report was announced in the Federal Register of August 11, 1987 (52 FR 29728; [Docket No. 87N-0254]). The Commissioner's authority to extend the provisional listings for FD&C Red No. 3 was thereafter challenged and sustained for a third time in Public Citizen v. Young, 831 F.2d 1108 (D.C. Cir. 1987).

The 1987 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 did state, however, that the color additive's tumorigenic effect is more likely to be the result of an indirect (secondary) mechanism. The 1987 Panel stated further that if it is assumed that the color additive poses a tumorigenic risk to humans, "the risk from ingesting [FD&C Red No. 3] containing food and drugs is small, that is, the number of people with [FD&C Red No. 3] induced tumors would be too small to be observed by epidemiologic or other human studies." The 1987 Panel suggested some studies that could be conducted to investigate further the mechanisms of action of FD&C Red No. 3.

In the Federal Register of November 3, 1987 (52 FR 42096), FDA announced that the agency was further extending the closing date for FD&C Red No. 3 and its lakes to May 2, 1988, to provide the agency with additional time to complete its review of the FD&C Red No. 3 toxicological data, as well as to consider the effect, if any, of the judicial decision in Public Citizen v. Young, supra, 831 F.2d 1108. (As discussed in section VI, in addition to ruling on the extension of the provisional listing, the court in Public Citizen v. Young addressed the applicability of the de minimis principle to color additives that are determined to be animal carcinogens.)

In a notice in the Federal Register of November 19, 1987 (52 FR 44485), FDA requested that all persons interested in the continued marketing of FD&C Red No. 3 and its lakes submit data concerning sale and use of the color additives in foods, drugs, and cosmetics. The agency stated that the requested data would be used to assess potential exposure to the color additive and to allocate the allowable safe uses of FD&C Red No. 3 among the current prevailing uses, if such allocation was determined to be necesary and appropriate based upon the agency's evaluation of available toxicological data. In a subsequent notice, FDA requested similar data concerning the use of FD&C Red No. 3 and its lakes in pet and animal food (52 FR 48326; December 21, 1987).

The closing date for the provisionally listed uses of FD&C Red No. 3 and its lakes was extended to July 2, 1988, by a final rule published in the Federal Register of May 2, 1988 (53 FR 15551) and to August 30, 1988, in a final rule published in the Federal Register of July 1, 1988 (53 FR 25127). The purpose of each of these extensions was to provide FDA with additional time to complete its evaluation of the data and to prepare the apropriate Federal Register documents.

In the Federal Register of August 30, 1988 (53 FR 33147), FDA proposed to extend the closing date for the ***3523** provisional listing of FD&C Red No. 3 and its lakes to June 30, 1989. In that proposal, the agency stated that the additional extension was needed to receive and review data from an on-going study of the hormonal effects of FD&C Red No. 3 in rats being conducted for CCMA. CCMA asserted that the results of this on-going study, when combined with other available data, would establish that FD&C Red No. 3 operates through a secondary mechanism. (These study results are discussed in section IV). In the Federal Register of October 28, 1988 (53 FR 43685), FDA responded to public comments on the August 30, 1988, proposal and published a final rule that postponed the closing date for FD&C Red No. 3 and its lakes to June 30, 1989. In a

final rule published in the Federal Register of June 30, 1989 (54 FR 27640), the agency extended the closing date for the provisionally listed uses of FD&C Red No. 3 and its lakes to August 29, 1989. In a final rule published in the Federal Register of August 29, 1989 (54 FR 35860), the agency extended the closing date for the provisionally listed uses of FD&C Red No. 3 and its lakes to October 30, 1989. The current closing date for the provisionally listed uses of FD&C Red No. 3 and its lakes is January 29, 1990, as established by the final rule of October 30, 1989 (54 FR 43961).

As the foregoing recitation of the regulatory history shows, the proponents of FD&C Red No. 3 have had the obligation since 1960 to establish the safety of the use of this color additive. Moreover, as shown above, 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 action. The proponents have not provided such data.

III. Chemistry

FD&C Red No. 3, a bluish red color of the xanthene class, is currently 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 now 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 salt 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. Section 74.303 provides specifications for the agency’s batch certification of FD&C Red No. 3. These specifications include limitations for sodium iodide, starting material-related impurities derived from resorcinol and phthalic anhydride, and lower-iodinated impurities. The specifications require that the color additive contain at least 87 percent total color, which is principally comprised of the disodium salt of 2′,4′,5′,7′-tetraiodofluorescein. Uncertified material is commonly called erythrosine or other names, including Colour Index (C.I.) Acid Red 51, C.I. No. 45430, or C.I. Food Red 14.45430, or C.I. Food Red 14.

IV. Toxicology

Although CTFA is the petitioner of record in this proceeding, other interested parties have submitted the results of studies and other information regarding the safety of FD&C Red No. 3. The pivotal study for this color additive is the chronic feeding study conducted in Charles River CD-1 rats, which, as discussed in detail below, establishes that FD&C Red No. 3 is an animal carcinogen. Most of the recently submitted studies, which have been submitted primarily by CCMA, address whether the color additive induces changes in thyroid/pituitary hormone levels that lead to formation of thyroid tumors in rats through a secondary mechanism. These studies were of short duration, with no exposure to FD&C Red No. 3 greater than 7 months, and none of them involved in utero exposure. Other studies, also submitted by CCMA, concern the effect of FD&C Red No. 3 on human thyroid physiology and how it is metabolized in man.

A. Summary of Toxicology Testing of FD&C Red No. 3

To establish that FD&C Red No. 3 is safe for use in cosmetics and externally applied drugs, CTFA, the petitioner, submitted reports of a number of animal toxicity studies conducted on the color additive prior to 1976. The external drug and cosmetic uses were not permanently listed on the basis of those submitted studies because studies specific to external application had not been completed.

As discussed above, on February 4, 1977 (42 FR 6992), FDA required the petitioners to perform additional long-term feeding studies in rats and mice as one of the conditions for the continued provisional listing of several color additives, including FD&C Red No. 3. The results of the new chronic study in rats showed an increased incidence of combined adenomas and carcinomas of the thyroid. Based upon these results, FDA concluded that FD&C Red No. 3 acted through a carcinogenic process to produce that response. That is, FDA concluded that FD&C Red No. 3 is an animal carcinogen. As noted above, FDA requested that the NTP Subcommittee review the data to determine whether it agreed with the agency’s findings and to consider whether the response was mediated through a secondary mechanism of carcinogenesis. As discussed in more detail below, the NTP Subcommittee concluded that results of the chronic study were convincing evidence of carcinogenicity for FD&C Red No. 3 in male rats.

All of the data submitted subsequent to the chronic rat feeding study have been designed to elucidate the mechanism of action of FD&C Red No. 3’s carcinogenic process and have not been designed or submitted to dispute the carcinogenic response observed in the chronic rat study. Thus, as detailed below, FDA’s conclusion that FD&C Red No. 3 causes cancer in animals

is unrefuted. The proponents of the color additive subsequently provided additional data from a short-term study, the Primate Research Institute (PRI) study, to support their initial contention that the thyroid tumors observed in the test animals were a response mediated by exposure to excess iodide supplied by FD&C Red No. 3. When the PRI results did not support the "iodide-mediated" hypothesis, the proponents then hypothesized that the thyroid tumors resulted from the operation of a secondary (or indirect) mechanism.

In particular, 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.

In support of this secondary mechanism hypothesis, the proponents submitted data from a 7-month exposure study in rats (the Hazleton study) and a 3-week study in rats to measure pituitary/thyroid effects (the Witorsch study). The proponents also submitted literature references pertinent to the ***3524** secondary mechanism hypothesis for FD&C Red No. 3.

Subsequently, in January 1989, the proponents submitted the results of a 60-day study (the Bio/dynamics I study) designed to provide evidence of the hormonal effects of FD&C Red No. 3 and to determine the threshold for these effects. In February 1989, the proponents submitted to FDA a final report on the absorption, distribution, and metabolism in rats of FD&C Red No. 3 (the ADME study). In April 1989, the proponents submitted additional information relating to the genotoxicity of FD&C Red. No. 3 and the secondary mechanism of carcinogenesis. In May and June of 1989, the proponents submitted protocols and preliminary results for a 60-day rat study and protocols for a 1-year study in rats (Bio/dynamics II study). In August 1989, the proponents submitted the final report for this most recent 60-day study. These studies were designed to provide additional support for the secondary mechanism hypothesis.

As discussed below, the agency has evaluated the earlier work and the proponents' more recent submissions.

B. Long-Term Rodent Studies

1. Experimental Design of the Long-Term Feeding Studies

Although the chronic feeding studies conducted prior to 1976 revealed no evidence of compound-related neoplastic responses, FDA concluded in 1976 that these earlier studies of FD&C Red. No. 3 were not adequate under current toxicologic testing standards to establish the safety of the color additive for the uses then provisionally listed. Thus, FDA's February 4, 1977, final rule required the petitioners to conduct additional chronic feeding studies on FD&C Red. No. 3. The studies were sponsored by CCMA and were conducted at the International Research and Development Corp. (IRDC), Mattawan, MI 49071. These studies included a long-term feeding study in mice and long-term feeding studies in rats with in utero exposure. (In utero exposure requires exposure of parent animals to the test substance prior to and during mating and exposure of their offspring during intrauterine development, lactation, and throughout their lifetime).

The experimental design for the IRDC studies of FD&C Red. No. 3 benefited from knowledge of the protocol deficiencies in previously conducted carcinogenesis bioassays and other chronic toxicity testing. Improvements in study design included: (1) The use of large numbers of animals of both sexes; (2) pilot studies to determine maximum tolerated dosages; (3) two control groups (thereby effectively doubling the number of controls); and (4) in utero exposure in one of two species tested. All of these protocol changes significantly increased the power of these tests to detect dose-related effects. For this reason, FDA believes that the results of the IRDC chronic feeding studies constitute a reliable basis for assessing the safety of FD&C Red. No. 3.

2. Long-Term Feeding Study in Mice

In the chronic feeding study conducted by the IRDC, Charles River CD(R)-1 mice of both sexes were randomly assigned to one of five treatment groups (120 animals per group with 60 animals per sex) that received FD&C Red No. 3 in dietary concentrations of 0, 0, 0.3, 1.0, and 3.0 percent for 24 months. (That is, there were two separate control groups of animals that did not receive FD&C Red No. 3 in their diet.) The final report for this study was submitted to the agency on May 11, 1982.

There were no adverse findings in this study that could be atttributed to the administration of the test compound. Thus, FDA concludes that the long-term exposure of Charles River CD(R)-1 mice to FD&C Red No. 3 did not produce a carcinogenic or other deleterious effect.

3. Long-Term Feeding Study in Rats

a. The IRDC studies. CCMA sponsored two long-term feeding studies in which FD&C Red No. 3 was administered in the diet of Sprague-Dawley Charles River Albino CD(R) rats in utero and for their lifetime (up to 28 months); these rat studies were also conducted by the IRDC.

In the first chronic feeding study, IRDC Study No. 410-002, the dosage levels of FD&C Red No. 3 were 0, 0, 0.1, 0.5, and 1.0 percent of the diet. (Again, two control groups received the diet without the test compound.) After this first study 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 an additional chronic feeding study in rats using the 4-percent dose level. This second rat study, IRDC Study No. 410-011, included two groups of rats: a control group given a standard diet without the test compound and a treated group that received 4-percent FD&C Red No. 3 in the diet. The data from IRDC Study No. 410-002 were submitted to the agency on May 11, 1982; the data from IRDC Study No. 410-011 were submitted on October 1, 1982.

In the first study (IRDC Study No. 410-002), the incidences of palpable masses were similar for both treated and control rats. Food consumption was only slightly higher in treated animals than in controls. Body weights were similar for control and treated animals from week 26 to the end of the study. Mean thyroid weight was higher in females in the 0.5-percent and 1-percent dose levels compared with controls.

Both FDA and CCMA performed microscopic examination of the animals on test for neoplastic lesions. Based upon that examination, CCMA contended that there were no significant results. FDA is not in agreement with this conclusion. The agency's microscopic examination revealed statistically significant, higher incidences of male rats with combined thyroid follicular cell adenomas and carcinomas in 0.1-percent, 0.5-percent, and 1-percent dose groups, compared with the combined control animals (p=0.016, 0.0007, 0.029, respectively). The interpretation of this finding is discussed below.

In the second study (IRDC Study No. 410-011), thyroid gland enlargement (as determined by increased weight) occurred in the male rats in the 4-percent treated group. CCMA also reported a considerable increase in the incidence of male rats with thyroid follicular cell adenoma in the 4-percent group (15/69 or 21.7 percent) compared to its concurrent control group (1/69 or 1.4 percent); this increase was statistically significant. The incidence of thyroid follicular cell hyperplasia in the treated animals was also higher than that in rats in the concurrent control group. CCMA also reported that the incidence of carcinomas was 2/69 (2.9 percent) in the male rat control group compared with 3/69 (4.3 percent) in the male rats dosed with 4-percent FD&C Red No. 3. CCMA concluded that there is no statistical difference ($p < 0.05$) between the incidences of carcinoma in these groups.

Based upon its own histopathology review of the second IRDC study, the agency disagrees with CCMA. FDA's review found 14/68 or 20.6 percent follicular cell adenomas in the 4-percent group compared with 1/68 or 1.5 percent in the controls. In addition, the agency's review found carcinomas in 5/68 or 7.4 percent of the 4-percent group compared with 1/68 or 1.5 percent in the controls. The agency's analysis of the incidence of combined adenomas and carcinomas demonstrated a statistically significant increase ($p < 0.0007$) in such tumors: 18/ ***3525** 68 (26.5 percent) in the 4-percent group compared with 2/68 (2.9 percent) in controls. The agency disagrees with CCMA's interpretation of these results, as discussed in detail below.

The agency also confirmed that there were a few more rats with parafollicular cell (C-cell) tumors in the 4-percent treated group compared with the control group. Given the variability in the spontaneous occurrence of C-cell 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.

Based on its evaluation of the data from the IRDC studies, the agency concludes that FD&C Red No. 3 caused cancer in male rats. Specifically, based upon its evaluation, the agency concludes that FD&C Red No. 3 caused an increased incidence of thyroid follicular cell hyperplasia and adenomas in males in the 4-percent dose group. In addition, the agency concludes that, in this same group of male rats, FD&C Red No. 3 caused an increased incidence of combined adenomas and carcinomas. For females, an increased incidence of adenomas was found in the 1-percent dose group but not in the 4-percent dose group, and thus, was not considered a dose-dependent effect in this dosage range. Notwithstanding this latter result in female rats, the results from the IRDC studies provide sufficient evidence to establish that FD&C Red No. 3 caused a carcinogenic effect in male rats.

b. The NTP Subcommittee review. 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, among other things, that there is convincing evidence from these chronic bioassays of the carcinogenicity of FD&C Red No. 3 in male rats. In particular, the NTP Subcommittee stated:

Long-term administration of FD&C Red No. 3 at a level of 4% in the diet in male Charles River CD rats resulted in significantly higher incidences of thyroid follicular cell adenomas, and combined follicular cell adenomas and carcinomas when compared to concurrent control rats. These findings were considered to be convincing evidence of carcinogenicity for FD&C Red No. 3 in male rats.

Additionally, there were significantly higher incidences of thyroid follicular cell adenomas, thyroid polymorphofollicular adenomas, and combined adenomas in female CD rats at a dietary level of 1%, and of thyroid C-cell adenomas in male rats at the 4% dose. Due to large variability in spontaneous occurrence of C-cell tumors in rats, the increase in incidence of C-cell adenomas was not judged to be biologically important. Exposed rats of both sexes had a higher incidence than controls of thyroid follicular hyperplasias. There was no evidence for a neoplastic effect of FD&C Red No. 3 in male or female Charles River CD-1 mice.

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. The Subcommittee recommended that additional studies be designed to elucidate the carcinogenic mechanisms including: (1) More definitive studies on the genotoxic potential of the color, not only in microbial systems but also in mammalian cells; (2) further clarification of apparent metabolic effects of the color as evidenced so far in [these studies] by increased food consumption, decreased body weight and alterations in levels of T3 and T4 and TSH, as well as determination of a no effect level for inhibition of T4 conversion to T3 ; and (3) studies on the pharmacokinetics of the color in male rats encompassing gastro-intestinal absorption, biotransformation, tissue binding and storage, and excretion.

Finally, the Subcommittee agreed that new data presented at the meeting by consultants for the sponsor, the Certified Color Manufacturers Association, did not change its conclusions.

Thus, both the NTP Subcommittee and FDA have concluded that FD&C Red No. 3 is an animal carcinogen.

c. The proponents' arguments. In a submission dated November 23, 1983, CCMA argued that the IRDC data did not provide evidence of carcinogenicity of FD&C Red No. 3. Specifically, CCMA contended that: (1) There were no significant pathological changes of the thyroid in mice or rats fed FD&C Red No. 3 at 0.1-, 0.5-, or 1-percent levels; (2) there was no dose-response relationship in the incidences of thyroid follicular cell hyperplasia or adenomas in male rats, and thyroid carcinomas were not compound-related; (3) male rats fed the color additive at the 4-percent level did not demonstrate decreased survival time; and (4) FD&C Red No. 3 did not possess the characteristics common to confirmed thyroid carcinogens. CCMA conceded, however, that the increased incidence of thyroid follicular cell adenomas and hyperplasia in male rats at the 4-percent feeding level as compared with controls "suggest that the compound may be oncogenic for the thyroid of Sprague-Dawley rats."

d. FDA's response to the proponents' arguments. FDA has considered CCMA's arguments and concludes that they do not alter the agency's conclusion, clearly supported by the NTP Subcommittee, that FD&C Red No. 3 at the 4-percent dose level caused a carcinogenic effect in male rats.

First, the agency agrees that there were no significant pathological changes to the thyroids of mice in the chronic mouse study. However, the agency disagrees with CCMA's general contention that there were no pathological changes in the rats in Study No. 410-002. This disagreement is based on the agency's findings of: (1) An increased incidence of female rats with adenomas at the 1-percent dose; and (2) an increased incidence of combined adenomas and carcinomas in all male rat groups as compared with controls.

CCMA's failure to find a significant tumorigenic effect was apparently because its statistical analysis treated adenomas and carcinomas as separate tumor classes. In other words, CCMA apparently distinguished between oncogenicity and carcinogenicity and between the ability of FD&C Red No. 3 to induce benign tumors (adenomas) and malignant tumors (carcinomas). CCMA also used this separation of tumors into adenomas and carcinomas as the basis for later testing for statistically significant differences of tumor incidence between treated and control groups. Although FDA also separately analyzes the incidences of adenomas and carcinomas, FDA extends it analysis further: the agency combines the incidences of adenomas and carcinomas and then statistically compares the combined incidence of tumors in treated animals with the control groups.

FDA believes that the agency's approach to tumor analysis is appropriate because it is entirely sound to interpret thyroid follicular cell adenomas as an earlier stage in a series of progressive proliferative changes leading to the expression of follicular cell carcinomas (Ref. 1). In conducting its review, the NTP Subcommittee also considered the combining of

carcinomas and adenomas to be an appropriate procedure. In IRDC Study No. 410-002, when the incidences of adenomas and carcinomas are combined, there is a statistically significant increase in the incidence of thyroid neoplasms in male rats at the 0.1-, 0.5--, and 1-percent dose levels.

Second, the agency concedes that while there is a positive carcinogenic response in each of these dose groups, there is not strong evidence for a dose-related carcinogenic response in this study. However, even if the findings in IRDC Study No. 410-002 are deemed inconclusive, the carcinogenic response in IRDC Study No. 410-011 was definitive. Thus, the lack of a dose-response effect in IRDC Study No. 410-002 would not alter the agency's conclusion that FD&C Red No. 3 was ***3526** demonstrated to be an animal carcinogen in IRDC Study No. 410-011.

Third, thyroid neoplasms are rarely fatal to the tumor-bearing animal (Ref. 2). Thus, the fact that male rats fed the color additive at the 4-percent level did not demonstrate decreased survival time is not inconsistent with FDA's conclusion that FD&C Red No. 3 caused a carcinogenic response in male rats in the 4-percent dose group. In fact, the finding that survival time was not decreased may indicate that the maximum tolerated dose (MTD) was not attained. (The MTD is the highest dose that can be tested without compromising the results of a carcinogenicity study. Use of the MTD assures the greatest sensitivity to detect a carcinogenic effect.)

Fourth, CCMA claims that FD&C Red No. 3 did not possess the characteristics common to confirmed thyroid carcinogens because thyroid carcinogens induce both malignant and benign tumors of the thyroid and tumors at other histologic sites. As discussed above, the agency concludes that in male rats fed 4-percent FD&C Red No. 3, the color additive did increase the incidence of follicular cell adenomas, as well as increase the combined incidence of follicular cell adenomas and follicular cell carcinomas. Thus, the data clearly demonstrate FD&C Red No. 3 is a thyroid carcinogen in animals. Furthermore, simply because some thyroid carcinogens do cause tumors at other histologic sites does not mean that all thyroid carcinogens must display this characteristic (Ref. 3).

In sum, FDA has reviewed the results of the two chronic feeding studies of FD&C Red No. 3 and has considered all of the arguments proffered by the proponents of the color additive. Based upon this review and consideration, FDA has concluded that the results of IRDC Study No. 410-011 firmly establish that FD&C Red No. 3 caused thyroid cancer in male rats when fed at the 4-percent dose level. The results of the second IRDC Study, No. 410-002, provide additional evidence to support the agency's conclusion that in a well-conducted scientific study (IRDC Study No. 410-011), FD&C Red No. 3 was shown to be an animal carcinogen.

C. Genotoxicity

The proponents of FD&C Red No. 3 contend that the color additive is not genotoxic and thus argue that a primary mechanism is not operating to induce rat thyroid follicular cell tumors. They have submitted published literature and reports to support their contention. The resolution of the potential genotoxicity of FD&C Red No. 3 is significant to the proponents' secondary mechanism hypothesis. If FD&C Red No. 3 is genotoxic, that would indicate that the color additive may interact directly with cellular deoxyribonucleic acid (DNA). This finding would be consistent with a primary carcinogenic mechanism and would contradict the proponents' contention that a secondary mechanism is exclusively producing the treatment-related effects. Importantly, however, the absence of positive mutagenicity findings, by itself, does not necessarily rule out a primary carcinogenic mechanism for FD&C Red No. 3.

FDA has evaluated the proponents' submissions concerning the potential genotoxicity of FD&C Red No. 3, as well as other available publications concerning the potential genotoxicity of FD&C Red No. 3 in various assay systems. As discussed in more detail below, there are negative results for the mutagenicity of FD&C Red No. 3 in bacteria and mitotic gene conversion in yeast. However, there are also reports of positive and weakly positive results from in vitro and in vivo assays of FD&C Red No. 3 for chromosomal effects. In addition, both positive and negative results have been reported from in vitro gene mutation assays in cultured mammalian cells. In view of these data, the agency concludes that unresolved issues concerning the genotoxicity of FD&C Red No. 3 remain. Thus, based on the available data, the agency is unable to conclude that this color additive is not genotoxic.

1. In vitro and In Vivo Chromosomal Effects

Four studies reviewed by the agency used cytogenetic endpoints in either in vivo or in vitro mammalian cell systems to evaluate the potential genotoxicity of FD&C Red No. 3. In the two in vivo studies, FD&C Red No. 3 was tested for its capacity to induce micronuclei in mouse bone marrow cells. The first study is one submitted to FDA by Litton on behalf of CCMA, which is part of the primary data published in a paper by Lin and Brusick; the second study is one in a publication by Godbole and Vaidya.

Lin and Brusick concluded that FD&C Red No. 3 was negative for micronuclei induction. However, the authors performed statistical analysis of these data only at the 1-percent level. The agency believes that the 5-percent level of statistical significance, a level generally used for micronucleus data, should have been used to determine whether a toxic effect on chromosomes (clastogenic effect) occurred in this study (REf. 4). In fact, in responding to FDA's evaluation of this study, CCMA's consultant and the study author, Dr. Brusick, acknowledged that the low dose results for the male mice would reach significance if the 5-percent level of significance were used.

In the second study, Godbole and Vaidya concluded that FD&C Red No. 3 was positive for induction of micronuclei. Although the authors performed no statistical analysis of their data, when the agency considers the results of the Lin and Brusick study and the Godbole and Vaidya study in combination, these two studies show that there is a possible induction of micronuclei in vivo by FD&C Red No. 3. This conclusion is based on the fact that: (1) In the study reported by Lin and Brusick, there were increased micronuclei frequencies in both male and female mice (3-fold and 8-fold, respectively), and (2) in the Godbole and Vaidya study, the increased frequencies were dose-related and, at the highest dose tested, the frequency was 8-fold higher than the negative control.

The agency reviewed two in vitro cytogenetic studies of FD&C Red No. 3; these studies provide supporting evidence for the clastogenic effect observed in vivo. Ishidate et al., reported that FD&C Red No. 3 induced a weak response for chromosomal aberrations in Chinese hamster cells. In the other in vitro study, Rogers et al., reported that there was a significant increase ($p<0.01$) in micronuclei frequency in V79 cells treated with 300 microgram/milliliter (g/ml) of FD&C Red No. 3.

CCMA rejects both of these apparently positive studies. Specifically, CCMA contends that the in vitro results obtained by Ishidate et al. do not necessarily indicate genotoxicity and may in fact be a technical artifact due to the high osmotic concentration of the color in the test system. FDA rejects this argument. First, the concentration of FD&C Red No. 3 used in this study was 0.6 milligram/milliliter (mg/ml), not 5 mg/ml as stated by CCMA. Second, the investigators in the Ishidate study were aware of the potential effect of osmotic pressure of the medium on the target cell population and considered such effects in setting a dose level. CCMA contends that Rogers' report on the increased micronuclei frequency in V79 cells cannot support the conclusion that FD&C Red No. 3 is clastogenic because it is inconsistent with the result of the in vivo micronucleus assay reported by Lin and Brusick discussed above. However, as shown above, when properly analyzed, the micronucleus test data reported in the Lin and Brusick paper demonstrated statistically significant positive clastogenic effects. Thus, ***3527** Rogers' report is not inconsistent with the results of other studies. The agency concludes, based on the combination of positive effects from the other studies and the lack of an adequate scientific basis for rejecting these positive results, that the available data indicate a possible genotoxic effect for FD&C Red No. 3.

2. Gene Mutation in Mammalian Cells in Culture

FDA reviewed three studies in which the color additive was tested for its capacity to induce gene mutation in mammalian cells in culture. In two studies, one conducted by Litton Bionetics and the other by Microbiological Associates and subsequently published by Cameron et al., L5178Y mouse lymphoma cells (TK locus) were used; in the third study, conducted by Rogers et al., the target line was V79 Chinese hamster lung cells using both the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and Na+, K+ -ATPase (ouabain) loci.

The results of the Litton study with FD&C Red No. 3 were evaluated as negative by both FDA and CCMA. In the V79 assays conducted and reported by Rogers et al., there was a greater than 4-fold increase in the number of TGR mutants for the HGPRT locus at the 100 ug/ml dose level of FD&C Red No. 3 in the absence of hepatocytes; negative responses were obtained at the ouabain locus both with and without hepatocytes for metabolic activation. In the Microbiological Associates study, erythrosine was tested in L5178Y mouse lymphoma cells. This study was evaluated as positive by FDA in that postitive responses were obtained both in the absence and in the presence of an S9 metabolic activation system. Positive responses in this assay are known to represent either gene mutations or chromosomal aberrations (Ref. 5). However, the authors did not evaluate the mutant colonies in this study to determine whether they were the result of gene mutations or chromosomal aberrations.

CCMA questions the validity of the positive results in the Microbiological Associates study on the basis of the potency of the response. CCMA contends that agents with potencies equivalent to that obtained with erythrosine in this mouse lymphoma assay are positive in virtually all tests for genotoxicity. Also, CCMA's consultant contends that with the potency reported, which was roughly equivalent to that of ethylmethane sulfonate (EMS), the positive control, the color additive should give at least a weak response in the Ames test, and that FD&C Red No. 3 is negative in the Ames test.

FDA has considered this argument as the basis for questioning the validity of Microbiological Associates' study but finds the argument unsubstantiated. First, there is another example of a highly potent response with FD&C Red No. 3. In particular,

Rogers et al., reported that the micronucleus frequency in V79 cells induced by FD&C Red No. 3 was equivalent to or somewhat higher than that obtained with the EMS positive control. Second, there are data that show that not every chemical that induces a positive response in the mouse lymphoma assay will be positive in the Ames test. For example, Fung et al., reported that both caffeic acid and chlorogenic acid were positive in the L5178Y mouse lymphoma assay and both chemicals were negative in the Ames test. The increase in mutant frequency over the solvent control for both of the these chemicals was of a magnitude similar to that observed with erythrosine (Ref. 6). Similarly, Rogers-Back et al., reported that acetaldehyde oxime, butanol oxime, 2-butanone oxime, and cyclohexanone oxime all induced positive responses in the L5178Y assay but were negative in the Ames test (Ref. 7).

The agency concludes that although there was a negative response in the Litton investigation using the L5178Y mouse lymphoma assay, the other positive response in this test system (the Microbiological Associates study), as well as the increase in mutants in the V79/HGPRT assay, indicate that FD&C Red No. 3 has the capacity to induce gene mutation in mammalian cells in culture.

3. Mutagencity in Bacteria

FDA reviewed a number of studies on the ability of FD&C Red No. 3 to induce mutagenic responses in Salmonella typhimurium and Escherichia coli. These studies were contained in a number of published papers as well as in a study by Litton Bionetics submitted to the agency. The agency agrees with CCMA that these various studies show that FD&C Red No. 3 does not induce a mutagenic response in either of these bacterial systems.

4. Genetic Studies in Yeast

FDA reviewed two studies of the genetic effects of FD&C Red No. 3 in yeast. First, the agency reviewed a study by Matula and Downie in which FD&C Red No. 3 was tested for its ability to induce reverse mutation in Saccharomyces cerevisiae; the authors concluded that the color additive induced a positive response. Based upon its review, the agency has concerns about various aspects of the methods used and considers the authors' conclusion tenuous.

The agency also evaluated data on mitotic gene conversion in S. cerevisiae by FD&C Red No. 3 which was contained in three other studies: a publication by Sankaranarayanan and Murphy, a report by Matula and Downie, and a study by Litton. With the exception of a reported positive response in strain D7 obtained by Matula and Downie, the data from these three studies were negative. Because of concerns about the methodology used in the study, the agency, based upon its own evaluation, finds the conclusion of Matula and Downie to be tenuous.

5. In Vitro Transformation

FDA evaluated a study by Price et al., who reported that FD&C Red No. 3 did not induce morphological transformation in Fischer rat embryo cells infected with rat C-type virus. The agency believes that these data are not reliable because the sample of FD&C Red No. 3 used in the study was autoclaved and thus may have undergone thermal degradation, possibly confounding the results.

6. DNA Damage in Bacteria

The agency reviewed a series of published reports on differential killing in bacteria that had been previously evaluated by the 1987 Panel. In such assays, killing of a repair-deficient strain of bacteria to a greater extent than the repair-proficient strain is deemed to be evidence that the compound may have DNA-damaging activity. The agency believes that the data from these tests are not reliable and should not be used in interpreting the genotoxicity of FD&C Red No. 3. This conclusion is based on the results from an international collaborative study in which short-term tests were evaluated as predictors for carcinogenicity. From the results of this collaborative study, it was recommended that the bacterial DNA-repair test not be used for carcinogen screening (Ref. 8). More importantly, FDA finds that it is impossible to assess transmitted genetic effects in these tests because the endpoint measured depends on the ability of the test chemical to kill the target cells.

7. Evaluation of Genotoxicity Data

Both the 1986 Panel and the 1987 Panel reviewed the available genotoxicity data for FD&C Red No. 3. In the course of considering the carinogenic risk associated with the use ***3528** of the color additive, the 1986 Panel found that "the results suggest that the dye is almost assuredly not a carcinogen acting directly on the genome." However, the 1986 Panel went on to say:

The question of how the toxic effects of the compound, especially the phototoxic effect and the release of iodine are involved in some of the short-term effects seen is a critical, unanswered one. The effect of toxicity in the assays for genetic damage should be clearly separated from a direct effect on the genome, which is difficult to do for many of these observations since the exposures which are effective seem to be toxic ones.

In considering the data that a possible secondary mechanism of action exists for FD&C Red No. 3, the 1987 Panel also evaluated the genotoxicity data available at that time. The 1987 Panel concluded that, as a whole, the short-term tests indicate that there was little reason to suggest any mechanism of direct interaction of FD&C Red No. 3 with DNA.

FDA believes that its conclusion that there are important, unresolved questions concerning the genotoxicity of FD&C Red No. 3 is not necessarily inconsistent with the conclusions of both the 1986 Panel and the 1987 Panel. Importantly, neither of these Panels had access to and reviewed the publications by Rogers et al., and Cameron et al. (the Microbiological Associates study). The results reported in these two publications provide the principal basis for the agency's conclusion regarding the potential genotoxicity of FD&C Red No. 3.

After evaluation of the results from different genetic endpoints, the agency concludes that the available data demonstrate that FD&C Red No. 3 is not mutagenic in bacteria and does not induce mitotic gene conversion in yeast. Importantly, however, FDA believes that the available data show that FD&C Red No. 3 induces chromosomal effects and gene mutations in mammalian cells. In particular, a weakly postive response for chromosomal aberrations was observed in Chinese hamster cells in vitro, and there were positive responses for micronuclei induction in V79 cells in vitro and in mouse bone marrow polychromatic erythrocytes. There was also an increase in the mutant frequency in V79 cells at the HGPRT locus. In addition, erythrosine was reported to induce gene mutations at the TK locus in L5178Y mouse lymphoma cells both in the absence and presence of an S9 metabolic activation system in one study; a different study gave negative results in this assay.

The results from each genetic assay must be judged individually. Negative results from a large number of studies in which the same assay was used, e.g., the Ames' salmonella test, do not outweigh or resolve concerns raised by single or replicated positive responses in other genetic assay systems, e.g., the positive response for micronucleus induction in V79 cells. As a result of the findings on chromosomal and gene mutational endpoints in mammalian cells, FDA concludes that FD&C Red No. 3 has not been shown to be nongenotoxic.

D. Skin Penetration Study

CTFA sponsored an in vitro percutaneous absorption study designed to measure the ability of FD&C Red No. 3 to penetrate excised human skin under conditions simulating the use of the color additive in cosmetics. Subsequently, CTFA used this information to estimate the systemic exposure to FD&C Red No. 3 from its use in topical applications. In addition, based upon the results of this study, CTFA conducted an assessment of the risks associated with the cosmetic uses of the color additive as discussed below in section VI.

The in vitro percutaneous absorption study of FD&C Red No. 3 was conducted for CTFA by Dr. T. Franz at the University of Washington. On April 25, 1984, CTFA submitted its final report for the study to FDA. In this study, skin sections were obtained from the abdominal region of cadavers within 24 hours of death, and used immediately or refrigerated and used within 20 hours. Subcutaneous fat tissue and about half of the dermis was removed from each section prior to the section's fit into a 1.0 cm2 Franz diffusion chamber. The penetration of 14 C-radiolabeled erythrosine, referred to as 14 C-FD&C Red No. 3 by the author, through human cadaver full thickness skin and isolated epidermis was determined for vehicles comprised of aqueous solutions buffered at ph=6 and 8; 50 percent ethanol/water; and oil in water emulsion. A lake preparation of the radiolabeled color additive was similarly tested in vehicles comprised of mineral oil, castor oil, and talc. The concentration of the color additive in the vehicle and the amount applied to the skin sample were chosen to approximate the usage of FD&C Red No. 3 in external cosmetics and drugs. Upon application of the vehicle containing the radiolabeled material to the surface of the skin sample, a receptor phase consisting of an isotonic saline solution of pH 7.4 at 37°C was sampled for radioactivity and replaced by fresh saline at intervals up to 96 hours. The report concluded that the greatest total percentage absorption of FD&C Red No. 3 results from use of the 50 percent ethanol/water vehicle and is approximately 0.9 percent of the applied dose for up to 72 hours after exposure.

CTFA's evaluation of the study pointed out deficiencies in the study. For example, the radioactivity measurements were near detection limits, and there was considerable variability observed in duplicate determinations with skin from the same donor. Nevertheless, CTFA used the study results to conduct risk assessments; for such assessments, CTFA estimated that the absorption of FD&C Red No. 3 was a maximum of one percent of the material in contact with the skin when applied in aqueous or ethanol/water solution or in oil/water emulsion.

FDA reviewed CTFA's percutaneous absorption study and determined that, although sound procedures were used in the study, the radiochemical purity of the test sample was not provided. Thus, the quantitative degree to which the 14 C moiety resided with 2′,4′,5′,7′-tetraiodofluorescein (or principal component), or a similarly radiolabeled contaminant, could not be evaluated. Based upon the results of this study, FDA concludes that the external use of FD&C Red No. 3 does result in penetration of human skin by some portion of the color additive. Although the failure to characterize the radiochemical purity

of the test sample precluded accurate measurement of the degree of penetration, the agency agrees with CTFA that, based upon measurements of the penetration of the 14 C moiety, FD&C Red No. 3 penetrates the human skin and does so at levels of less than 1 percent. However, the study provided little information as to the exact nature of the components of FD&C Red No. 3 that actually penetrate the skin.

The 1986 Panel also reviewed CTFA's study and concluded that the lack of information about radiochemical purity made the study of limited use in determining the degree to which FD&C Red No. 3 penetrates the skin.

E. The Secondary Mechanism of Carcinogenesis

As discussed extensively above, in the second chronic feeding study of FD&C Red No. 3 (IRDC Study No. 410-011), the color additive was shown to be a thyroid carcinogen in male rats. The proponents of FD&C Red No. 3 have not sought to dispute the results of the second chronic feeding study with data from yet another chronic study that purports to show an absence of toxic effects. Instead, the proponents have conducted a number of studies in an ***3529** attempt to establish the mechanism by which the color additive FD&C Red No. 3 exerts its carcinogenic effect. Although these studies have been many and have varied in their design, as shown in detail below, the data submitted by the proponents fails to establish a process by which FD&C Red No. 3 operates as a carcinogen.

The carcinogenic process is recognized as a complex, long-term, multi-step process that results from numerous causes (Ref. 9). Exposure to chemical substances or certain experimental conditions may result in the expression of neoplasia from either a primary (direct) effect, a secondary (indirect) effect, or both (Ref. 2). Unless sufficient evidence is provided to the contrary, FDA must assume that a carcinogenic effect at a given organ site is mediated by a direct effect of the test substance.

The proponents of FD&C Red No. 3 have hypothesized that this color additive mediates thyroid neoplasia or oncogenesis by a secondary effect; i.e., by disrupting the hormonal relationships that normally exist between the pituitary and the thyroid. As discussed below in section VI, the proponents contend that if FD&C Red No. 3 is shown to exert its oncogenic effect by a secondary mechanism, then the Delaney clause of the Color Additive Amendments (21 U.S.C. 376(b)(1)(B)) would not preclude the permanent listing of the color additive for the petitioned uses.

FDA has evaluated the studies, published literature, and reports from experts on thyroid physiology that were submitted by the proponents in support of their secondary mechanism hypothesis. FDA believes that such a secondary mechanism hypothesis has merit from a scientific perspective. However, based upon a fair evaluation of the data and information filed to date, FDA concludes that the existing scientific data are not adequate to demonstrate that such a mechanism is operating to produce the carcinogenic response associated with exposure to FD&C Red No. 3. Of course, 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.

In order to facilitate an understanding and evaluation of the proponents' hypothesis, the agency has included below a discussion of the hormonal relationships that operate to sustain normal thyroid gland function and an explanation of how dysfunctions in various portions of the thyroid/pituitary axis can mediate changes in thyroid function and structure. This description is based on information available in the general literature (Refs. 10 and 11).

1. Normal Thyroid Gland Physiology

The thyroid is a small double-lobed gland in the human as well as the rat that lies at the base of the frontal portion of the neck on either side of the trachea. The thyroid gland secretes two hormones, thyroxine or 3,5,3,′5,′ ‘-tetraiodothryonine (T4) and small amounts of 3,5,3,′-triiodothyronine (T3). In the body, all of the T4 and a small portion of the T3 are synthesized in the thyroid. Most of the T3 produced in the body is derived peripherally by conversion of T4 to T3 . The biological activity of T3 is considerably greater than T4 . These two hormones are supportive of many physiological functions in the body. For example, they are essential to normal intrauterine development of the central nervous system and skeleton of the fetus. These hormones also have an important influence on the rate of cellular metabolism so that food is converted into energy to support many cellular functions.

Within the thyroid, there are two types of functional cells: parafollicular or C-cells and follicular cells. The C-cells are responsible for secreting calcitonin, a hormone that is important in the control of calcium metabolism. The follicular cells form a single layer of cells lining each follicle and are responsible for the synthesis, storage, and secretion of T4 and T3 . In the center of each follicle is a cavity filled with colloid. Colloid is composed of a glycoprotein, thyroglobulin, and is the storage site of T4 and T3 .

The level of thyroid gland activity is principally regulated by the secretion of a third hormone, thyrotropin (or TSH), from the

anterior pituitary. The body automatically adjusts the synthesis and release of the thyroid hormones, T4 and T3 , based on the circulating level of T3 . If the levels of T3 drop so low that the thyroid cannot supply its normal functions, then the anterior pituitary releases additional quantities of TSH. The thyroid gland responds to the additional TSH by releasing more T4 and T3 , and at the same time synthesizes more T4 and T3 .

This increased level of thyroid activity, also known as thyroid activation, usually results in a decrease both in the amount of colloid and in the size of the follicles. In this more active state, the follicular cells change size from short and cuboidal to tall and columnar; this increase in cell size is referred to as hypertrophy. Hypertrophied follicular cells often show an increase in certain organelles indicative of increased functional activity.

If the thyroid stimulation continues for a prolonged period of time, the number of cells lining the follicles increases; in time, multiple layers of follicular cells develop. This increase in numbers of follicular cells is referred to as cellular hyperplasia. If the degree of hypertrophy and/or hyperplasia is great enough, the thyroid gland itself will increase in size and weight; this condition is referred to as glandular hypertrophy.

Under normal conditions, as the increased secretion of TSH causes increased synthesis and release of T4 and T3 , the T4 is converted to T3 and a portion of the new T3 is absorbed by the anterior pituitary. This absorption of T3 lowers the release of TSH until the level of T3 again falls to lower than required levels. This physiological response of increased glandular secretion of hormones mediated by a hormone from the pituitary, TSH, that is subsequently inhibited by the hormones whose release it stimulates, is a common mechanism to control glandular function in the endocrine system and is referred to as a "negative feedback" mechanism.

The thyroid hormones, T4 and T3 , are synthesized in the follicular cells of the thyroid gland in a series of five steps.

(1) Inorganic iodide is actively concentrated in the thyroid gland.

(2) The trapped iodide is oxidized by the thyroid peroxidase enzyme.

(3) The oxidized iodide binds to tyrosyl residues (derived from the amino acid, tyrosine) with a specific thyroprotein, thyroglobulin, to form hormonally inactive precursor molecules, mono- and diiodotyrosine.

(4) The mono- and diiodotyrosine molecules couple to form either T4 or T3 .

(5) The release of T4 and T3 occurs following the hydrolysis of the colloid protein, thyroglobulin, by lysosomal enzymes, proteases, and peptidases. (Lysosomes and subcellular structures that contain various types of hydrolytic enzymes that are involved in intracellular digestive processes).

The enzyme, 5′-monedeiodinase converts T4 to T3 by removal of iodine from the 5′ position; this process occurs peripherally (outside the thyroid gland) in the kidneys and liver. Another peripherally located enzyme mediates the removal of iodine from the 5 position on the T4 molecule, resulting in the formation of a biologically inactive form of T3 , known as reverse T3 or rT3 . When the peripheral conversion of T4 to T3 is blocked by inhibition of 5′'- ***3530** monodeiodinase, reverse T3 , along with T4 , accumulates.

Administration of goitrogens (antithyroid agents) as well as many kinds of environmental stimuli can affect the release of hormones from the thyroid gland. Antithyroid agents interfere with the synthesis of thyroid hormones and have the common property of producing a decrease in serum T4 and T3 levels, thereby causing the pituitary to increase production of TSH. This TSH then stimulates the thyroid gland. If release of TSH continues unabated at high enough levels for a sufficiently long time, hyperplasia will occur and the thyroid gland will hypertrophy (become enlarged), develop follicular adenomas and, in some cases, carcinomas (Ref. 12).

2. The Secondary Mechanism Hypothesis

The proponents' submission of May 9, 1988, contained a detailed discussion of their secondary mechanism hypothesis. In that and subsequent submissions dated January 12, 1989, and April 3, 1989, the proponents presented the following argument:

(1) The administration of 4.0 percent FD&C Red No. 3 inhibits the 5′ '-monodeiodinase in the rat liver and kidney, thereby decreasing serum T3 concentrations and increasing serum T4 and rT3 concentrations.

(2) Decreased serum T3 concentrations increase the responsiveness of the pituitary thyrotrophic (TSH-secreting) cells to

endogenous thyrotropin releasing hormone (TRH) resulting in the increased secretion of TSH by the pituitary.

(3) The thyroid is stimulated by the increased levels of TSH to produce more T4 and more T3 to compensate for the continued deficit of T3 caused by the inhibition of 5′-monodeiodination of T4 by 4.0 percent FD&C Red No. 3. The continued inhibition of 5′-monodeiodination prevents a restoration of normal serum T3 concentrations.

(4) If the inhibition of 5′-monodeiodination of T4 by 4-percent FD&C Red No. 3 and the subsequent hyperstimulation of the thyroid by TSH continue for extended periods of time, follicular cell hypertrophy, hyperplasia, and adenoma may develop. The duration of inhibition is critical to the degree to which changes in follicular cell morphology occur. There is a progression of changes in follicular cell morphology resulting from sustained, increased stimulation of the rat thyroid

(5) If FD&C Red No. 3 operates through a secondary mechanism, a threshold dose, i.e., a dose having no effect on thyroid economy, can be established. Based upon the IRDC study, a threshold for the increased incidence of follicular cell hyperplasia was established at a dietary concentration (in male rates) of 0.5 percent (251 milligrams per kilogram per day (mg/kg/day)).

(6) FD&C Red No. 3 is not genotoxic, and thus, is not a direct-acting carcinogen.

(7) There are other substances, such as amiodarone, that act through a secondary mechanism, to produce effects similar to those observed for FD&C Red No. 3.

In sum, according to the proponents' secondary mechanism hypothesis, TSH, the endogenous harmone, mediates the oncogenic effect observed in the IRDC study in rats. Furthermore, there is likely to be a dose level of FD&C Red No. 3 that would not inhibit the conversion of T4 to T3 ; with adequate conversion of T4 to T3 , the pituitary would not be stimulated to synthesize and release excess TSH. Thus, FD&C Red No. 3 could be safely used in products at levels below this "threshold" effect because it would not induce a TSH-medicated excess stimulation of follicular cells.

The sections below describe data from a number of short-term studies which the proponents believe support their conclusions regarding the proposed effects of FD&C Red No. 3 on thyroid/pituitary hormone levels and thyroid morphology. The proponents claim that the Bio/dynamics I study and the Hazleton study demonstrate the sustained elevations in serum TSH that ultimately resulted in adenomas at the end of the IRDC study. They postulate that if the Hazleton study had been continued to a 2-year termination, the TSH levels of the treated animals would have remained significantly above control levels. Thus, by inference, a tumorigenic response would not have developed in the IRDC study had TSH levels returned to normal or near normal.

The proponents also contend that the morphological evidence from the four principal studies (the IRDC, PRI, Hazleton, and Bio/dynamics I studies), in combination, show the progression of changes in thyroid follicular cell morphology in male rats fed the 4-percent dose. Specifically, at the end of the 2-month Bio/dynamics I study, the thyroid showed subtle evidence of follicular cell hypertrophy; in the 7-month Hazleton and 6-month PRI studies, the follicular cell hypertrophy was more pronounced; and finally, in the IRDC study, hypertrophy and hyperplasia were seen at the 1-year interim sacrifice and, after 2 years of treatment, follicular cell adenomas were found. The proponents further contend that this progression of changes in follicular cell morphology is consistent with a progression of changes caused by sustained elevation of serum TSH. In addition, the proponents contend that the results of the four major studies, when analyzed together, demonstrate that FD&C Red No. 3 is a weak goitrogen, a conclusion supported by the absence of significant changes in thyroid morphology until after 60 days exposure, even though serum TSH levels were elevated.

The proponents assert that the validity of their hypothesis is further supported by the existence of a wide variety of agents that induce rat thyroid follicular cell adenomas allegedly through a TSH-mediated secondary mechanism. In particular, they contend that amiodarone, a drug approved by FDA for human use, is most similar to FD&C Red No. 3 terms of its mechanism of thyroid impairment and eventual effects on the rat thyroid, because neither is genotoxic and both inhibit the peripheral metabolism of T4 in rats, resulting in increased serum TSH concentrations. The proponents assert that differences in absorption or bioavailability explain why amiodarone induces follicular cell adenomas at much lower doses and in shorter time periods than FD&C Red No. 3 and, thus, appears to be more potent than FD&C Red No. 3.

The proponents also rely upon the fact that in the IRDC study, there was no significant increase in follicular cell hypertrophy, hyperplasia, or adenomas among female rats fed 4-percent FD&C Red No. 3. The proponents contend that this lack of a tumorigenic effect in females is further supported by published results showing that serum TSH concentrations in male rats are normally much higher than in female rats. Male rats are also known to demonstrate higher spontaneous background rates

of thyroid follicular cell tumors than female rats. The proponents agree with the conclusion of the publication and attribute these increased incidences to the effects of the male hormone, testosterone, on serum TSH concentrations.

On the basis of the foregoing, the proponents conclude that the weight of the scientific evidence, including the findings of the four principal studies mentioned above, supports the hypothesis that, in the IRDC study, the follicular cell adenomas observed in the high-dose (4 percent) male rats resulted from a TSH-mediated secondary mechanism.

***3531 3. Short-term Studies in Rats**

a. PRI study. The first study conducted to determine whether a change in hormonal mechanisms was responsible for the carcinogenicity of FD&C Red No. 3 was conducted under contract for CCMA by PRI. The objective of this study was to determine whether the thyroid follicular cell tumors found in the IRDC study were caused by (1) iodide resulting from the contamination of FD&C Red No. 3; (2) iodide available from the metabolism of FD&C Red No. 3; or (3) some other noniodide-related property of the color additive itself. This study was submitted to FDA in October 1983. As discussed below, FDA has concluded that the data from the PRI study are inconclusive with regard to the mechanism of action of FD&C Red No. 3.

The PRI study was 27 weeks long and contained seven treatment groups of rats. These groups received FD&C Red No. 3, a purified erythrosine, sodium iodide, FD&C Red No. 3 with added sodium iodide at two levels, control diet, or control diet with ethanol; the dietary level of FD&C Red No. 3 and erythrosine was 4 percent. Following administration of the test diet, serum concentrations of the thyroid hormones, as well as other parameters, were measured. Duplicate analyses were made: one analysis was performed on fresh blood shortly after withdrawal (“in life”); the other was performed on blood samples frozen until after the compound administration was completed (“serial”).

PRI performed a statistical analysis of the data from this study and reported elevated serum levels of TSH and depressed T3 serum levels. Apparently, the authors selectively relied on results of the “in life” serum sampling as the basis for these conclusions because their statistical analyses of the results of the “serial” serum sampling for TSH levels do not support these conclusions.

FDA’s statistical analyses of the “in life” samples of the treated male animals demonstrated that after 27 weeks, there was a statistically signfiicant increase in the level of T4 ($p=0.0001$), compared with controls, only for those animals receiving FD&C Red No. 3 or erythrosine. There was also an increase in the level of TSH that was of borderline statistical signifiance ($p=0.04$) and a slight decrease in the level of T3 . The “serial” assays of these same serum samples showed no elevation of TSH ($p=0.316$) or depression of T3 ($p=0.316$); however, the levels of T4 were still elevated to a statistically significant degree ($p<0.0001$), compared with controls. The agency also determined that neither the sodium iodide nor the diet plus ethanol groups demonstrated comparable effects on T3 , T4 , or TSH. Thus, the data from this study do not sustain the proponents’ hypothesis that sodium iodide mediated the response observed in the IRDC study.

The proponents of FD&C Red No. 3 claim that morphological data from the PRI study support their hypothesis of an operative secondary mechanism. Specifically, the proponents assert that there was evidence of thyroid gland activation as indicated by follicular cell hypertrophy in the thyroids of animals that received FD&C Red No. 3 in their diet.

FDA evaluated electron micrographs of follicular cells from the thyroid glands of male and femals rats fed the control diet and those fed the 4-percent FD&C Red No. 3 diet in this study. That examination revealed no conspicuous or consistent treatment-related differences in the ultrastructural appearance of these organelles, other than an increased concentration of lysosomes. The significance of these results is discussed below in section V.

b. Hazleton study. Hazleton Laboratories conducted a second major study under contract for CCMA (Project No. 6145-101). The study was designed to determine the influence of 7 months of continuous exposure to FD&C Red No. 3 on thyroid function in rats. The initial report for this study was submitted to the agency in December 1984; the final report on the ultrastructural morphometric evaluation was submitted in April 1988.

One primary objective of this study was to determine whether the changes in thyroid physiology and morphology induced by FD&C Red No. 3 could be reversed by the administration of T3 . According to the secondary mechanism hypothesis, administration of T3 to rats receiving FD&C Red No. 3 should mediate a reversal of both the TSH-stimulated thyroid proliferative changes and of the hormonal changes (that is, administration of T3 should result in decreased serum levels of T4 , rT3 , and TSH). The hypothesis posits that excess TSH causes follicular cell hypertrophy; the hypothesis further predicts that administration of T3 would cause the follicular cells to return to a normal, unhypertrophied state.

In the Hazleton study, serum concentrations of the thyroid hormones and iodine excretion were measured after administration of FD&C Red No. 3 to rats. There were 6 groups of animals, with 15 rats of each sex in each group; the dietary levels of the color additive were 0.0, 0.25, 0.5, 1.0, 2.0, and 4.0 percent. During the last month of the study, five rats per sex from each treatment group received exogenous T3 by injection. At the study's conclusion, thyroid glands of all animals on test were examined by electron microscopy. Also, as mentioned below in the discussion on metabolism, in vitro deiodination studies in liver and pituitary were also performed.

The proponents and the agency agree that male rats in the 4-percent dose group had decreased mean body weights, a greater food consumption, a greater excretion of total iodine, and greater mean thyroid weights. In terms of the hormonal results, the agency and the proponents agree that the male rats showed an increase in serum T4 , a decrease in serum T3 , and an increase in serum rT3 , compared with controls. FDA and the proponents also agree that the mean serum TSH values were higher in treated animals than in control animals, although the difference was not statistically significant for the entire period of the study.

The proponents claim that the results of this study support a conclusion that administration of FD&C Red No. 3 results in an increase in serum TSH. The proponents further assert that the increase was difficult to confirm statistically because of diurnal variation or compensation by the thyroid gland from animal to animal. The significance of the proponents' conclusion is discussed below in section V.

In this same study, morphological evidence of thyroid activation induced by FD&C Red No. 3 was evaluated by examination of ultrastructural changes in the thyroid follicular cells. Specific parameters, including cellular hypertrophy and increased numbers of lysosomes, provide evidence of functional stimulation of the thyroid gland. The proponents concluded that the feeding of FD&C Red No. 3 for 7 months resulted in a dose-dependent hypertrophy of the thyroid follicular cells.

FDA reviewed the electron micrographs from this study and was unable to confirm that administration of the color additive for 7 months resulted in cellular hypertrophy. Subsequently, the proponents submitted quantitative measures of the cells. The agency agrees that these measurements of cell size support an interpretation of cellular hypertrophy, provided that the sampling of tissue for the electron micrographs was unbiased. However, the proponents' submission did not describe the manner of selecting regions for the electron micrographs. Thus, the agency cannot assume that the sections chosen were ***3532** representative of each thyroid gland as a whole for either the control or the FD&C Red No. 3-treated groups. In addition, no evidence of any further progressive proliferative changes, such as hyperplasia that would be expected to lead to tumorigenesis were presented by the proponents. The significance of these findings is discussed below in section V.

The proponents and the agency agree that in this study, the follicular cell hypertrophy regressed upon administration of T3 to a selected group of rats. However, based upon its review, the agency concludes that the follicular cells did not fully regress to a normal state because additional lysosomal bodies remained in the follicular cells at the termination of the study. The interpretive significance of these conclusions is also discussed below in section V.

c. Witorsch study. The proponents submitted an unpublished study by Witorsch et al., in November 1984. The purpose of this study 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 in rats that was hyperresponsive to TRH. The proponents postulated that FD&C Red No. 3 would lower the production of T3 and would reduce the feedback inhibition of the pituitary that is mediated by normal levels of T3 and result in pituitary hyperresponsiveness to TRH. Such evidence would support the hypothesis of raised TSH levels following administration of the color additive.

In this study, the animals were fed dietary FD&C Red No. 3 at levels of 0.0, 0.5, 1.0, and 4 percent (2464 mg/kg/day); sodium iodide at 100 mg/kg/day; or fluorescein at 1000 mg/kg/day for 3 weeks. The study measured TSH, T3 , T4 , and T3 resin uptake before and after an intravenous bolus of TRH.

Both the proponents and FDA agree that these results support the conclusion that neither sodium iodide nor fluorescein produced hormonal changes in the animals on test. The proponents claim that the results in the 4-percent FD&C Red No. 3 group showed an increase in serum T4 , an enhanced TSH response to administration of TRH, an increase in the level of basal serum TSH that was not statistically significant, and no significant changes in the free T3 index. The proponents minimize the significance of these results and assert that moving the animals during the study confounded the study's results. The agency is not convinced by this claim because there are no data or arguments presented to support it.

In addition, the agency analyzed these same data. However, unlike the proponents, the agency compared the changes mediated by FD&C Red No. 3 only after correcting each hormone measure for its baseline (or starting) value. Following this

correction, the agency compared serum T3 , free T3 , and T3 resin uptake for differences between control and treated groups and found that serum T3 and free T3 were increased slightly after administration of FD&C Red No. 3. Thus, the data from this study demonstrate that, although there is a very slight increase in serum T4 levels, there is also an increase in serum T3 in both free and bound forms. Also, comparison of TSH values between the control and 4-percent-treated groups demonstrates no significant increase in TSH with TRH provocation. Thus, the agency concludes that TRH provocation does not result in a hypersecretion of TSH associated with feeding FD&C Red No. 3. The significance of these findings is discussed below in section V.

d. Bio/dynamics I study. In January 1989, CCMA submitted the results of a 60-day study conducted under contract by Bio/dynamics (Project No. 88-3320). This study was designed to show that administration of FD&C Red No. 3 alters thyroid hormone economy in the male rat and results in increased stimulation of the thyroid by TSH. The study design sought to establish a dose level at which no compound-related effects could be measured. The study was also designed to minimize the animal-to-animal variations that allegedly accounted for the lack of significance in the changes in TSH values in the Hazleton study.

Male Sprague-Dawley rats (160 per treatment group) were fed 0.0-, 0.25-, or 4-percent FD&C Red No. 3 for up to 60 days. The proponents concluded that administration of FD&C Red No. 3 at the 4-percent level resulted in (1) a significant elevation of TSH, rT3 , and T4 levels above the control values throughout most of the study, and (2) a significant decrease in serum T3 throughout the study as compared with controls. The report claimed that the hormonal levels for the 0.25-percent group were unaffected by the experimental treatment.

FDA believes that the proponents used inappropriate methods of statistical analysis to evaluate these hormonal results. Specifically, the proponents used an analysis of variance (ANOVA); this approach is appropriate where there are equal population variances for all dose-day combinations. Such equal population variances were not present in this study. For example, at day 60, there is a 37-fold greater variance in rT3 for the 4-percent group as compared with the control group. This large variance due to thyroid and pituitary hormonal changes induced in the 4-percent group reduces the probability of detecting a statistically significant difference in the levels of these same hormones when comparing the 0.25-percent and control groups.

The agency reanalyzed the results from this study, using logarithmic transformations of the data, a statistical method that accounts for the unequal population variances. In addition, the agency's analysis compared only the 0.25-percent and control groups using an ANOVA. Based on these analyses, the agency concludes that administration of FD&C Red No. 3 at the 0.25-percent as well as the 4-percent levels resulted in (1) a statistically significant increase of TSH, rT3 , and T4 levels above the control values throughout most of the study, and (2) a statistically significant decrease in serum T3 as compared with controls at day 30 ($p < 0.05$) for the 0.25-percent dose and throughout the study for the 4-percent dose. These effects were dose-related. Furthermore, the qualitative response patterns of these effects for the two dose levels were similar across time. The qualitatively similar pattern of hormonal responses supports the conclusion that both the 0.25-percent and the 4-percent doses mediated changes in thyroid hormones.

Morphologic changes were also evaluated in this study. CCMA reported and FDA concurs that morphometric data show that, in the period 0 to 30 days, the effects observed in the thyroid were decreased follicle size, decreased colloidal area, and decreased follicular cell height compared with the control group. At 60 days, these observed effects were reversed, as evidenced by increased follicle size, increased colloidal area, and increased follicular cell height compared with the control group.

FDA concludes that the results from this study are paradoxical, and, thus inconclusive. The proponents attempt to explain the lack of morphological changes at 60 days by arguing that 60 days was apparently not sufficient time to permit the development of the changes in follicular cells that could be detected by light microscopic techniques. They further suggest that the observations may be due in part to the effects of the iodide available from the color additive because an increase in the organic iodine content of the thyroid decreases the thyroid's responsiveness to TSH. The significance of these results is discussed below in section V.

***3533 4. Metabolism Studies**

The proponents submitted several studies to support that part of the secondary mechanism hypothesis that FD&C Red No. 3 interferes with the conversion of T4 to T3 , thus causing reduced serum T3 levels and elevated serum T4 levels. These studies included data on the absorption, distribution, and metabolic fate of FD&C Red No. 3 when administered to rats.

a. Ruiz and Ingbar rat liver study. The proponents submitted a 1982 publication by Ruiz and Ingbar that was designed to

show that administration of FD&C Red No. 3 to rats causes a dose-related inhibition of T4 metabolism (i.e., an inhibition of the 5′-monodeiodination of T4 to yield T3). The degree of inhibition of the conversion of T4 to T3 was determined by measuring the relative rates of conversion of 125 I-T4 to 125 I-T3 . (125 I is a radioisotope of iodine.)

Two sets of experiments were conducted, one without tissues and the other utilizing rat liver homogenates derived from rats treated in vivo with FD&C Red No. 3. In the set of experiments without tissues, the tissue-free control showed no 125 I-T4 degradation. A zero-time sample with erythrosine underwent extensive degradation (21 percent), while erythrosine added to another sample with a longer, but unspecified, time of exposure demonstrated marked degradation that was decreased by protection from light or addition of serotonin.

Due to an apparent light-activated effect in the nontissue experiments, a second set of experiments was performed using erythrosine or fluorescein and rat liver homogenates. In this study, male rats were administered erythrosine by intraperitoneal (i.p.) injection. Only the liver homogenates from rats treated with i.p. erythrosine demonstrated a dose-dependent reduction in the rate of conversion of 125 I-T4 to 125 I-T3 and 125 I. The proponents claimed that this result demonstrates that erythrosine, not fluorescein, is the active component affecting the conversion of T4 to T3 and thus, that this study shows that rats that receive FD&C Red No. 3 have a dose-related inhibition of T4 metabolism through inhibition of the 5′-monodeiodiation of T4 to T3 .

The agency concludes that the second phase of this study offers some limited support for the postulate that FD&C Red No. 3 inhibits the peripheral conversion of T4 to T3 . However, this evidence is not definitive for the following reasons. First, in this study, FD&C Red No. 3 was administered intraperitoneally (i.p.). This difference is significant because, for a given dose, i.p. administration results in substantially larger systemic exposure than the oral route. Thus, the study does not provide evidence that FD&C Red No. 3, when administered orally, effectively inhibits T4 metabolism. Second, this study does not provide evidence regarding effects on T4 metabolism with continuous, prolonged exposure to FD&C Red No. 3 and thus, does not account for any physiologic compensatory mechanisms available to the animal.

b. Metabolism segment of the Hazleton study. The Hazleton study discussed above (Project No. 6145-101) included a segment designed to establish the stimulation mechanism of TSH release from the rat pituitary and to show the manner in which the peripheral metabolism of T4 may influence circulating levels of T4 to T3 following administration of FD&C Red No. 3. In particular, the study measured the conversion of 125 I-T4 to 125 I-T3 in pituitary and liver homogenates of rats fed 0.0-, 0.5-, 1.0-, 2.0-, and 4.0-percent FD&C Red No. 3 for 7 months.

The proponents concluded that the liver homogenate data support a dose-dependent inhibition of the formation of T3 from T4 . Further, they claimed that the lack of inhibition of T4 metabolism in the pituitary is due to analytical problems associated with the small quantity of pituitary tissue available.

The agency does not agree with the proponents' overall conclusion about this portion of the Hazleton study. Although the agency agrees with the proponents' conclusion concerning the liver homogenate data, FDA believes that the proponents have applied these data selectively in that the data from the pituitary portion of the experiment have not been explained or used by the proponents. This failure is significant because the pituitary results contradict the proponents' hypothesis. The proponents rejected the pituitary results for methodological reasons alone. It is not clear that the methodological shortcomings raised by the proponents are sufficient to invalidate the pituitary results. Thus, the study provides only limited evidence that FD&C Red No. 3 inhibits the conversion of T4 to T3 .

c. ADME study. CCMA sponsored a study of the bioavailability of FD&C Red No. 3 in rats. This study was also conducted by Hazleton Laboratories America, Inc. On March 4, 1986, CCMA submitted a preliminary report on this study. The final report, called the ADME study (absorption, distribution, metabolism, and excretion), was not submitted to the agency until February 27, 1989.

In this study, the tissue distribution and urinary and fecal excretion of 14 C-labeled erythrosine and 125 I-labeled erythrosine were studied after oral administration to rats. Male and female adult Sprague-Dawley rats received 14 C- or 125 I-labeled erythrosine by gavage after consuming pretreatment (induction) diets containing 0.0-, 0.5-, or 4-percent FD&C Red No. 3.

The study results showed that most of the radioactivity was eliminated in the feces. The liver contained the highest level of radioactivity; low levels of radioactivity were found in blood and tissues. No detectable levels of 14 C were found in the thyroid gland, but the gland did contain measurable residues of 125 I. The excretion patterns and the 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 of the ADME study suggest that the thyroid gland was saturated with iodide prior to administration of the radioactive material. This conclusion is evidenced by the observation that 125 I residues in the thyroid gland in the 4-percent (high dose) radiolabeled erythrosine group were not significantly higher than those in the 0.5-percent (low dose) radiolabeled erythrosine group. Therefore, the percent of 125 I residue/mg of thyroid decreased with increasing amounts of FD&C Red No. 3 administered in the diet prior to radiolabeled test dose. Small amounts of lower halogenated fluoresceins were detected in the urine, plasma, kidney, and liver.

The proponents asserted that these results show that less than 5 percent of the ingested FD&C Red No. 3 entered into the enterohepatic circulation, with none accumulating in the thyroid, 0.2 percent accumulating in the liver, and 0.02 percent accumulating in the kidneys. Thus, the proponents concluded that the pattern of distribution demonstrates that FD&C Red No. 3 is primarily metabolized in the liver and kidney. The proponents further concluded that the nonabsorbed portion of FD&C Red No. 3 is stable after ingestion as evidenced by the fact that there was little deiodination of the erythrosine to lower iodinated fluoresceins and iodide in the feces.

The agency notes that the analytical data suggest that the radiolabeled material is qualitatively similar to FD&C Red No. 3. Moreover, based upon these results, the agency concludes that, in rats, (1) less than 25 percent of an administered dose of FD&C Red No. 3 is absorbed, with the remainder being ***3534** excreted in the feces; (2) the absorbed material enters the enterohepatic circulation, where a small portion of the tetraiodofluorescein component is deiodinated to di- and triiodofluoresceins; (3) the iodine removed from the tertaiodofluorescein ultimately accumulates in the thyroid gland; (4) less than 0.1 percent, if any, of an administered dose of FD&C Red No. 3 is absorbed by the thyroid; (5) the fate of organic impurities related to the resorcinol intermediate in the administered FD&C Red No. 3 is not known; and (6) the absorption, distribution, and metabolism of FD&C Red No. 3 is not sex-dependent.

Thus, the agency and the proponents agree that the amount of FD&C Red No. 3 absorbed by the gastrointestinal system is limited, with most of the material excreted unchanged in the feces. Of the limited amount of FD&C Red No. 3 that is absorbed, some is deiodinated. The deiodinated products are excreted primarily in the urine.

The agency is aware that the 1987 Panel reviewed a report for the ADME study. However, because much of the raw data was not available for the Panel's review, the Panel's evaluation is not discussed here.

The significance of the results of the ADME study are discussed below in section V.

5. IRDC Study Interim Sacrifice Data

According to the proponents' hypothesis of a secondary mechanism, follicular cell hyperplasia, a precursor of tumor formation, should be demonstrated in a chronic study. Because the IRDC study included a 1-year interim sacrifice of 10 rats of each sex, the proponents recently offered evidence from that interim sacrifice to support their hypothesis of a secondary carcinogenic mechanism. In particular, in April 1989, CCMA asserted that 9 of 10 male rats in the 4.0-percent dose group that were sacrificed at 1 year had proliferative changes of thyroid follicular cells ranging from moderate to severe hypertrophy and hyperplasia. This assertion was based upon a September 1982 report authored by a consultant pathologist.

FDA disagrees with CCMA's findings on this point. Specifically, in its own histopathological examination of the thyroid glands of the rats from the interim sacrifice, FDA observed no thyroid hyperplasia. Moreover, the thyroid weights of the interim sacrifice animals were not increased. This finding suggests the absence of widespread cellular hyperplasia.

The agency's conclusion on this point is consistent with the previous position taken by CCMA on the interim sacrifice data. In particular, as late as May 1988, CCMA's interpretation of the interim sacrifice reported only hypertrophy (and no hyperplasia) in the high-dose male rats sacrificed during the course of the second IRDC study. Furthermore, in a 1987 publication, Borzelleca, Capen, and Hallagan reported that, in both IRDC studies, there were no compound-related gross or microscopic changes in the 10 rats of each sex from each group sacrificed and necropsied at 1 year. 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. The significance of these data is discussed below in section V.

6. Human Studies

The proponents submitted four studies to show the absorption and metabolism of FD&C Red No. 3 and its effect on thyroid function in humans. Based on their interpretations of the results of these studies, the proponents claim that there is no basis for inferring a risk of thyroid oncogenicity in humans from FD&C Red No. 3. The agency has evaluated these submissions as discussed below and concludes that these studies offer no evidence that would alter the agency's conclusion that FD&C Red No. 3 has not been shown to be safe.

a. Bioavailability of FD&C Red No. 3. The proponents submitted two unpublished and undated studies on the bioavailability of erythrosine in humans: "Studies of the Bioavailability and Metabolism of Ingested Erythrosine in Man," by S. Ingbar et al. (Ingbar I study), and "Further Studies of the Absorption and Metabolism of Ingested Erythrosine in Man," by S. Ingbar et al. (Ingbar II study). These are reviewed below; the significance of these studies is discussed below.

i. Ingbar I study. Ingbar et al., conducted a study to determine the extent of absorption and metabolism of FD&C Red No. 3 in humans. In the study, five healthy volunteers (four males and a female) hospitalized in a metabolic ward were given a daily milk shake preparation containing FD&C Red No. 3 at doses of 0 milligram-per-day (mg/day) for the first week, 5 mg/day for the second week, 10 mg/day for the third week, and 25 mg/day for the fourth week. Blood was drawn every other day and analyzed for T4 , T3 , TSH, resin T3 uptake, total iodine, protein-bound iodine (PBI), and erythrosine. Additional blood samples were obtained every fourth day for the measurement of various serum chemistries (blood urea nitrogen, creatinine, glucose, sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase, lactic dehydrogenase, bilirubin, uric acid, total protein, albumin, and cholesterol). Throughout the study, complete 24-hour urine samples were collected to measure total iodine and erythrosine excretion.

The authors reported an increase in serum total iodine and serum PBI accompanied by little or no effect on urinary iodine excretion. The authors concluded that the most plausible explanation for these results was that a small portion of orally administered FD&C Red No. 3 is absorbed and that this absorbed fraction deiodinates slowly. The proponents agree with these conclusions.

The agency agrees with the authors' interpretation but notes that the test material used in the study was not tested with respect to its chemical identity and purity. Absent such information, the agency is unable to determine whether or not the administered erythrosine was equivalent to certified FD&C Red No. 3.

Based upon this study, FDA further concludes that when erythrosine in a dose of 10 mg/kg in an aqueous solution is administered for a few days, at least a small amount of the erythrosine is absorbed by the body, resulting in an elevation of the PBI concentration. It is unknown to what extent this erythrosine is being deiodinated. The significance of these results is discussed below.

ii. Ingbar II study. Ingbar et al., conducted a second study using radiolabeled erythrosine to investigate the absorption and metabolism of FD&C Red No. 3 in humans. In this study, a single dose of 131 I erythrosine was given to five human subjects using several different dosing protocols, which varied in the amount of administered materials and the vehicles used. Throughout the study, all subjects received potassium iodide to saturate the thyroid with iodide and thereby block the uptake of 131 I by the thyroid. Three subjects were given 75 or 80 mg of erythrosine containing 50 microcuries (mCi) of 131 I in the same milkshake preparation used in the Ingbar I study. Administered erythrosine consisted of unlabeled erythrosine as a component of the drink mix, with added 131 I labeled erythrosine. Because there was some concern that erythrosine might bind to protein in the milkshake preparation, three subjects were given 75 mg of erythrosine containing 50m Ci of 131 I in lemonade. At the outset of the study, the subjects were placed in a whole body radiation counter in order to obtain a ***3535** zero time radiation level. Total 131 I body content was obtained at intervals of 1 to 3 days.

In all five individuals (or six cases, because one individual participated in both aspects of the study), whole body 131 I content dropped to 1 percent of the administered dose in 7 days. In the four subjects monitored for 14 days, the authors found that there was a fast phase and a slow phase of disappearance of the radioactive iodine. After an initial 24-hour delay, the majority of fecal excretion of 131 I occurred between days 2 and 3; recoveries ranged between 80 and 103 percent of the administered dose. Levels of radioactivity in serum were only slightly above background. The maximum cumulative urinary excretion was no more than 0.4 percent for any subject; the majority of the urinary excretion of radioiodine occurred in the first 48 hours for all but one subject. The authors calculated that the potential initial body retention of erythrosine was 1.2 0.4 percent and that there was residual radioactivity at 14 days which was concentrated in the area of the liver. The authors calculated a half time for the slow phase of disappearance to be 8.4 2.1 days. The authors noted no differences in the absorption of erythrosine between the milk shake and lemonade groups. The authors also reported that there were no effects from the eryhthrosine on serum T4 , T3 , rT3 , or TSH concentrations.

Based upon these results, the proponents concluded that only a very small fraction of ingested erythrosine, on the order of 1 percent or less, was absorbed from the gastrointestinal tract of man, and that there were no effects on serum T4 , T3 , rT3 , or TSH concentrations.

For three separate reasons, FDA believes that these data cannot be used to assess the potential biological effects of FD&C

Red No. 3. First, as with the Ingbar I study, the authors did not provide the data necessary to characterize adequately either the radiolabeled or unlabeled components of the test sample. However, based on the combined observations of (1) increased PBI; (2) accumulation of 131 I in the region of the liver; and (3) low levels of urinary output of iodide, the agency concludes that it is likely that both organic and inorganic forms of iodine are absorbed. Second, the study did not characterize which specific chemical species were absorbed, how the absorbed species were modified in the body, which chemical species remained in the region of the liver, or how the absorbed material interacted with normal thyroid functions, e.g., the conversion of T4 and T3 , or the binding of T3 to specific pituitary receptors. Third, the saturation of the thyroid with iodide prior to administration of erythrosine prevents a determination of the hormonal effects that may be attributed solely to uptake of iodide from erythrosine by the thyroid. Thus, the form and absolute amount of organically bound iodine, after absorption, and its potential to alter functions of the pituitary-thyroid axis, cannot be determined from these experiments.

The agency notes that the thyroid hormones, T4 and T3 , are biologically effective in microgram quantities per day and that very low concentrations of compounds having structural similarities to thyroid hormone may interfere with the action or metabolism of thyroid hormone. Thus, even if the authors are correct that only about one percent of the administered dose of an uncharacterized radiolabeled erythrosine preparation was absorbed, these results do not rule out the potential for a biological effect of FD&C Red No. 3.

b. Effects of administration of FD&C Red No. 3. The proponents submitted two publications that address the effects of ingestion of FD&C Red No. 3 on the human thyroid: Gardner et al. (1987), "Effects of Oral Erythrosine (2′,4′,5′, 7′'-Tetraiodofluorescein) on Thyroid Function in Normal Men" (the Gardner studies) and Paul et al. (1988), "The Effect of Small Increases in Dietary Iodine on Thyroid Function in Euthyroid Subjects" (the Paul study). These studies are discussed below.

i. The Gardner studies. These two separate studies were designed to assess the effects of oral administration of FD&C Red No. 3 (identified as erythrosine by the authors). One study was designed to determine whether the 200-mg/day dose of FD&C Red No. 3 affects the pituitary-thyroid axis; the second study was designed to determine the no-observable-effect level (NOEL) for FD&C Red No. 3 in man. (The proponents refer to these two investigations as the single "Gardner study".) Three groups (10 subjects each) of apparently healthy men between the ages of 22 and 38 with mean age of 27 years received FD&C Red No. 3 orally in single doses of 200, 60, or 20 mg/day for 14 days. The study with the 20- and 60-mg doses was done about 4 months after the 200-mg dose study. Serum T4 , T3 , rT3 , TSH, PBI, total iodide, serum T3 ,-charcoal uptake, and 24-hour urinary iodide excretion were measured on days 1, 8, and 15. TRH stimulation tests of TSH secretion were performed on days 1 and 15.

FDA received two versions of the Gardner studies. The first submission was an unpublished report received in April 1985 that properly presents these data as the results of two separate studies. The second submission was a 1987 publication received in April 1988 that states that 30 men were equally divided into three treatment groups, thus implying a single study designed to investigate the effects of FD&C Red No. 3 in human subjects.

The authors concluded that there were no significant changes in serum T4 , T3 , rT3 , and T3 -charcoal uptake values at any dose. Significant dose-related increases in serum total iodide and PBI concentrations occurred with all three doses; significant dose-related increases in urinary iodide excretion occurred with the 60- and 200-mg/day doses. The authors also reported that the mean basal serum TSH concentration in men receiving 200 mg/day erythrosine increased significantly ($p < 0.05$) and the mean peak TSH increment after TRH stimulation increased significantly ($p < 0.05$). They attributed this increase in TSH secretion to the antithyroid effect of increased serum iodide concentrations, rather than to a direct effect of the color on thyroid hormone secretion or peripheral metabolism. The authors concluded that there is an effect at the 200-mg/day dose and that the 60-mg/day dose level is a NOEL.

The proponents agree with most of the authors' conclusions; however, they dispute the authors' conclusion about TSH levels. Specifically, although the proponents agree that there was an increase in TRH-stimulated TSH secretion in the 200-mg/day group, the proponents do not agree that there was a corresponding increase in basal TSH levels. This disagreement hinges on the appropriateness of the statistical methods used for assessing changes in basal TSH levels. In support of their position, the proponents rely upon an analysis of the data by Crump and Farrar whose analysis employed the method of Mantel-Haenzel modified to analyze continuous variables.

As discussed below, FDA reevaluated the design of this study and the methods of statistical analysis and concludes that there was a significant increase in both basal and TRH-stimulated TSH secretion in the 200-mg/day group. In addition, the agency finds that 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.

***3536** ("Power" refers to the ability of a test to obtain a statistical significance of the difference observed in the study (Refs. 13 and 14).) If the experimental and control groups do not in fact have different outcomes, an apparent difference may nevertheless be observed by chance. This phenomenon is known as Type I error or "alpha". In most cases, the agency requires an alpha value of at least 0.05 to ensure that the probability of the event occurring by chance is not more than 5 out of 100. There is also a possibility that a real difference between experimental and control groups may go undetected by statistical analyses; this is a Type II error or "beta". In most cases, the agency requires that the power calculation have a value of 80 percent in order to ensure that an effect would be detected by statistical analysis.)

(1) Changes in basal TSH. Because the data in the Gardner study were collected four months apart and the serum samples were analyzed by different laboratories, FDA believes that the 200-mg dose experiment must be treated as an independent study, separate from the study of 20-mg/day and 60-mg/day doses. Different dosages are usually administered simultaneously so as to minimize confounding effects in a study. If all doses are administered at the same time, one test for differences between dose groups with an analysis such as the one used by Crump and Farrar. The agency rejects the use of this approach here because, as discussed above, FDA considers the 200-mg/day dose group to be a separate study. Aside from the differences in dose, there may be other, unobserved factors that differed between the 200-mg/day dose group and the other two dose groups. Therefore, the agency believes that a test comparing the 200-mg/day dose group to the other dose groups is meaningless. A paired t-test analysis (a statistical method used by the authors) tests for an effect within a dose group. This is a more appropriate method for analyzing data from the 200-mg dose group. Accordingly, FDA agrees with the authors' conclusion that there was a significant increase in basal TSH concentration. Furthermore, the agency believes that the statistical test used by Crump and Farrar does not have sufficient statistical power to give meaningful results. FDA calculated the statistical power corresponding to a test similar to that used by Crump and Farrar, and found that the chance of obtaining statistical significance (at the 0.05 level) is less than 20 percent. Therefore, the agency accepts the conclusion reached by the study authors that the 200-mg/day dose of FD&C Red No. 3 has a statistically significant effect on basal TSH, and rejects the contrary conclusion of the proponents.

(2) No-Observable-Effect Level. Although FDA agrees that the results of the Gardner studies do not show statistically significant changes in either TSH or TRH-evoked TSH for doses of 60 and 20-mg/day, the agency believes that these studies do not have adequate statistical power to establish the lack of an effect of FD&C Red No. 3 on thyroid functions at doses lower than 200 mg/day. Based upon its calculations, FDA determined that the chance of detecting a statistically significant (at the 0.05 level) difference between the 20-mg/day and the 60-mg/day dose group for TRH-evoked TSH is less than 20 percent. As a consequence, a reliable NOEL cannot be established with these data. In addition, the Gardner studies do not have sufficient statistical power to rule out the possibility of an effect on the peripheral metabolism of thyroid hormone.

Because of the interrelationship between the results of the Paul and Gardner studies, the agency's overall findings regarding the physiological effects of administration of FD&C Red No. 3 to humans are presented after the agency's discussion of the Paul study, which is set out below.

ii. The Paul study. This study was designed to investigate the effect of a small increase in dietary iodine on thyroid function and to determine whether the increase in TSH observed in the Gardner studies was due to the intact dye or to iodide originating either as a contaminant of the color additive or from its deiodination. Nine men with normal thyroid functions (enthyroid subjects) between the ages of 26 and 56 years (34 3 mean SE) and 23 euthyroid women between the ages of 23 and 44 years (32 2) received 0.25 mg, 0.5 mg, or 1.5 mg of supplemental iodide for 14 days. Serum T4 , T3 , resin T3 uptake, TSH, PBI, total iodine, and free T4 index were measured on days 0 and 15. TRH tests were also performed on days 0 and 15. A 24-hour urine collection was obtained for measurement of iodine and creatinine content on day 7 and day 14; serum iodine analysis was done on blood taken on day 8.

No changes in thyroid metabolism were observed among subjects receiving 0.25 or 0.5 mg of sodium iodide daily. Subjects receiving 1.5 mg of sodium iodide exhibited urinary iodine excretion levels equivalent to the subjects in the Gardner study that received 200 mg of FD&C Red No. 3 daily. Further, subjects receiving 1.5 mg of sodium iodide exhibited small but significant decreases in serum T4 and T3 concentrations and small increases in serum basal TSH and TRH-stimulated TSH levels. All values remained within the normal range.

The proponents claim that when urinary iodine excretion is increased by dietary iodide to a level that is equivalent to that which occurred after the administration of 200 mg/day of FD&C Red No. 3, there are changes in pituitary and thyroid functions that are similar to those that occurred following the administration of FD&C Red No. 3. Therefore, the proponents contend that the effects of FD&C Red No. 3 observed in the Gardner study were due to the ingestion of iodide and were not attributable to the absorption of an organically bound iodine component of the color additive.

FDA rejects this contention. While the Gardner and Paul studies show similar urinary excretions of iodine (total iodine per gram creatinine) for 200 mg/day of FD&C Red No. 3 and 1.5 mg/day of sodium iodide, respectively, the changes in the pituitary and thyroid functions were not shown to be the same. In particular, while the effects on TSH appear to be similar, changes in thyroid hormones were not concordant. Specifically, the color additive caused a 30-percent increase in basal TSH levels and a 67-percent increase in TRH-stimulated TSH levels without change in T3 and T4 levels. The iodide caused no significant increase in basal TSH and a 44-percent increase in TRH-stimulated TSH levels associated with a 7.5-percent decrease in T3 and an 11-percent decrease in T4 levels. Thus, the agency believes that the data do not support the proponents' conclusion that the effects of FD&C Red No. 3 on pituitary and thyroid functions are explained in full by iodide present in or released from the color additive and are not caused by the color additive itself.

c. Interpretation of human data and conclusions. The proponents claim that these studies in humans demonstrate that FD&C Red No. 3 is poorly absorbed (<1 percent) from the gastrointestinal tract of humans and that the color additive does not produce effects on the peripheral metabolism of thyroid hormone in humans even at 200 mg/day for 2 weeks. They further contend that iodide made available by repeated 200-mg/day doses of FD&C Red No. 3 may result in an increased response to TRH stimulation by decreasing thyroid hormone release. Despite this effect, serum T4 , T3 , rT3 , and TSH concentrations, as well as the increment ***3537** in serum TSH that follows TRH administration, remained within the normal range in subjects ingesting 200 mg/day. Thus, the proponents conclude that the four studies described above, together with data demonstrating substantial differences between rat and human thyroid economy and oncogenesis, demonstrate that there is no basis for inferring a risk of thyroid oncogenicity in humans from the consumption of FD&C Red No. 3.

Because of the methodological limitations of these four human studies discussed above, the agency finds that the results do not provide conclusive evidence concerning the bioavailability of FD&C Red No. 3 or the hormonal effects of ingestion of the color additive in humans. Regarding bioavailability, the agency finds that the results suggest that (1) ingestion for a few days of 10 to 25 mg/day of FD&C Red No. 3, in an aqueous solution, may result in some (about 1 percent) absorption of the color additive into the body and a consequential elevation of the PBI concentration; (2) the fate of the absorbed FD&C Red No. 3 is unknown, but both organic and inorganic components appear to be present in the body after ingestion; (3) some radioactivity of 131 I labeled erythrosine was detected in experimental subjects 14 days after a one-time administration of 80 mg of the radiolabeled compound; and that (4) a sizable portion of the accumulated iodine is in the organic form. The agency also finds that the proponents have not addressed the bioavailability or hormonal effects that might result from the administration of FD&C Red No. 3 in a nonaqueous food media for an extended period of time.

Contrary to the proponents' position, the agency finds that these studies demonstrate that the administration of FD&C Red No. 3 at levels of 200 mg/day appears to have an effect on human thyroid hormonal functions, specifically on pituitary stimulation (TSH) of thyroid metabolism. Further, this effect has not been shown to be identical to the effects attributable to inorganic iodide, and the potential effects at levels of administration of FD&C Red No. 3 at less than 200 mg/day cannot be determined from the submitted data. Thus, the agency concludes that a no-effect level on thyroid hormonal function for FD&C Red No. 3 in humans has not been established. In any case, the agency concludes that in the absence of adequate data to establish the hypothesized secondary mechanism in rats, the results of these clinical studies do not alter the agency's decision as to the safety of the petitioned uses of FD&C Red No. 3.

V. Evaluation of the Secondary Mechanism

The proponents of FD&C Red No. 3 agree with the agency that FD&C Red No. 3 caused follicular cell neoplasms in the thyroid glands of male rats fed FD&C Red No. 3 at a dose level of 4 percent. However, in their May 1988 submission, the proponents contend that "there is no evidence that FD&C Red No. 3 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.

FDA has reviewed all of the data and information submitted by the proponents to support its secondary mechanism hypothesis and finds that this evidence does not demonstrate that the carcinogenic effects of FD&C Red No. 3 observed in male rats are the result of the hypothesized TSH-mediated mechanism. The available evidence is inadequate for two principal reasons. First, this evidence does not demonstrate that TSH levels remain elevated for the duration of a study that results in thyroid tumors near termination. Second, this evidence does not adequately demonstrate the full sequence of morphological events that are expected to result from a prolonged elevation of TSH. FDA's evaluation of the secondary mechanism hypothesis is complicated by the short duration of the studies provided. The proponents have known since at least 1982 that FD&C Red No. 3 is an animal carcinogen. Nevertheless, all of the studies subsequently conducted by the proponents cover the effects of FD&C Red No. 3 administration only through 7 months, despite the fact that the carcinogenic response is

observed near the end of a 28-month study. As discussed below, the agency finds, that, even when pieced together, the evidence from the short-term studies fails to establish the secondary mechanism hypothesis and does not rule out the possibility that the tumor induction is a direct response to exposure to FD&C Red No. 3. Furthermore, the agency finds that the proponents' data do not adequately define the no-effect dose for FD&C Red No. 3. Importantly, however, evidence of a no-effect dose is of no significance until operation of the secondary mechanism has been established. The agency's conclusions with respect to the components of the secondary mechanism hypothesis are discussed in detail below.

A. Inhibition of 5'-Monodeiodinase in the Rat Liver and Kidney

The proponents postulate, based on the experimental data they have provided, that FD&C Red No. 3 inhibits an enzyme in the liver and kidneys, 5′ '-monodeiodinase. As a result, their hypothesis predicts that under the influence of FD&C Red No. 3, serum T3 levels should decrease, and serum T4 and rT3 levels should increase. The proponents' evidence of inhibition of 5′'-monodeiodinase is provided in the publication by Ruiz and Ingbar, a portion of the Hazleton study, and the ADME study.

As set forth above, FDA has evaluated this information. Despite the shortcomings of these studies that are delineated above, the agency concludes that collectively, the results of all of the short-term studies, including the Bio/dynamics I study, provide limited evidence to support the proponents' claim that, at least initially, FD&C Red No. 3 impairs the conversion of T4 to T3 by blocking the action of the peripheral 5′-mondeiodinase. The proponents have clearly acknowledged that the duration of inhibition of 5′-monodeiodinase is critical to the degree to which changes in follicular cell morphology occur. Significantly, however, none of their studies provides data to establish the long-term effect of FD&C Red No. 3 on the 5′-monodeiodinase enzyme. Thus, the agency concludes that there is no evidence that this enzyme is chronically inhibited by FD&C Red No. 3.

B. Increased TSH in Response to Decreased T3

If the proponents' hypothesis is correct, the inhibition of 5′-monodeiodinase should result in decreased T3 levels which then result in secretion of excess TSH by the pituitary. However, based upon the agency's review of the available data, the proponents have not established through data that TSH levels are continuously elevated during chronic administration of FD&C Red No. 3. First, the 28-month IRDC studies themselves provided no evidence that the levels of TSH are elevated above normal because TSH and T3 levels were not measured.

Second, although there is some evidence of elevated TSH levels from the remaining short-term studies, those studies were all of limited duration. In particular, in the Bio/dynamics I study, there is evidence of increased levels of ***3538** TSH for the duration of the study, which was only 60 days. In the PRI study, there is evidence of significantly elevated levels of TSH but only in one set of analyses (the "in-life" phase) of the study. Moreover, the fact that, in the PRI study, there were different results on the same set of blood samples tested at different times indicates that not all relevant experimental procedures were under adequate control; this limits confidence in the results of this study.

The results of the Hazleton study also do not establish the hormonal changes necessary to support the proponents' hypothesis. In particular, there is no evidence of sustained, statistically significant differences in the TSH levels between the treated and control animals throughout the course of the study. Although the TSH means for rats treated with FD&C Red No. 3, compared with control animals, increased to borderline statistical significance ($p = 0.04$) at the 30-day time point, the overall difference in TSH means throughout the 7-month course of study was not statistically significant. The 1987 Panel reached the same conclusion about the Hazleton study results. That Panel concluded that administration of FD&C Red No. 3 caused an increase in TSH levels based on a comparison of group means values between treated and control groups, but also recognized the lack of statistical significance between the male rat groups.

Finally, the Witorsch study results do not provide acceptable evidence of increased TSH secretion. In the Witorsch study, the proponents tested whether there was an increase in the responsiveness of pituitary thyrothropic (TSH-secreting) cells to exogenous TRH. In fact, the results of this study demonstrate no such increase in pituitary responsiveness because there was no difference in the proportion of increase in TSH between animals treated with FD&C Red No. 3 and the control animals when both groups were injected with TRH.

Accordingly, the proponents have not demonstrated a key portion of the secondary mechanism hypothesis: that TSH levels are chronically higher than normal for the portion of the life of the rat necessary to produce thyroid follicular neoplasms. Moreover, the proponents have not demonstrated that FD&C Red No. 3 induces increased pituitary responsiveness.

C. TSH Induced Thyroid Stimulation to Increase Production of Thyroid Hormones

According to the proponents' hypothesis, the increased levels of TSH resulting from administration of FD&C Red No. 3 should stimulate the thyroid to produce more T4 and T3 . Quantitatively, thyroid production of T3 is small compared with that produced by peripheral conversion of T4 to T3 . Thus, the thyroid would be stimulated to attempt to compensate for the

continued deficit of T3 caused by the inhibition of the 5′-monodeiodination of T4 by FD&C Red No. 3. However, even with the increased stimulation of the thyroid by excess TSH, the continued inhibition of 5′-monodeiodination would prevent restoration of T3 levels to normal. Thus, under these conditions, only levels of T4 and rT3 would increase.

The agency concludes that the results of the Hazleton and Bio/dynamics I studies support the predicted changes in T3 , T4 , and rT3 . Significantly, however, the results of the Witorsch study show an increase in T3 , rather than the predicted decrease. Moreover, there were no measurements of T4 , T3 , rT3 , or TSH at any time during the conduct of the chronic IRDC study. Thus, there are no data on the thyroid hormone changes beyond 7 months. Therefore, the proponents' data do not demonstrate that the administration of FD&C Red No. 3 results in the long-term hormonal changes predicted by their hypothesis.

D. Progression of Changes in Follicular Cell Morphology

If the proponents' hypothesis is valid, the thyroid glands of rats on test should manifest evidence of stimulation not only in terms of excess levels of TSH, T4 , and rT3 , and reduced levels of T3 , but also in terms of morphologic changes. Hypothetically, the proposed increases in TSH should increase thyroid activity. A thyroid gland undergoing increased activity should show increased size (enlargement) and increased weight. Over time, prolonged stimulation of thyroid activity is associated with increased numbers of follicular cells (hyperplasia), which may progress to a nodular proliferation of follicular cells and eventually to neoplasia. This pattern of progressive morphological change (follicular cell hypertrophy, follicular cell hyperplasia, thyroid gland hypertrophy, nodular hyperplasia, follicular cell adenoma, and, possibly, follicular cell carcinoma) is similar, irrespective of the causal agent (Refs. 3, 12, and 15). Thus, there should be a pattern of progressive change in the proliferative lesions present in the thyroid glands of animals continuously fed FD&C Red No. 3.

Based on its review, FDA has concluded that the data from the studies submitted do not establish the progressive morphologic changes that would be the expected result of thyroid gland stimulation. First, there is inconsistent evidence of cellular hypertrophy. Although the electron micrographs from the Hazleton study provide evidence of thyroid follicular cell hypertrophy, the results of the PRI study showed no such hypertrophy.

Moreover, the morphologic changes that were observed in the Bio/dynamics I study are paradoxical or, at least, inconclusive. In the Bio/dynamics I study, the thyroids of animals exposed to FD&C Red No. 3 apparently did show some early evidence of activation by TSH because follicle size and colloid area in the gland were decreased early in the study. However, the cells lining the follicles were decreased in size instead of being increased or hypertrophied. Data from the termination of this study are also paradoxical. At that point, the follicular cells had become larger than those of the control animals, as the hypothesis would predict. However, at termination, the follicle size and colloid area also had become larger. Such increased follicle size and colloid area both suggest decreased thyroid activity and thus, conflict with the proponents' hypothesis.

The proponents' data also fail to establish cellular hyperplasia in rats fed FD&C Red No. 3. In particular, the proponents' claim of hyperplasia occurring in the rats sacrificed at 1 year in the IRDC study has not been substantiated. Indeed, as late as May 1988, the proponents claimed that no hyperplasia was observed at 1 year in this study. In addition, in the context of the proponents' hypothesis, one would also expect to observe thyroid gland hypertrophy at 1 year. However, there was no other evidence to confirm this glandular hypertrophy resulting from cellular hyperplasia (such as increased thyroid weights).

Finally, CCMA developed no morphologic data for the 16-month period between the 1-year interim sacrifice and the terminal sacrifice in the IRDC study. Thus, there are no data to illustrate the progressive proliferative changes from the alleged hyperplasia at 1 year to the adenomas and carcinomas observed at the conclusion of the IRDC study.

Based upon the foregoing, the agency concludes that the proponents have not demonstrated the full sequence of morphological events that are necessary to support their contention that elevated levels of TSH mediate a series of progressive proliferative lesions of the thyroid that ultimately lead to the expression of thyroid tumors.

3539** ***E. A No-Effect Level

The proponents hypothesize that if FD&C Red No. 3 operates through a secondary mechanism, a dose having no effect on thyroid economy can be established. This "no-effect" level is based on measurements of TSH levels in response to administration of FD&C Red No. 3. One objective of the Bio/dynamics I study was to establish such a dosage level. However, as discussed above in section IV, the agency concludes that the Bio/dynamics I study results do not support the proponents' claim that the 0.25-percent dose level is a "no-effect" dose in rats.

Apparently, the proponents agree with the agency's conclusion about the Bio/dynamics I study because on May 10, 1989, they submitted to the agency a protocol for another "no-effect" level study, Bio/dynamics Project No. 88-3378 (Bio/dynamics

II study). On May 19, 1989, CCMA submitted a report describing preliminary results after 30 days, of the Bio/dynamics II study, and on June 19, 1989, they submitted the TSH assay for the study. On August 4, 1989, CCMA submitted a final report for the study and concluded that a dietary concentration of 0.06-percent FD&C Red No. 3 (approximately 36 mg/kg/day) is a no-effect level for male rats.

The agency is currently evaluating the portion of the results of the Bio/dynamics II study that has been submitted. However, because the agency has concluded that the proponents have not offered sufficient evidence to support the secondary mechanism hypothesis, this additional information related to a no-effect level cannot alter the agency's decision concerning the provisionally listed uses of FD&C Red No. 3.

F. Genotoxicity

In evaluating the possible mechanism underlying the carcinogenicity of FD&C Red No. 3, the agency also considered issues related to the genotoxicity of the color additive. The agency does not accept the proponents' conclusion that the available data establish that FD&C Red No. 3 is not genotoxic. In particular, as discussed above in section IV, the agency has concluded that there are still issues regarding the potential of FD&C Red No. 3 to interact with and damage genetic material. These questions are based upon results of tests of FD&C Red No. 3 in systems used to assess the interaction of the color additive with mammalian genetic material. These results include those on chromosomal aberrations and micronuclei formation in hamster cells in vitro and micronuclei formation in mouse bone marrow and gene mutation in the mouse lymphoma TK+/-assay. The results from these studies indicate that FD&C Red No. 3 has the potential to interact with cellular DNA; the evidence of such interaction prevents the agency from concluding that FD&C Red No. 3 acts solely through a secondary mechanism in the induction of thyroid tumors. Furthermore, even if FD&C Red No. 3 was established not to be genotoxic, this lack of genotoxicity would not be sufficient evidence by itself to establish the hypothetical secondary mechanism.

G. Other Evidence

1. Other Goitrogens

In two recent publications, the authors contend that there is a developing body of experimental data that supports the concept of a secondary mechanism mediating the expression of thyroid carcinogenesis by a number of substances (Refs. 3 and 12). These authors state that a diversity of experimental procedures can elicit thyroid tumors in experimental animal models. For example, physical procedures, such as development of experimental iodine deficiency, removal of a portion of the thyroid gland, transplantation of tumors secreting TSH, and exposure to certain synthetic chemicals (goitrogenic agents) result in the experimental animal being exposed to elevated levels of TSH for prolonged periods with the eventual occurrence of thyroid tumors.

For certain of these goitrogenic agents (e.g., propylthiouracil and sulfamethoxazole), there is a reasonably clear-cut correlation between the duration of exposure to the test substance and evidence of progressive proliferative changes, such as thyroid follicular cell hypertrophy, hyperplasia, and neoplasia. However, for these particular agents, there is no satisfactory series of hormonal assays that conclusively establish that TSH was indeed elevated over the period of time required for tumor development (Ref. 12).

There are other substances, e.g., 4,4′-methylenedianiline and 4,4′ '-methylenebis(N,N-dimethylbenzenamine), that, like the agents discussed above, have been determined to be thyroid carcinogens that interfere with the thyroid-pituitary axis. However, these other substances also have carcinogenic effects at other organ sites and possess significant genotoxicity (Ref. 3). The data available on these latter two substances suggest that a substance might (depending on organ site occurrences) have both a direct- and an indirect-acting carcinogenic effect.

In support of their secondary mechanism hypothesis, the proponents submitted evidence from the literature and a discussion of other agents that allegedly induce rat thyroid follicular cell adenomas through a TSH-mediated secondary mechanism. Among these goitrogenic agents, the proponents suggest that amiodarone is most similar to FD&C Red No. 3.

The agency acknowledges that there is accumulating evidence to support the hypothesis that exposure to certain synthetic chemicals, including amiodarone, may produce thyroid tumors by a secondary mechanism of hormonal disruption of the thyroid-pituitary axis. At this time, however, for any individual agent, the data needed to establish the secondary mechanism hypothesis does not exist. Even if such data were available, however, they would not prove the proponents' contention that only a secondary mechanism is operating for FD&C Red No. 3. That is, while the available information on other goitrogens lends credibility to the hypothesis that FD&C Red No. 3 acts through a secondary mechanism, such information does not constitute direct evidence to support the claim that FD&C Red No. 3 operates solely through such a mechanism.

2. Effects of Testosterone

As further support of a TSH-medicated secondary mechanism, the proponents suggested that, in the IRDC study, the male rats, but not the female rats, developed adenomas because testosterone causes an elevation of serum TSH levels in male rats. The agency acknowledges that male rats apparently do show higher levels of TSH than female rats and that this may lead to higher levels of spontaneous thyroid follicular tumors (Refs. 16 and 17). However, it is not clear to the agency how this difference in spontaneous tumor rates between untreated male and female rats influences the finding of greater tumor rates in FD&C Red No. 3-treated male rats compared with control male rats in the IRDC Study No. 410-011. The agency is not aware of any study that definitively connects the increased levels of TSH in male rats with increased rates of tumor formation. The levels of endogenously secreted testosterone would be expected to be the same in the male controls and the treated males. Thus, the agency concludes that the relevance of this argument remains to be established.

3540 *H. Summary

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.

Although FDA has acknowledged the scientific plausibility of the proponents' hypothesis that FD&C Red No. 3 operates through a secondary mechanism to produce a carcinogenic response, the proponents have been given adequate time to establish this hypothesis, but have failed to do so. Based upon the studies to date, FDA believes that even if the proponents were given an opportunity to conduct an additional study, there are many uncertainties that could affect the timing and outcome of such a study. Because the proponents have failed to meet their burden under the act to show that FD&C Red No. 3 is safe to a reasonable certainty, despite adequate time to do so, FDA has, as announced elsewhere in this issue of the Federal Register, determined that there will be no additional extension of the closing date for the provisional listings of FD&C Red No. 3 to permit additional study. Because the proponents' data are clearly insufficient, the hypothesis cannot be used to support the continued safe use of FD&C Red No. 3 in cosmetics and externally applied drugs.

VI. The Legal Standard Applicable to FD&C Red No. 3

A. The Statutory Standard of 21 U.S.C. 376(b)

Under section 706(b)(4) of the act (21 U.S.C. 376(b)(4)), the "general safety provisions" for color additives, the Secretary is prohibited from listing a color additive for a particular use unless the data presented to FDA establish that the color additive is safe for such use. The act's legislative history makes clear that safety requires proof to a reasonable certainty that no harm will result from the proposed use of an additive. FDA's color additive regulations incorporate this definition of safety. ("Safe" means that there is convincing evidence that establishes with reasonable certainty that no harm will result from the intended use of the color additive." (21 CFR 70.3(i)).)

The color additives anticancer clause (also referred to as the Delaney clause), section 706(b)(5)(B) of the act, states in part:

A color additive (i) 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, and (ii) shall be deemed unsafe, and shall not be listed, for any use which will not result in ingestion of any part of such additive, if, after tests which are appropriate for the evaluation of the safety of additives for such use, or after other relevant exposure of man or animal to such additive, it is found by the Secretary to induce cancer in man or animal * * *.

Thus, under the act (21 U.S.C. 376(b)(5)(B)(i)), a color additive intended for ingested uses is deemed unsafe and may not be listed if it is an animal carcinogen. Likewise, a color additive intended for noningested uses is deemed unsafe and may not be listed (21 U.S.C. 375(b)(5)(B)(ii)) if the color additive is determined to be an animal carcinogen in tests "appropriate for the

evaluation of the safety of additives" for the particular use or uses under review.

B. Application of the Legal Standard

The pending petition requests the permanent listing of FD&C Red No. 3 for externally applied drug uses and for cosmetic uses. As discussed in detail above, FDA has concluded that the chronic rat feeding studies demonstrate that FD&C Red No. 3 is an animal carcinogen. In view of the finding of animal carcinogenicity, the color additive Delaney clause (21 U.S.C. 376(b)(5)(i)) requires that CTFA's petition be denied to the extent that it requests the permanent listing of the color additive for ingested cosmetic uses.

In addition, to the extent that CTFA's petition requests the permanent listing of FD&C Red No. 3 for external drug and external cosmetic uses, it must also be denied. The Delaney clause (21 U.S.C. 376(b)(5)(B)(ii)) deems unsafe and prohibits the listing of a color additive for noningested uses if the color additive is shown to be an animal carcinogen in "appropriate tests."

As set out below, after careful evaluation of the evidence, including these ingestion studies and the skin penetration study submitted by the petitioner, FDA concludes that the ingestion studies are appropriate tests (21 U.S.C. 376(b)(5)(B)(ii)) for evaluating FD&C Red No. 3 for use in externally applied drugs and cosmetics.

As discussed above in section IV D, in the skin penetration study submitted by CTFA, radiolabeled erythrosine, containing the radiolabeled 2′,4′,5′,7′ '-tetraiodofluorescein as the principal component, was applied to excised pieces of human skin. The penetration of the material from the skin's surface through the layers of skin into a receptor fluid was measured by the increase in radioactivity of the receptor fluid. Small percentages of the radiolabel were measured after penetrating the skin. Thus, CTFA's skin penetration study does support the agency's position that some portion of FD&C Red No. 3 is absorbed through the skin and distributed throughout the body. In addition, the animal feeding studies establish that FD&C Red No. 3 induces cancer at a site remote from the alimentary tract; this indicates that the color additive is systemically absorbed before acting as a carcinogen.

FDA has consistently held that ingestion studies are appropriate for evaluating the safety of a color additive that is to be applied to the skin if the additive is shown to penetrate skin and be absorbed by the body (see, e.g., 43 FR 1101 at 1103, January 6, 1978; 51 FR 28331 at 28342, August 7, 1986). A color additive that penetrates the skin can be distributed to remote sites in a manner analogous to the distribution that occurs when an ingested color additive enters the circulatory system from the gastrointestinal tract. Therefore, FDA has concluded that the FD&C Red No. 3 ingestion studies are appropriate for evaluating the safety of the externally applied uses of the color additive. Because FD&C Red No. 3 has been shown to induce cancer in appropriate tests, under the color additive Delaney ***3541** clause (21 U.S.C. 376(b)(5)(B)(ii)), FD&C Red No. 3 is unsafe for use in externally applied drugs and externally applied cosmetics and cannot be listed.

For the foregoing reasons, CTFA's petition must be denied in its entirety.

C. CTFA's Legal Arguments Based on its Risk Assessments

In 1984, CTFA conducted an assessment of the risks associated with the use of FD&C Red No. 3 in cosmetics and externally applied drug products. CTFA concluded that the risk of cancer from the use of such products is well below the range of significance. Specifically, CTFA estimates that the maximum additional risk of cancer to humans from the use of FD&C Red No. 3 from external cosmetic and drug products ranges from 1 in 32 million to 1 in 17 million, depending upon the risk assessment procedures used. For lip products, CTFA calculates a risk from 1 in 2.3 million to 1 in 1.2 million. In view of the allegedly limited risks proposed by exposure to FD&C Red No. 3, CTFA argues that, for four separate reasons, the Delaney clause of the color additive amendments (21 U.S.C. 376(b)(5)(B)) should not operate to ban FD&C Red No. 3 for use in cosmetics and externally applied drugs.

CTFA's risk assessments for FD&C Red No. 3 depend upon a number of assumptions. For example, CTFA assumes that the principal component of the color additive is the carcinogenic agent and that all of the carcinogenic agent is absorbed in the animal feeding studies. FDA does not believe that the available information and data provide a basis for making this assumption, as well as other assumptions made by CTFA. However, even if CTFA's risk assessments are accepted as valid and accurate, FDA has concluded, as set forth in detail below, that under the applicable statutory standards, FD&C Red No. 3 cannot be permanently listed for use in cosmetics and externally applied drugs.

CTFA first argues that FD&C Red No. 3 is a secondary carcinogen with an extremely low level of risk associated with its use. In such circumstances, CTFA claims that the color additive Delaney clause (21 U.S.C. 376(b)(5)(B)) does not apply.

As discussed in detail above, FDA has concluded that the data presented by the proponents fail to establish that FD&C Red No. 3 mediates thyroid carcinogenesis by a secondary effect through disruption of the hormonal relationships that normally exist between the pituitary and thyroid glands. Thus, FDA has not found it necessary as part of its decision to determine whether the color additive Delaney clause would, as a legal matter, bar the listing of a carcinogenic color additive shown to operate by a secondary mechanism.

CTFA also argues that, under the principle of de minimis non curat lex ("the law does not concern itself with trifles"), the color additive Delaney clause (21 U.S.C. 376(b)(5)(B)) should not operate to ban FD&C Red No. 3 for use in cosmetics, including lipsticks, and externally applied drugs, because the risks associated with the petitioned uses of the color additive are so insignificant. To the contrary, the U.S. Court of Appeals for the District of Columbia Circuit has expressly held that "the Delaney Clause of the Color Additive Amendments does not contain an implicit de minimis exception for carcinogenic dyes with trivial risks to humans." Public Citizen v. Young, 831 F.2d 1108, 1122 (D.C. Cir. 1987), cert. denied, 108 S. Ct. 1470 (1988).

Thus, even if CTFA's 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 (21 U.S.C. 376(b)(5)(B)) under the principle of de minimis.

Third, CTFA argues that under section 306 of the act (21 U.S.C. 336), FDA is not required to take action to ban FD&C Red No. 3. The agency concludes that section 306 is inapplicable here for two reasons. First, section 306 grants FDA the discretion to determine whether a matter should be referred to the Department of Justice for the institution of a civil or criminal enforcement action. (Section 306 states that FDA is not required to report for "prosecution, or for the institution of libel or injunction proceedings, minor violations of this Act * * *.") At issue here is whether, under the act, FD&C Red No. 3 may be permanently listed for certain uses, not whether the agency has the discretion to decide whether to refer an enforcement action concerning the color additive. Second, the prosecutorial discretion granted FDA by section 306 cannot be used to modify the express statutory standard of 21 U.S.C. 376(b)(5)(B). Indeed, to do so would be contrary to the decision in Public Citizen v. Young, supra. As discussed above, the D.C. Circuit held that the color additive Delaney clause establishes an "extraordinarily rigid" standard for FDA: if a color additive induces cancer, then it cannot be permanently listed. 831 F.2d at 1112, 1122.

Finally, CTFA argues that the results of the FD&C Red No. 3 animal feeding studies should not trigger the operation of the Delaney clause here because such studies are not "appropriate" tests (21 U.S.C. 376(b)(5)(B)(ii)) to assess the safety of externally applied color additives. In so arguing, the petitioner relies heavily on the agency's decision on the color additive lead acetate (45 FR 72112, October 31, 1980; 46 FR 15500, March 6, 1981).

In particular, CTFA argues that under the portion of the Delaney clause that is applicable to external uses of color additives (section 706(b)(5)(B)(ii) of the act), an animal ingestion study demonstrating carcinogenicity is not an absolute bar to the approval of a color additive for noningested use. The petitioner asserts that, to find a substance to be a carcinogen under this portion of the Delaney clause, the test that shows carcinogenicity must be "appropriate" for the evaluation of the safety of the additive or must involve some other exposure that is "relevant" to the use of the substance. CTFA argues that in the decision to list lead acetate, FDA concluded that feeding studies showing lead acetate to be carcinogenic were not relevant or appropriate under the Delaney clause because a risk assessment demonstrated that use of lead acetate presented an insignificant risk. The petitioner claims that the same is true of FD&C Red No. 3.

FDA has considered this argument and has concluded that it must fail for two reasons. First, as discussed above, the animal feeding studies of FD&C Red No. 3 are appropriate tests for evaluating the safety of the external uses of the color additive.

Second, the petitioner misinterprets FDA's decision concerning lead acetate. In deciding to list permanently lead acetate, FDA concluded that ingestion studies showing lead to be carcinogenic in animals were not appropriate for the evaluation of the safety of lead acetate for external uses. This conclusion was "based upon the unusual combination of scientific facts peculiar to lead acetate in hair dyes, a combination which will rarely, if ever, be presented again in this context" (45 FR 72112 at 72115; October 31, 1980). One of the principal factors that influenced FDA's conclusion that the Delaney clause did not apply to lead acetate was the fact that a background level of lead is always present in the human bloodstream, a background level much greater than the possible increase in lead burden resulting from use of lead acetate in hair dyes. In contrast, there is no background level of FD&C Red No. 3 in humans. Thus, the agency's decision regarding lead acetate does not require FDA to grant CTFA's petition for ***3542** cosmetic and externally applied drug uses.

For these reasons, FDA has concluded that, because FD&C Red No. 3 has been shown to be an animal carcinogen in

appropriate tests, FD&C Red No. 3 cannot be listed permanently for use in externally applied drugs and cosmetics.

VII. References

The following references have been placed on display in the Dockets Management Branch (address above) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. McConnell, E. E. et al., "Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies," Journal of the National Cancer Institute, 76:283-289, 1986.

2. Cristov, K., and Raichev, R., "Experimental Thyroid Carcinogenesis," Current Topics in Pathology, 56:79-114, 1972.

3. Hill, R. N. et al., "Thyroid Follicular Cell Carcinogenesis," Fundamental and Applied Toxicology, 12:629-697, 1989.

4. Margolin, B. H. et al., "Statistical Analyses for In Vitro Cytogenetic Assays Using Chinese Hamster Ovary Cells," Environmental Mutagenesis, 8:183-204, 1986.

5. Clive, D. et al., "Guide for Performing the Mouse Lymphoma Assay for Mammalian Cell Mutagenicity,"Mutation Research, 189:143-156, 1987.

6. Fung, V. A. et al., "Mutagenic Activity of Some Coffee Flavor Ingredients," Mutation Research, 204-219-228, 1988.

7. Rogers-Back, A. M. et al., "Genotoxicity of 6 Oxime Compounds in the Salmonella/mammalian-microsome Assay and Mouse Lymphoma TK+/-Assay," Mutation Research, 204:149-162, 1988.

8. Brookes, P., and de Serres, F. J., "Overview of Assay System Performance," in Vol. 1 of Progress in Mutation Research: Evaluation of Short-Term Tests for Carcinogens, Elsevier/North-Holland, NY, 98-111, 1981.

9. Federal Register of March 14, 1985 (50 FR 10371-10442).

10. Genuth, S. M., "The Thyroid Gland," Physiology, Berne, R. M., and Levy, M. N., ed., C. V. Mosby Co., St. Louis, MO, 1013-1032, 1983.

11. Larsen, P. R., "The Thyroid," in Cecil's Textbook of Medicine, Wyngarden, J. B., and Smith L. H., Jr., ed., W. B. Saunders Co., Philadelphia, PA, 1315-1340, 1988.

12. Paynter, O. E. et al., "Goitrogens and Thyroid Follicular Cell Neoplasia: Evidence for a Threshold Process," Regulatory Toxicology and Pharmacology, 8:102-119, 1988.

13. Mendenhall, W. et al., Mathematical Statistics with Applications, Duxbury Press, Boston, MA, 403-404, 1981.

14. Bailar, J. C., and Mosteller, F., ed., Medical Uses of Statistics, NEJM Books, Waltham, MA, 163-165, 1986.

15. Zbinden, G., "Assessment of Hyperplastic and Neoplastic Lesions of the Thyroid Gland," Trends Pharmacological Sciences, 8:511-514, 1987.

16. Farbota, L. et al., "Sex Hormone Modulation of Serum TSH Levels," Surgery, 102:1081-1087, 1987.

17. Christianson, D. et al., "The Sex-related Difference in Serum Thyrotropin Concentration is Androgen Mediated," Endocrinology, 108:529-535, 1981.

VIII. Environmental Impact

The agency has carefully considered the potential environmental effects of this action. FDA has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding, contained in an environmental assessment, may be seen in the Dockets Management Branch (address above) between 9 a.m. and 4 p.m., Monday through Friday.

IX. Regulatory and Economic Impact

Although this action is exempt from Executive Order 12291 and the Regulatory Flexibility Act, the agency has analyzed the

economic effects of this action and has determined it is not a major rule as defined by Executive Order 12291. Further, FDA, in accordance with the Regulatory Flexibility Act, has considered the effect of this action on small entities including small businesses and has determined that no significant adverse affect will derive from this action. A copy of the agency's economic assessment is on file with the Dockets Management Branch.

X. Conclusion

Under section 706 of the act, a petitioner seeking approval for permanent listing of a color additive has the burden of proof to demonstrate by adequate tests that the color additive is safe. Therefore, FDA is precluded from permanently listing a color additive when the petitioner has not established "with reasonable certainty that no harm will result from the intended use of the color additive." In addition, the act (21 U.S.C. 376 (b)(5)(B)) prohibits the permanent listing of a color additive when such color additive has been shown by appropriate tests to be an animal carcinogen.

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 their hypothesis that the observed carcinogenic effect of FD&C Red No. 3 is a result of a hormonally induced secondary mechanism.

Therefore, FDA concludes that the available data on FD&C Red No. 3 do not establish that its use in coloring cosmetics and externally applied drugs is safe within the meaning of section 706 of the act. Based on this finding, FDA is now denying CAP 9C0096 and is denying the permanent listing of FD&C Red No. 3 for use in cosmetics and externally applied drugs.

XI. Objections

The toxicity study reports, the agency's evaluations of these studies, and all other information relied upon by the agency in reaching its decisions are on file in Docket No. 76C-0044 at the Dockets Management Branch and may be reviewed between 9 a.m. and 4 p.m., Monday through Friday. To facilitate the use of the administrative record of this petition, the agency has prepared an index of the data and other information relied upon by the agency in this proceeding; this index is also on file in Docket No. 76C-0044.

Any person who will be adversely affected by the foregoing order may at any time on or before March 5, 1990, submit to the Dockets Management Branch (address above) written objections thereto. Objections shall show how the person filing will be adversely affected by the order, specify with particularity the provisions of the order deemed objectionable, and state the grounds for the objections. Objections shall be filed in accordance with requirements of § 71.30 (21 CFR 71.30). If a hearing is requested, the objections shall state the issue for the hearing and shall be supported by grounds factually and legally sufficient to justify the relief sought, and shall include a detailed description and analysis of the factual information intended to be presented in support of the objections in the event that a hearing is held. Three copies of all documents shall be filed and shall be identified with the docket number found in brackets in the heading of this document. Any objections received in response to the order may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under section 706 ***3543** of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 376), and the transitional provisions of the Color Additive Amendments of 1960 (74 Stat. 404-407 (21 U.S.C. 376, note)), and under the authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10).

Dated: January 26, 1990.

James S. Benson,

Acting Commissioner of Food and Drugs.
[FR Doc. 90-2264 Filed 1-29-90; 11:20 am]

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