REPORT OF THE COMPTROLLER GENERAL OF THE UNITED STATES

Need To Establish The Safety Of Color Additive FD&C Red No. 2

Food and Drug Administration

Department of Health, Education, and Welfare

The Food and Drug Administration has permitted the use of FD&C Red No. 2, a color additive, in food, drugs, and cosmetics for 15 years without making a final determination of its safety although the Federal Food, Drug, and Cosmetic Act requires that color additives used in such products be determined to be safe.

During this period, scientific studies have raised questions about the safety of FD&C Red No. 2. Permitting continued use of the additive before resolving the safety questions exposes the public to unnecessary risk.

MWD-76-40

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OCT. 20, 1975

COMPTROLLER GENERAL OF THE UNITED STATES
WASHINGTON, D.C. 20548

B-164031(2)

The Honorable Gaylord Nelson
United States Senate

Dear Senator Nelson:

In response to your January 30, 1975, request, this is our report on the need for the Food and Drug Administration to establish the safety of color additive Food, Drug, and Cosmetic Red No. 2. As requested, reports on saccharin and aspartame will be forwarded separately as our reviews concerning them are completed.

The Administration is part of the Department of Health, Education, and Welfare. As requested by your office, we have not obtained the Department's written comments on the matters in the report. However, we have discussed these matters with Administration officials and have considered their comments in the report.

We invite your attention to the fact that this report contains a recommendation to the Secretary of Health, Education, and Welfare. As you know, section 236 of the Legislative Reorganization Act of 1970 requires the head of a Federal agency to submit a written statement on actions he has taken on recommendations to the House and Senate Committees on Government Operations not later than 60 days after the date of the report, and the House and Senate Committees on Appropriations with the agency's first request for appropriations made more than 60 days after the date of the report.

We will be in touch with your office in the near future to arrange for copies of this report to be sent to the Secretary of Health, Education, and Welfare and the four Committees to set in motion the requirements of section 236.

Sincerely yours,

Comptroller General
of the United States

Contents

ABBREVIATIONS

FDA	Food and Drug Administration
FD&C	Food, Drug, and Cosmetic
FD&C Act	Federal Food, Drug, and Cosmetic Act, as amended
GAO	General Accounting Office
HEW	Department of Health, Education, and Welfare
NCTR	National Center for Toxicological Research

COMPTROLLER GENERAL'S REPORT
TO THE HONORABLE GAYLORD NELSON
UNITED STATES SENATE

NEED TO ESTABLISH THE
SAFETY OF COLOR ADDITIVE
FD&C RED NO. 2
Department of Health, Education, and Welfare

D I G E S T

Red No. 2--officially designated Food, Drug, and Cosmetic Red No. 2--is a color additive used to improve the appearance and promote the marketing of a variety of foods, drugs, and cosmetics. Red No. 2 is the name given to a certified lot of the dye generically known as amaranth.

The composition and purity of amaranth varies. The Food and Drug Administration has established composition and purity specifications that amaranth must meet before it can qualify for use in food, drugs, and cosmetics. Only amaranth meeting such specifications is classified as Red No. 2.

For 15 years the Food and Drug Administration has permitted the use of Red No. 2 without making a final determination of its safety, although the law requires such determination for color additives, and scientific studies have raised questions about the safety of Red No. 2.

Continued use of the additive before resolving the safety questions exposes the public to unnecessary risk. The Secretary, Department of Health, Education, and Welfare, should direct the Commissioner of the Food and Drug Administration to promptly establish the safety of Red No. 2 or prevent its use in food, drugs, and cosmetics. (See p. 25.)

Since July 12, 1960, the Federal Food, Drug and Cosmetic Act has required the Administration to review the safety of color additives used in food, drugs, and cosmetics and to issue regulations prescribing their safe use. Color additives commercially established at that time, such as Red No. 2, could continue in use, on an interim basis for a reasonable period, pending completion of scientific

investigations to determine their safety. (See pp. 1 to 3.)

The Food and Drug Administration has repeatedly extended the interim period for Red No. 2 on the basis of requests from manufacturer or industry associations to allow time to complete scientific investigations concerning its safety. In some cases the requests did not identify investigations that were being conducted or indicate when they were expected to be completed. (See pp. 5 to 8 .)

Since 1970 several scientific studies involving animals, including some performed or sponsored by the Administration, have raised questions concerning the safety of Red No. 2 in food. In some of these studies Red No. 2 or amaranth was shown in test animals to be toxic to reproductive systems or to be carcinogenic. (See ch. 3.)

Because of its concern about the safety of Red No. 2, the Food and Drug Administration in July 1972 issued a proposal to limit human exposure to the color additive. As of September 1, 1975, the Food and Drug Administration had not made a final determination of the safety of Red No. 2 or taken action to implement its proposal to restrict its use in food, drugs, and cosmetics. (See p. 9.)

CHAPTER 1

INTRODUCTION

By letter dated January 30, 1975, Senator Gaylord Nelson requested us to review the Food and Drug Administration's (FDA's) methods for determining the safety of three additives --Food, Drug, and Cosmetic (FD&C) Red No. 2 (hereafter referred to as Red No. 2), saccharin, and aspartame--for use in food. The Senator requested separate reports on each of the additives, focusing on

--the history of FDA's regulation of the additives, including in-house and outside tests leading to a change in their regulated status,

--the current status of testing the additives and FDA activities affecting their status,

--the extent to which FDA has examined alternatives to the additives in the event their safety is questioned, and

--whether the regulatory action taken by FDA on these three additives, based on the scientific evidence available, complies with the Federal Food, Drug, and Cosmetic Act, as amended (FD&C Act) (21 U.S.C. 301).

In addition, we were requested to furnish information on the annual sales volume of Red No. 2, the names of Red No. 2 manufacturers and purchasers, and the number of manufacturers and purchasers considered to be small businesses. This report on Red No. 2 is the first of the three reports to be issued.

REGULATION OF COLOR ADDITIVES

Since July 12, 1960, the Color Additive Amendments to the FD&C Act (Public Law 86-618) have required FDA to establish regulations listing color additives that are safe for use in food, drugs, or cosmetics. Such regulations may list color additives for use generally in food, drugs, or cosmetics or may prescribe the conditions under which the color additives may be safely used. In determining whether a proposed use is safe, the act requires that consideration be given to

--the probable consumption of the additive and of any substance formed in food, drugs, or cosmetics because of the use of the additive,

--the cumulative effect, if any, of such additive in the diet of man or animal, taking into account the same or any chemically or pharmacologically related substance in such diet,

--safety factors which are generally recognized by qualified experts as appropriate for the use of animal experimentation data, and

--the availability of practicable methods of analysis for determining the identity and quantity of (1) the pure dye and all intermediates and other impurities contained in the color additive, (2) the additive in food, drugs, or cosmetics, and (3) any substance formed in such products because of the use of the additive.

The FD&C Act states that a color additive is deemed unsafe and should not be listed in a regulation permitting its use in food, drugs, or cosmetics if it is found by FDA to induce cancer in man or animal.

FDA regulations (21 CFR 8.4) specify that any interested person may submit a petition to FDA proposing that a color additive be listed for use in a food, drug, or cosmetic. The petition must include

--the name and all pertinent information concerning the color additive, including its physical, chemical, and biological properties,

--the amount of the color additive proposed for use; the color effect intended to be achieved; and all directions, recommendations, and suggestions for its proposed use,

--a description of methods for determining the pure color and all intermediates, subsidiary colors, and other components of the color additive; the amount of the additive used in raw, processed, or finished products; and the substances formed as a result of the additive's use,

--full reports of investigations made with respect to the safety of the color additive,

--complete data on the probable consumption of and/or relevant exposure to the additive and of any substance formed because of the use of such additive, and

--proposed tolerances and other limitations on the use of the additive that may be required to insure its safety.

Under the 1960 amendments to the FD&C Act, color additives which were commercially established before July 12, 1960, were provisionally listed in FDA regulations to make possible their use on an interim basis for "a reasonable period" pending the completion of the scientific investigations needed for making a determination as to their safety. As of July 1975, about 90 color additives, including Red No. 2, were provisionally listed for use in food, drugs, and cosmetics.

WHAT IS RED NO. 2?

Red No. 2 is a color additive used to improve the appearance and promote the marketing of products. Red No. 2 is the name given to a certified lot of the dye generically known as amaranth. The composition and purity of amaranth varies. FDA has established composition and purity specifications that amaranth must meet before it can qualify for use in food, drugs, and cosmetics. Only amaranth meeting such specifications is classified as Red No. 2. FDA requires the certification of each batch of amaranth to establish that its composition meets FDA specifications.

Red No. 2 has been used for many years in a wide variety of foods, drugs, and cosmetics. Included are candies, beverages, dessert powders, cereals, maraschino cherries, pet foods, ice creams, snack foods, tablets, capsules, and lipsticks. In addition, Red No. 2 is an ingredient used in producing over 200 other food color additive mixtures.

Six U.S. companies--Allied Chemical Corporation, Morristown, New Jersey; Crompton and Knowles Corporation, New York; H. Kohnstamm and Company, Incorporated, New York; Stange Company, Chicago; Sterling Drug, Incorporated, New York; and Warner-Jenkinson Manufacturing Company, St. Louis--manufacture Red No. 2. In 1973, the latest year for which Red No. 2 sales data was available from the U.S. International Trade Commission, about 1.1 million pounds of Red No. 2 valued at $2.9 million were sold in the United States.

The Small Business Administration has categorized businesses by type of industry and has established the criteria for small businesses in each category. The manufacturers of Red No. 2 are categorized as chemical companies which, under Small Business Administration's criteria, must have fewer than 750 employees to be considered small businesses. Only one of the six manufacturers of Red No. 2--H.

Kohnstamm and Company, Incorporated--has fewer than 750 employees.

Red No. 2 is also imported into the United States from England, France, Germany, Japan, and Mexico, but the amount imported was not available as import data on Red No. 2 is not separately maintained. According to an FDA official, Japan is the major source of Red No. 2 imported into the United States. Information on specific purchasers of Red No. 2 was not readily available.

CHAPTER 2

SAFETY OF RED NO. 2 NOT ESTABLISHED

The FD&C Act permitted the use of commercially established color additives for a reasonable period of time, pending the completion of scientific investigations to determine their safety. Use of Red No. 2 has been allowed under such authority for 15 years.

The FD&C Act, as amended in 1960, placed all color additives commercially established at that time, including Red No. 2, on a provisional list to allow their use for a reasonable period until their safety could be reviewed and regulations for their use could be issued. The 1960 amendments provided that the provisional listing was to terminate no later than 2-1/2 years from the effective date of enactment (July 12, 1960), or January 12, 1963. The amendments also provided, however, that FDA could postpone the termination date if such action was consistent with the objective of carrying to completion, in good faith, as soon as reasonably practicable, the scientific investigations necessary for making a determination as to the additive's safety.

Since January 12, 1963, the termination of the provisional listing of Red No. 2 has been postponed 14 times to allow for completing scientific investigations concerning its safety. As of September 1, 1975, about 15 years after Red No. 2 was provisionally listed for use in food, drugs, and cosmetics, a final determination of its safety had not been made.

INITIAL POSTPONEMENTS IN TERMINATING PROVISIONAL LISTING

On July 12, 1960, Red No. 2 was provisionally listed.

On November 13, 1962, The Toilet Goods Association, Inc., requested that the termination date of the provisional listing for Red No. 2 be postponed to August 1964. The request indicated that two skin tests on animals were being conducted to evaluate the additive's safety for external uses. One study involved the additive's toxic effects when applied to the skin of rabbits while the other involved the additive's carcinogenic potential when applied to the skin of mice. On November 21 and December 14, 1962, the Pharmaceutical Manufacturers Association and the Certified Color Industry Committee, respectively, also requested that the termination date of the provisional listing for Red No. 2 be postponed. The Pharmaceutical Manufacturers Association requested the

postponement to allow for the completion of the animal skin tests; however, the Certified Color Industry Committee's request made no statement as to the specific scientific investigations being conducted on Red No. 2. Based on these requests, FDA postponed the termination date to August 1, 1964.

On May 15, 1964, the Pharmaceutical Manufacturers Association requested that FDA again postpone termination of the provisional listing for Red No. 2. The request, however, did not indicate the length of time to be covered by the postponement or the reason for it.

On May 28, 1964, The Toilet Goods Association, Inc., transmitted to FDA a status report on the evaluation of certain colors. The report indicated that the Red No. 2 skin tests on the rabbits and mice had been completed but that Red No. 2 was still "being pharmacologically evaluated."

On June 3, 1964, FDA postponed the termination date for the provisional listing of Red No. 2 to July 1, 1965.

On April 15 and May 13, 1965, the Certified Color Industry Committee and The Toilet Goods Association, Inc., respectively, requested a third postponement, to July 1, 1966, of the termination dates of the provisional listings for several color additives including Red No. 2. The Committee requested the postponement "to permit completion of the scientific work necessary to support petitions for permanent listing." The Association's request stated that safety studies had been completed but that analytical methods applicable to the studies needed to be "corroborated." Neither request identified specific studies that were being conducted nor stated when the indicated work would be completed.

On June 25, 1965, FDA granted the requested postponement.

PETITION FOR APPROVING SAFETY OF RED NO. 2

On September 17, 1965, the Certified Color Industry Committee (known since 1973 as the Certified Color Manufacturers' Association, Inc.) submitted to FDA a petition requesting the issuance of regulations permanently listing Red No. 2 as safe for use in food, drugs, and cosmetics. The petition made reference to several studies supporting its safety claim for Red No. 2.

In March 1966, FDA advised the petitioner that the petition was incomplete and could not be accepted because it

failed to "establish safe conditions of use of Red No. 2 in cosmetics." FDA stated that, at "a minimum, quantitative formulations of the cosmetics and manufacturing operations should be" included in the petition. In September 1968 the petition was amended to add The Toilet Goods Association, Inc., and the Pharmaceutical Manufacturers Association as petitioners. The petitioners, however, refused to provide the data which FDA had indicated was necessary because they believed the petition, as submitted, adequately established the safety of Red No. 2 for the cosmetic uses indicated.

FDA's authority to require formulation data on cosmetics was the subject of a court case initiated in 1963 by The Toilet Goods Association, Inc. As a result, FDA deferred consideration of the 1968 amended Red No. 2 petition pending final disposition of the court case. On August 25, 1969, the court ruled that FDA did not have authority to require, in all cases, formulation data for cosmetics but that it could request such data in special situations (U.S. Court of Appeals, Second Circuit in Toilet Goods Association vs. Finch 419 F 2nd 21 (1969)).

During the approximately 4-year period between the time the petition was submitted in 1965 and the 1969 court decision, the petitioners requested five further postponements of the termination date of the provisional listing of Red No. 2. In most cases postponements were requested to allow time to either (1) complete scientific work necessary to support the petition, (2) develop use level data to supplement information in the petition, or (3) complete FDA's review of the petition. Although various reasons were cited in the requests for postponements, FDA postponed the provisional listing of Red No. 2 five times until December 31, 1969, to allow, according to the Federal Register notices, time for completing scientific investigations necessary for determining its safety.

In granting the postponement of the provisional listing to March 31, 1968, for food, and to June 30, 1968, for drugs and cosmetics, the Commissioner of FDA, in a letter dated July 17, 1967, to The Toilet Goods Association, Inc., stated

> "I would be less than candid if I did not tell you that this action is taken without enthusiasm on my part. The law is now some seven years old, and it seems high time that these provisional listings should be over and done with. Certainly I hope that by next year at this time there will be very few extensions needed."

In October 1968, FDA agreed to review the petition to list Red No. 2 as safe even though the question regarding cosmetic formulation data was under litigation. FDA's March 5, 1969, final evaluation report on its review of the petition concluded that toxicity data was adequate to support a regulation listing Red No. 2 for general use in coloring food, dietary supplements, and drugs. The report concluded that limits on the use of Red No. 2 were not necessary but stated that resolution of the cosmetic formulation issue was necessary before Red No. 2 could be listed for cosmetics.

FDA postponed the termination date of the provisional listing of Red No. 2 for the ninth time to December 31, 1970, to allow time to resolve the issue concerning cosmetic formulation data.

In a January 4, 1971, memorandum, the HEW Assistant General Counsel, Food, Drugs, and Environmental Health Division, presented his legal opinion of the cosmetic formulation debate. In his memorandum he stated that, while the 1969 Court of Appeals opinion went in part against FDA, it allowed FDA to insist on scientific proof that any color additive would be safe under conditions of proposed use. Specifically, the HEW Assistant General Counsel stated that:

> "I cannot approve legally a regulation which purports to carry out our statutory responsibility 'to assure the safety of the use or uses for which a particular color additive is listed'; 'to prescribe the conditions under which such additive may be safely employed for such use or uses'; and to provide 'specifications as to the manner in which such additive may be added to or used in or on' cosmetics, without addressing those questions."

The Assistant General Counsel believed cosmetic formulation data was necessary for FDA to determine that any color additive would be safe for its intended uses.

SAFETY OF RED NO. 2 QUESTIONED

In 1970 before the cosmetic formulation issue could be resolved, FDA learned of studies in a foreign country which indicated that amaranth was carcinogenic and might be toxic to the reproductive system of animals. (See p. 11.) Therefore, FDA initiated or sponsored a number of studies on Red No. 2, concerning these safety questions. During the approximately 5-year period since the safety questions were raised, five additional postponements have been requested by the petitioners and others to allow time for a final decision regarding the safety of Red No. 2 and to complete

scientific investigations on the safety questions raised in 1970. Pursuant to these requests, FDA postponed the termination of the provisional listing for Red No. 2 until December 31, 1975.

Although FDA had granted postponements that extended the provisional listing of Red No. 2 through 1975, as early as 1971, FDA expressed concern about the safety of Red No. 2 and was considering a restriction on its use. In January 1972, FDA contracted with the National Academy of Sciences to review available toxicological data concerning the safety of Red No. 2, and to evaluate the need to limit human exposure.

The Academy, through its Committee on Food Protection, Food and Nutrition Board, reviewed data from published and unpublished studies on the toxicity of Red No. 2 and, in its June 1972 report to FDA, stated that:

1. Study data available prior to 1970 indicated that normal use of Red No. 2 was not potentially hazardous to human health.

2. More recent data indicating adverse reproductive effects relating to Red No. 2 were inconclusive because such effects were not confirmed in other laboratory studies.

The Committee report stated that a proposal to limit human exposure to Red No. 2 was unnecessary at that time. The Committee recommended, however, more long-range studies because some of the data reported on Red No. 2 needed further study.

Contrary to the Committee's opinion, FDA published a proposal in the July 4, 1972, Federal Register, to establish a tolerance of 1.5 milligrams per kilogram of body weight per day as a safe level of consumption. FDA's proposal was based on its research which showed that 15 milligrams per kilogram of body weight was the highest level of exposure for rats in which no statistically significant effects were noted. FDA applied a 10-fold safety factor and arrived at 1.5 milligrams per kilogram. As of September 1, 1975, no action had been taken to implement this proposal and the petition to issue a regulation to permanently list Red No. 2 remained outstanding.

The Acting Director, Bureau of Foods, in a memorandum dated July 14, 1975, to the FDA Associate Commissioners for Compliance and Science, stated that final regulatory decisions on Red No. 2 could not be made until safety questions

concerning the carcinogenicity and embryotoxicity (poisonous effects on an embryo) were answered. He stated that the carcinogenicity and embryotoxicity issues could be pursued independently and that interim action on tolerance levels could be based on embryotoxicity data. (The carcinogenicity and embryotoxicity questions are being considered in FDA's chronic study, see pages 20 to 23; a Canadian reproductive cat study, see page 17; and an FDA-industry reproductive collaborative study, see pages 19 and 20.)

CHAPTER 3

STUDIES RELATING TO THE SAFETY OF RED NO. 2

In 1970 FDA became aware of the results of investigations by Russian scientists which raised questions concerning the safety of amaranth as an additive in food. Since that time a number of animal studies have been made to evaluate the safety of Red No. 2 or amaranth. We reviewed studies conducted in foreign countries, FDA and U.S. research laboratories, and industry (see app. I). The studies were directed at the additive's (1) physiological effects on body functions, (2) effects on the reproductive system, and (3) chronic (long-term) effects. Some of these studies associated problems found in test animals with the use of Red No. 2 or amaranth; some indicated no problems; others were inconclusive.

EARLY RUSSIAN STUDIES

In 1964 the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives indicated the need for more work on several food color additives to determine more accurately their toxicological effects. The Committee indicated that additional investigations were particularly needed concerning the effects of certain color additives, including amaranth, on reproduction. Russian scientists initiated three research studies to evaluate these effects. These studies, which FDA officials said became available to them in 1970, raised questions concerning the safety of Red No. 2 as a food additive.

A report on one of the Russian studies indicated that amaranth was gonadotoxic (poisonous to reproductive organs) and possibly embryotoxic. The report stated that, compared to rats in the control group (a group of rats not fed the additive), rats fed amaranth showed a decrease in fertility, a decrease in the number of live births, and an increase in the number of offspring which failed to survive 1 month after birth.

The other two studies concluded that amaranth is carcinogenic. One study report stated that "chemically pure amaranth possesses carcinogenic activity of medium strength and should not be used in the food industry." According to the report, amaranth was fed to 50 rats over a 33-month period. Thirteen of the rats developed malignant tumors while none of the rats in the control group developed such tumors.

FDA scientists stated that information on the purity of the amaranth used in the Russian studies is not available and it may not have conformed to FDA specifications for Red No. 2.

PHYSIOLOGICAL STUDIES

Physiological studies trace the movement of a chemical in the body, examine the body's disposition of the chemical, and determine possible changes in body functions and processes resulting from its use. These studies help to identify potential health problems associated with the use of a chemical and provide a basis for analyzing the results of other studies such as reproductive and carcinogenic studies.

We reviewed reports on five physiological studies on Red No. 2. These studies examined its metabolism in the body, its mutagenicity, and its effects on the endocrine glands. The results of these studies are briefly discussed below.

Metabolism studies

Metabolism studies determine what compounds, or metabolites, a substance may break down to in the body; how fast and into which organs the components are dispersed; and how fast they are eliminated. Metabolism studies aid in understanding results of studies, such as reproductive and carcinogenic studies, by determining which body organs receive the greatest exposure to the substance or its metabolites. We reviewed two studies initiated or reported since 1970 on the metabolism of Red No. 2 in rats.

An industry study published in June 1974 evaluated body absorption and elimination rates for naphthionic acid, one of the metabolites of Red No. 2. The study report states that the metabolite is rapidly absorbed from the intestinal tract of rats and eliminated from the body in a relatively short period of time. The report noted also that naphthionic acid appeared to break down further into other metabolites in the body and that this should be explored in future experiments.

An FDA metabolism study, initiated in March 1972 but not published as of July 1975, was directed at identifying the metabolites of Red No. 2 and determining the body's disposition of each. The study showed that Red No. 2 is broken down into three metabolites.[1] These metabolites

[1]The three metabolites of Red No. 2 are: naphthionic acid; 1- amino- 2- naphthol- 3,6- disulfonic acid; and 1,2- naphthoquinone- 3,6- disulfonic acid.

were determined to rapidly cross the intestinal wall and migrate into various body organs. Naphthionic acid was shown to migrate more completely than the other two. One or more of the metabolites was observed in the urinary bladder, kidney, liver, adrenal glands, pancreas, spleen, and lung. All three metabolites were found to be excreted almost entirely within 24 hours after the color additive was ingested.

Mutagenicity studies

Mutagenicity studies are designed to determine if a substance causes mutations. A mutation is any heritable change such as a chemical transformation of an individual gene which may alter its functions, or a rearrangement of the structure of or a gain or loss of parts of a chromosome. Mutations may cause abortion, genetic disease, shorter life span, infertility, mental retardation, senility, and cancer. We identified two studies initiated since 1970 in this area. Both studies were sponsored by FDA. One was performed by the Stanford Research Institute under an FDA contract and the other by an FDA laboratory. These studies indicated that Red No. 2 causes genetic damage and is gonadotoxic.

In June 1971 FDA awarded a contract to Stanford Research Institute to examine the mutagenicity of 14 selected chemical compounds. One of the compounds studied was Red No. 2. The Institute evaluated the genetic hazards of the test compounds by several methods. These included the cytogenetic and dominant lethal methods.

The cytogenetic test determines if a specific material is potentially capable of causing heritable genetic damage by altering the structure of chromosomes and thus is potentially mutagenic. This is determined by microscopic examination of the chromosome structure. The effects of chromosomal alteration on reproductive functions can be determined through dominant lethal tests.

The dominant lethal test determines if a compound is mutagenic or possibly gonadotoxic. In this test, treated male animals are mated to untreated female animals and the number of fertilized eggs and early fetal deaths are counted. If the number of deaths is higher in the treated group than in the control group, there is direct evidence that the specific material is a mutagen. However, if there are fewer fertilized eggs implanted in the uteri of the treated group than the control group, the compound is not mutagenic but can be considered gonadotoxic.

The results of the Stanford Research Institute study were reported to FDA in January 1972. According to an FDA scientist, the results from the dominant lethal test showed that Red No. 2 caused a reduction in the number of fertilized eggs implanted in the female rats' uteri, indicating gonadotoxicity. The FDA scientist believed there was no evidence from the dominant lethal test that Red No. 2 was mutagenic since there was no difference in the number of early fetal deaths between the treated and control groups. However, because the results of the Stanford cytogenetic test showed chromosomal alteration, he believed that the mutagenic potential of Red No. 2 should be further studied.

In April 1972 the Genetic Toxicology Branch of FDA's Bureau of Foods completed a cytogenetic test on animals to further evaluate the potential mutagenicity of Red No. 2. As of March 1975, FDA had not analyzed the test data. We discussed this matter with an FDA Bureau of Foods' scientist who subsequently made a statistical analysis of the test data which indicated that Red No. 2 and one of its metabolites were potentially mutagenic in that they caused changes in the chromosomal structure of the test animals.

We asked the scientist about the failure to promptly evaluate data from its cytogenetic tests on Red No. 2. He told us that it was an oversight which could be attributed, in part, to the fact that, at the time of the test in 1972, the Bureau of Foods did not have a centralized reporting system to accumulate information on individual studies being performed within FDA or a requirement for formal progress reports on such studies.

In October 1974 the Bureau of Foods established (1) a computerized information system to store data on ongoing experiments, and (2) procedures requiring personnel responsible for ongoing experiments to submit annual progress reports on the purpose, protocol, and results of their studies to a technical reports editor. The editor is required to account for each experiment underway until completed and to insure that research results are reported. The new control system applies only to research that was ongoing at the time the controls were established or to future studies; therefore, the oversight involving the cytogenetic test would not have been discovered by the new controls.

For fiscal year 1976, FDA plans two additional mutagenicity studies on a number of additives, including Red No. 2. In one study it will be retested to evaluate the results of previous studies to determine if it causes heritable genetic damage. An FDA Bureau of Foods scientist

stated that this study is designed to further evaluate the mutagenicity of Red No. 2, using a different method of analysis.

The second study will attempt to further evaluate the mutagenicity of Red No. 2 by studying the effects of its three metabolites in a laboratory test tube culture.

Endocrinology studies

Endocrinology is a branch of biological science which studies the endocrine glands (such as the thyroid and pituitary glands) and their secretions, in relation to body processes and functions. Secretions of the endocrine glands pass into the blood or lymph where they are carried to the body organs whose functions they regulate or control.

Since 1970 one endocrinology study has been conducted on Red No. 2. This study was performed by FDA to investigate the effects of Red No. 2 and two of its metabolites on the endocrines. FDA investigators reported in July 1972 that these metabolites are causative factors in the enlargement of certain female rat reproductive organs. The report stated that Red No. 2 metabolites increased production of certain hormones, resulting in an imbalance in the endocrine system, which in turn caused an accumulation of fluid in certain reproductive organs. One of the participating scientists concluded that there was little doubt that Red No. 2 had gonadotoxic effects on rats. (The gonadotoxicity of Red No. 2 and amaranth has been further evaluated under reproductive studies discussed below.)

REPRODUCTIVE STUDIES

Reproductive studies determine the effects various chemicals or other substances have on the reproductive processes. We reviewed 21 studies on the effects of Red No. 2 or amaranth on the reproductive systems of test animals which have been reported or initiated since the 1970 Russian study. These studies were conducted by FDA, industry, or foreign countries. Four of the studies showed detrimental effects to the animal's reproductive functions indicating that Red No. 2 or amaranth is embryotoxic. Nine studies showed no adverse effects from the use of Red No. 2 or amaranth, and three indicated further studies were needed to determine the safety of Red No. 2 or amaranth. As of July 1975, the results of the five remaining studies had not been reported.

During these studies Red No. 2 or amaranth was administered to animals or embryos by injection, direct feeding, or gavage. Gavage is the oral administration of a substance

directly to the stomach through a feeding tube.

Results of studies using injection

In February 1971 FDA began an evaluation of the embryotoxicity of Red No. 2 by injecting the color additive or its metabolites into chicken eggs. In June 1971 FDA also contracted with the University of Arizona for a similar study. The results of both studies were reported in 1972.

The FDA in-house study showed that chick embryos subjected to Red No. 2 or two of its metabolites (naphthionic acid and 1- amino- 2- naphthol- 3,6- disulfonic acid) had an increased mortality rate over chick embryos in the control group, thus indicating the substances were embryotoxic. The University of Arizona reported that the results of its study provided equivocal evidence that Red No. 2 was embryotoxic and that further investigation was warranted.

There was general agreement among FDA scientists we interviewed that chick embryo studies are valuable for identifying possible problems but regulatory action should not be taken solely on their results. The scientists indicated that, when chick embryo tests show a substance is embryotoxic, it should be evaluated in other animals before final conclusions are drawn.

Results of studies using direct feeding

Five reproductive studies were completed or were being conducted as of July 1975 on Red No. 2 or amaranth using direct feeding. In addition, two studies were completed or were being conducted in which test animals were fed Red No. 2 in capsule form. (An additional reproductive study which used both direct feeding and gavage to administer Red No. 2 is included as one of five gavage studies discussed on page 18.)

Two studies--an industry study reported in October 1973 and an FDA study reported in January 1975--indicated that Red No. 2 continuously ingested by three generations of rats had no adverse effects.

One industry-sponsored teratology study (study of serious malformations of the normal structure) assessed the effects of Red No. 2 administered in gelatin capsules on the offspring of rabbits. Another industry teratology study involved direct feeding to rabbits. The results, reported during February and June 1972, respectively, indicated no adverse effects attributable to Red No. 2.

The three remaining studies evaluated the safety of Red No. 2 as used in pet food. One study, using dogs as test animals, reported in March 1974, showed that puppies receiving Red No. 2 gained less weight over a given period than puppies from the control group but that this effect was not statistically significant.

Another pet food study, using cats as test animals, reported in April 1974, indicated adverse reproductive effects when female cats were fed Red No. 2. The results of the study were questioned by FDA because it believed the experiment was poorly conducted and records were not adequately maintained. For example, the number of mated females was less than that required by the protocol for two different feeding levels, and information was not maintained on males used for breeding. FDA's Division of Toxicology concluded, therefore, that another study was needed to resolve the question of the potential reproductive effects of Red No. 2 on cats.

The third pet food study, according to a July 14, 1975, memorandum to the FDA Associate Commissioners for Compliance and Science, from the Acting Director, Bureau of Foods, FDA, involved cats and was being conducted by the Health Protection Branch of the Canadian Government. In this study amaranth was being administered by capsule. In his July memorandum the Acting Director stated that the results of this study should resolve the questions raised by the earlier cat study; however, he stated that it would appear inappropriate for FDA to take action on the safety of Red No. 2 until those questions have been resolved.

Results of studies using gavage

After their 1970 study on the reproductive effects of amaranth, Russian scientists conducted another study concerning the reproductive system of rats administered amaranth by gavage. The study report published in 1972 noted that amaranth reduced the effectiveness of the male rat's sperm in fertilizing eggs and inhibited the recurrent period of the female rat's sexual activity (known as heat). In addition, an embryotoxic effect was noted when pregnant females were administered amaranth. Based on these findings, the report concluded that amaranth is embryotoxic and gonadotoxic.

Also, FDA teratology studies published in October 1972 and June 1973 indicated that Red No. 2, one of its metabolites, and an intermediate compound of Red No. 2, administered to rats by gavage, produced toxic effects. In the first study, which began in 1971, Red No. 2 was administered

to female rats by gavage beginning with day 0 through day 19 of pregnancy at dose levels of 7.5, 15, 30, 100, and 200 milligrams per kilogram of body weight per day. On day 20 the rats were sacrificed and observations were made of both the fetuses and the rat's reproductive systems. No gross malformations of the fetuses were noted. However, a statistically significant number of resorptions (fetal deaths) was observed in the rats fed Red No. 2 at the three highest dose levels compared with the control rats. This study indicated that 15 milligrams per kilogram of body weight was the highest level of exposure for rats in which no statistically significant effects were noted for Red No. 2.

FDA's second study on Red No. 2 followed a protocol similar to that used for the first study, except that the rats were gavaged with two Red No. 2 metabolites and an intermediate compound commonly resulting from the Red No. 2 manufacturing process. A 1973 report on this study stated that there was a statistically significant number of rats with resorptions which received one of the metabolites and the intermediate compound compared with the control group. On the basis of this study, FDA determined that the no-effect dose levels (the levels where no statistically significant adverse effects are noted) for the metabolite and intermediate chemical were 100 and 30 milligrams per kilogram of body weight, respectively.

In addition, we identified five other teratology and reproductive studies reported between February 1972 and January 1975 which used the gavage technique for administering Red No. 2 or amaranth to test animals. Unlike the FDA in-house studies, however, animals in each of these studies were gavaged from day 6 of pregnancy rather than from day 0. All of these studies reported no significant difference in the effects demonstrated between the control animals and the animals receiving Red No. 2. However, FDA determined that its contracted teratology study on rats performed by the Food and Drug Research Laboratories, Incorporated, in Maspeth, New York, demonstrated resorptions that were statistically significant. The FDA statistical analysis report on this study concluded that the significant number of resorptions noted during the study supported the effects demonstrated in the October 1972 FDA gavage study.

On May 14, 1973, FDA established an Ad Hoc Advisory Group to evaluate, among other things, the appropriateness of gavage testing and the safety of Red No. 2. The Group concluded that gavage was an appropriate method for testing the additive, provided a standardized and relatively non-traumatic method of gavage was used. It noted, however,

that, because FDA's October 1972 report showed that rats gavaged with Red No. 2 from days 0 through 19 of pregnancy experienced a significant number of early fetal deaths and other studies generally showed that animals gavaged from day 6 through 15 of pregnancy experienced no adverse effects, a comparative study of these two gavage procedures should be undertaken.

The Ad Hoc Advisory Group designed a protocol for a Government-industry study of the two gavage procedures. The protocol called for tests to determine the embryotoxicity of Red No. 2. Tests were performed by FDA's Bureau of Foods and its National Center for Toxicological Research (NCTR) and Industrial Bio-Test Laboratories, an independent laboratory. Each organization performed statistical reviews of its test data which NCTR consolidated and analyzed. The test data and NCTR's preliminary analysis were presented to the Ad Hoc Advisory Group on June 6, 1974. The Group's report, dated December 20, 1974, concluded that the collaborative test results showed no adverse effects from Red No. 2 by either gavage procedure. The report noted that the adverse effects previously demonstrated by gavage in the October 1972 FDA study had not been duplicated during the collaborative study. The Ad Hoc Advisory Group therefore concluded that the scientific data indicated Red No. 2 was not embryotoxic when fed to rats by gavage.

In March 1975, after reviewing the summary data on the tests made by Industrial Bio-Test Laboratories and NCTR, we questioned the Group's conclusion concerning the tests' indications of embryotoxicity.

We noted that one strain of rats (Charles River strain) used in the tests, experienced increased resorptions when gavaged from days 0 through 19 of pregnancy. In addition, it appeared that NCTR, in making its consolidated analysis, might have used different statistical parameters than the Bureau of Foods study reported in October 1972.

An FDA Bureau of Foods scientist agreed to reevaluate the collaborative study using the same parameters used in the FDA study reported in October 1972. The reevaluation showed a statistically significant number of litters with two or more resorptions in the Charles River strain of rats when gavaged with Red No. 2 from day 0 through day 19 of pregnancy in both the NCTR and Industrial Bio-Test studies. Similar effects were not noted in the Charles River rats gavaged from day 6 through day 15 of pregnancy.

Although a statistically significant number of resorptions had occurred in the Osborne-Mendel strain of rats in

the Bureau of Foods study reported in October 1972, similar findings were not noted for that strain in the collaborative study. Also the collaborative study results indicated that Red No. 2 caused statistically significant preimplantation losses (failure of a fertilized egg to implant in the uterus) in both strains of rats. Such a finding was not noted in the Bureau of Foods' October 1972 study report.

Based on the Bureau of Foods' reevaluation of the collaborative study, an FDA official said the Ad Hoc Advisory Group's conclusion that Red No. 2 is not embryotoxic is not supportable.

According to a July 14, 1975, memorandum from the Acting Director of the Bureau of Foods to the FDA Associate Commissioners for Compliance and Science, the final report on the FDA-industry collaborative study on reproduction had not been completed and the question concerning Red No. 2's effects on reproduction must await completion of the report. The memorandum stated that, if an adverse effect is noted by the collaborative study, it would have to be considered as confirming the October 1972 FDA study. A tolerance level of 0.15 milligrams per kilogram of body weight per day would then be established by FDA for Red No. 2, which would limit the use of the color additive in soft drinks. This tolerance level would be based on a 100-fold safety factor and on data from the October 1972 FDA teratology study. The collaborative study protocol included testing at only a single dose level, thus preventing determination of a no-effect dose level. If the results of the collaborative study do not answer the question of embryotoxicity, then according to the July 14, 1975, memorandum, the question will be referred to HEW's Toxicology Advisory Committee.

CHRONIC STUDIES

Chronic studies on the long-term toxicity of a substance can include assessments of its cancer-producing potential. Two such studies on Red No. 2 or amaranth have been initiated or reported since 1970 when FDA became aware of the Russian studies which raised questions concerning the potential of amaranth to cause cancer. (See pp. 11 and 12.) One long-term study was conducted in Rumania. The report on this study is in French and has not been officially translated by FDA. However, a Bureau of Foods scientist showed us his rough translation of the study indicating that amaranth is toxic and has adverse effects on the livers of rats.

The other study was a 30-month effort initiated in March 1972 by FDA to evaluate the chronic toxicity and cancer-producing potential of Red No. 2 in rats. This study used 500 rats divided into 10 groups--5 groups of 50 males and 5 groups of 50 females. Two control groups, one male and one

female, were not fed Red No. 2. The remaining groups were fed diets containing Red No. 2 levels of either 0.003, 0.03, 0.3, or 3 percent. During the study, however, several rats were inadvertently switched from one feeding level to another. In addition, a large number of rats in the study were not available at the completion of the study for full pathological examination (examination of body tissues for abnormal effects). As of July 1975, FDA had not completed its evaluation of the study; however, in view of the discrepancies encountered during the study, its results appear to be of questionable validity.

An apparent switch of test animals among feeding levels was discovered on January 17, 1974, by a laboratory technician, when he noticed a rat's identification number did not correspond to its cage number. He told us that he reported this mixup to the Acting Chief of the Chronic Toxicity Branch the same day it was discovered because the Chief of the Branch was on vacation. The laboratory technician, however, did not document his finding until January 3, 1975, when he prepared a memorandum to the file noting that rats at all feeding levels except the highest (3.0 percent) had been placed in the wrong cages. The memorandum stated that "An example of the extent of the mixup was some rats at the 0.3 percent level were found in the 0.003 percent cages. It took me 3 hours to straighten the mixup." Unfortunately, the technician did not keep a record of which animals were misplaced.

The Chief of the Chronic Toxicity Branch said she did not learn of the mixup until it had been reported by the news media in December 1974, almost a year after it was discovered. She stated that, for 15 of the 30 months of the study, no professional staff member was assigned as project investigator; therefore, the daily routine of monitoring the study went unattended. Other management responsibilities prevented her from being actively involved in the study.

At the time the news media reported the mixup, the Chief of the Chronic Toxicity Branch requested that an effort be made to reconstruct what happened. The project investigator for the latter phase of the study attempted to reconstruct the events leading to the mixup. She reported her findings in a February 25, 1975, memorandum to the Acting Director, Division of Toxicology. In the memorandum she stated that the available records indicated that two rats may have been placed on the wrong feeding diet during January 1974. The project investigator also reported that information on the rats' weights for a period in January 1974 could not be found. She stated that, if this

information were available, it would be possible to determine more conclusively which rats had been misplaced.

We reviewed individual animal laboratory history records for the 500 rats involved in the FDA study. Although we were unable to determine the extent of the rat mixup, we noted several discrepancies that occurred at various times during the study. For example, four rats were apparently transferred to empty cages which were previously occupied by rats that had died. The weights of the transferred rats were then recorded as the weights of the dead rats. Also, one pair of rats was apparently exchanged twice between each other's cages. These discrepancies were not detected until the rats died or were sacrificed. In another instance, a pathologist noted that a rat being sacrificed at the end of the study had previously been listed as dead.

FDA's general protocol for chronic oral toxicity studies entitled "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics," states that, in studies using rats, usable tissues from a minimum of 25 animals for each sex at each feeding level is required for pathological examination. We found that the pathologists were able to collect the required number of usable tissues of 25 rats for only 2 of the 10 groups in FDA's cancer study. (See app. II.) Of the 500 rats in the study, FDA pathologists examined 405 rats which died during the feeding phase of the study and found that only 103 were adequately preserved to provide the required tissues for pathological examination. According to the pathologists, the tissues of the remaining 95 rats sacrificed at the end of the feeding phase were adequate for pathological examination. An FDA pathologist told us that if the objective of this study was to collect enough tissues to analyze the tumor incidence over the life cycle of the rats, the study fell "grievously" short of meeting the objective.

A July 14, 1975, memorandum from the FDA Acting Director, Bureau of Foods, to the FDA Associate Commissioners for Compliance and Science states that:

> "* * * there is a possibility that a mix-up may have occurred among the rats being fed FD&C Red No. 2 such that the level and duration at which individual animals were dosed can no longer be identified with certainty. In our opinion, this places severe limitations on the usefulness of the data derived from the study. The only observations which may be possible from this study would concern the presence or absence of tumors relative to historical control data for

this strain of rat. However, for this reason, it is our judgment that the study may retain some value and that its evaluation should be completed."

According to the memorandum, FDA will submit the question concerning carcinogenicity of Red No. 2 to HEW's Toxicology Advisory Committee after the results of the FDA chronic study are available. The Committee will be asked, according to the memorandum, to resolve this question by evaluating the FDA study results together with other experimental results on the subject.

CHAPTER 4

ALTERNATIVES TO RED NO. 2

When FDA questions the safety of a color additive, such as Red No. 2, the usage, marketability, and possible substitutes must also be considered. The FD&C Act requires that, if the Secretary of HEW finds that the data submitted on the quantity of color additive likely to be consumed in the diet or applied to the human body fails to show that it would be safe and otherwise permissible to list a color additive for all uses and concentrations proposed,

> " * * * the Secretary shall, in determining for which use or uses such additive * * * shall be or remain listed, * * * take into account * * * (A) the relative marketability of the articles involved as affected by the proposed uses of the color additive * * *, the relative dependence of the industries concerned on such uses; (B) * * * and (C) the availability, if any, of other color additives suitable and safe for one or more of the uses proposed."

In 1971 FDA requested all interested persons to submit usage data for Red No. 2. FDA met with trade associations whose members used the color additive in their products and requested data on available substitutes for Red No. 2 and on the marketability of products without Red No. 2 or with a substitute.

According to an FDA official, many food manufacturing companies indicated various reasons why they could not market their products without Red No. 2. They indicated that, for several of their products, either no substitute for Red No. 2 could be found or available substitutes produced an inferior color.

A possible substitute for Red No. 2 is FD&C Red No. 40 which was approved as safe for use in food and drugs, effective June 9, 1971, and for use in cosmetics, effective February 21, 1975. An FDA official told us, however, that FD&C Red No. 40 has two major disadvantages. According to this official, FD&C Red No. 40 costs about twice as much as Red No. 2 and it produces a less desirable color than Red No. 2 in some products. The official said that many food industry representatives believe that discontinuing the use of Red No. 2 will reduce the marketing appeal of their products because the coloring effect Red No. 2 produces in food products is hard to duplicate.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Since July 12, 1960, the FD&C Act has required FDA to review the safety of color additives used in food, drugs, and cosmetics and to issue regulations prescribing their safe use. Color additives, such as Red No. 2, that were commercially established at that time could continue to be used in these products on an interim basis for a reasonable period, pending completion of scientific investigations needed to determine their safety.

FDA, however, has permitted the use of Red No. 2 in food, drugs, and cosmetics for 15 years without making a final determination of its safety. FDA has repeatedly extended the interim period for using Red No. 2 in food, drugs, and cosmetics on the basis of requests from manufacturer or industry associations to allow time to complete scientific investigations concerning its safety. In some cases, however, the requests did not identify investigations underway or indicate when they were to be completed.

Moreover, since 1970 several scientific studies involving animals, including some performed or sponsored by FDA, raised questions concerning the safety of Red No. 2 in food. In some of these animal studies Red No. 2 or amaranth was shown to be either toxic to reproductive systems or carcinogenic. Because of its concern about the safety of Red No. 2, FDA in July 1972 issued a proposal to limit human exposure to the color additive. As of September 1, 1975, FDA had not made a final determination on the safety of Red No. 2 or restricted its use in food, drugs, and cosmetics. Permitting its continued use for an extended period while questions concerning its safety remain unresolved results in unnecessary risks to the public health. To minimize such risk, FDA should act promptly to establish the safety of Red No. 2 or take appropriate regulatory action.

RECOMMENDATIONS

We recommend that the Secretary, HEW, direct the Commissioner, FDA, to act promptly to establish the safety of Red No. 2 or take appropriate regulatory action to prevent its use in food, drugs, and cosmetics.

CHAPTER 6

SCOPE OF REVIEW

We reviewed pertinent legislation, regulations, and practices relating to FDA's regulation of color additives; examined FDA records relating to the past and present regulatory status of Red No. 2; and reviewed reports of scientific studies on the safety of Red No. 2.

We also obtained information from officials of FDA's Bureau of Foods, Washington, D.C., and National Center for Toxicological Research, Jefferson, Arkansas; the U.S. International Trade Commission, Washington, D.C.; Health and Welfare, Canadian Government, Ottawa, Canada; and other organizations.

Our review of the regulatory status was confined to the period since 1960 when amendments to the FD&C Act required a determination of the safety of each color additive used in food, drugs, and cosmetics. We reviewed reports on scientific studies initiated or reported in or after 1970.

APPENDIX I

SCIENTIFIC STUDIES RELATED TO THE SAFETY OF RED NO. 2 REPORTED OR INITIATED SINCE 1970

Studies Reporting Effects Attributable to the Use of Red No. 2 or Amaranth

Title	Date reported or initiated	Author(s) or investigator(s)	Type of study	Effects reported
1. Carcinogenic Properties of the Amaranth Paste	- a/1970	M. M. Baigusheva Moscow, Russia	Chronic (early Russian)	Carcinogenicity
2. Carcinogenic Properties of the Red Food Dyes Amaranth, Ponceaux 5X, and Ponceaux 4R	- 1970	M. M. Andrianova Laboratory for Carcinogens Institute for Nutrition Moscow, Russia	Chronic (early Russian)	Carcinogenicity
3. Effect of Amaranth Food Dye on Reproductive Function and Progeny Development in Experiments with Albino Rats	- 1970	A. I. Shtenberg and Ye. V. Gavrilenko Pesticide Toxicology Laboratory Nutrition Institute, U.S.S.R. Academy of Medical Sciences Moscow, Russia	Reproductive--gavage (early Russian)	Gonadotoxicity and possible embryotoxicity
4. Toxicological Research on the Coloring Amaranth (note b)	May - June 1972	V. Gales et al Institut de Medecine et de Pharmacie et Institut Oncologique de CLUJ, Roumanie (Rumania)	Chronic	Toxic to the liver
5. Analysis of Chick Assay Data on Red No. 2 and Metabolites	February 1972	Jacqueline Verrett, FDA	Reproductive--injection	Embryotoxicity
6. Teratology Studies on Food Colourings. Part I. Embryotoxicity of Amaranth (FD&C Red No. 2) in Rats	October 1972	T. F. X. Collins and J. McLaughlin, FDA	Reproductive--gavage	Fetal deaths
7. The Effect of Amaranth (FD&C Red No. 2) and Its Metabolites on the Endocrine and Reproductive Systems of the Rat	July 1972	Bowie, Banks, Brouwer, and Lindstrom, FDA	Endocrinology	Gonadotoxicity
8. Teratology Studies on Food Colourings. Part II. Embroyotoxicity of R Salt and Metabolites of Amaranth (FD&C Red No. 2) in Rats	June 1973	T. F. X. Collins and J. McLaughlin, FDA	Reproductive--gavage	Fetal deaths
9. The Gonadotoxic and Embryotoxic Effect of the Food Dye Amaranth	November 1972	A. I. Shtenberg and Ye. V. Gavrilenko Pesticide Toxicology Laboratory Nutrition Institute, U.S.S.R. Academy of Medical Sciences Moscow, Russia	Reproductive--gavage	Gonadotoxicity and embryotoxicity
10. Analysis of Cell Aberrations in Rats Given Red No. 2 and its Metabolites (note c)	April 1975	Sidney Green, FDA F. Moreland, FDA	Mutagenicity	Potential mutagenicity

Studies Reporting No Effects Attributable to the Use of Red No. 2 or Amaranth

11. Teratogenic Study with FD&C Red No. 2 in Albino Rats	February 1972	M. L. Keplinger et al Industrial Bio-Test Laboratories, Inc. Northbrook, Illinois	Reproductive--gavage	-
12. Teratogenic Study with FD&C Red No. 2 in Albino Rabbits	February 1972	M. L. Keplinger et al Industrial Bio-Test Laboratories, Inc. Northbrook, Illinois	Reproductive--capsule	-
13. FD&C Red No. 2 and Red No. 3: Comparison of Oral Intubation versus Dietary Feeding in the Pregnant Rabbit	March 1972	Woodard Research Corporation	Reproductive--gavage and direct feeding	-
14. Teratogenic Study in Rats with FD&C Red No. 2	June 1972	C. Burnett, Revlon, Incorporated	Reproductive--gavage	-
15. Teratogenic Study in Rabbits with FD&C Red No. 2	June 1972	C. Burnett, Revlon, Incorporated	Reproductive--direct feeding	-
16. Three-Generation Reproduction Study with FD&C Red No. 2 in Albino Rats	October 1973	M. L. Keplinger et al Industrial Bio-Test Laboratories, Inc. Northbrook, Illinois	Reproductive--direct feeding	-
17. Long-Term Effects of Dietary Amaranth in Rats				
I. Effects on Reproduction	January 1975	T. F. X. Collins et al, FDA	Reproductive--direct feeding	-
II. Effects on Fetal Development	January 1975	T. F. X. Collins et al, FDA	Reproductive--direct feeding	-
18. A Teratologic Study with the Dyes Amaranth and Ponceau 4R in Mice	January 1975	K. Sune Larsson Laboratory of Teratology Karolinska Institutet Stockholm, Sweden	Reproductive--gavage	-
19. Food and Drug Research Laboratories Experiment on Red No. 2 in Rats, Mice, Hamsters, and Rabbits (note c)	February 1972	Food and Drug Research Laboratories, Incorporated Maspeth, New York (FDA contract)	Reproductive--gavage	(note d)

APPENDIX I

Studies Reporting Inconclusive Results from the Use of Red No. 2 or Amaranth

Title	Date reported or initiated	Author(s) or investigator(s)	Type of study	Effects reported
20. Teratogenic and Reproduction Studies in Beagle Dogs	March 1974	Industrial Bio-Test Laboratories Northbrook, Illinois Sponsor--Pet Food Institute Washington, D.C.	Reproductive--direct feeding	-
21. Teratogenic and Reproduction Study in Cats	April 1974	Industrial Bio-Test Laboratories Northbrook, Illinois, and Quaker Oats Company Barrington, Illinois Sponsor--Pet Food Institute Washington, D.C.	Reproductive--direct feeding	-
22. Effect on Three Routes of Administration on the Metabolism of FD&C Red No. 2	June 1974	A. B. Pritchard and P. A. Holmes Central Research, General Foods Corp. Technical Center, Tarrytown, New York	Metabolism	-
23. Study of Mutagenic Effects of FD&C Red No. 2	January 1972	Stanford Research Institute Menlo Park, California (FDA contract)	Mutagenicity	-
24. Amaranth Toxicity and Teratogenicity Studies in Avian Embryos	March 1972	B. L. Reid University of Arizona (FDA contract)	Reproductive--injection	-

Ongoing Studies as of July 1975 on Effects from the Use of Red No. 2 or Amaranth (note c)

25. Chronic Study of Red No. 2 in Rats	March 1972	Jean Taylor, FDA	Chronic	-
26. Embryotoxicity of Red No. 2 Administered by Gavage or in Drinking Water to Charles River Rats (note e)	November 1973	FDA's NCTR Jefferson, Arkansas	Reproductive--gavage	-
27. Industrial Bio-Test Laboratories Test of Red No. 2 Administered by Gavage or in Drinking Water to Charles River Rats (note e)	November 1973	Industrial Bio-Test Laboratories, Inc. Northbrook, Illinois	Reproductive--gavage	-
28. FDA Test of Red No. 2 Administered by Gavage or in Drinking Water to Osborne-Mendel Rats (note e)	January 1974	T. F. X. Collins et al, FDA	Reproductive--gavage	-
29. Embryotoxicity of Red No. 2 Administered by Gavage or in Drinking Water to Osborne-Mendel Rats (note e)	November 1973	FDA's NCTR Jefferson, Arkansas	Reproductive--gavage	-
30. Metabolism of Red No. 2	March 1972	E. J. Lethco, FDA	Metabolism	-
31. Embryotoxicity of Amaranth in Cats	May 1975	K. Khera Health Protection Branch Health and Welfare Canadian Government	Reproductive--capsule	-

a/Reported in Russia in 1968. FDA did not learn of study until 1970.

b/Report is in French and GAO's translation of the title may not be exact.

c/Subject matter of the study because report has not been published.

d/FDRL reported no effects on any of the four species of animals tested. In January 1973, however, FDA reviewed the portion of this experiment using rats and determined that the data showed Red No. 2 had toxic effects on those test animals. In January 1973 FDRL revised its report to FDA.

e/Part of collaborative study run simultaneously by FDA; Industrial Bio-Test Laboratories, Inc.; and NCTR.

FDA CHRONIC STUDY OF RED NO. 2 IN RATS

SUMMARY OF NUMBER OF RATS WITH USABLE AND UNUSABLE TISSUES

FOR PATHOLOGICAL ANALYSIS

Percentage of Red No. 2 in the diet	Total rats at beginning of study	Rats with usable tissues		Rats with unusable tissues
		Rats with required number of tissues available (note a)	Rats with required number of tissues unavailable (notes a and b)	
0 percent (control):				
Male	50	17	8	25
Female	50	20	14	16
0.003 percent:				
Male	50	15	4	31
Female	50	35	5	10
0.03 percent:				
Male	50	16	4	30
Female	50	20	8	22
0.3 percent:				
Male	50	10	8	32
Female	50	22	8	20
3.0 percent:				
Male	50	17	8	25
Female	50	26	4	20
Total	500	198	71	231
Percent of total rats	100	39.6	14.2	46.2

a/For dosage levels 0.003 percent, 0.03 percent, and 0.3 percent pathologists examine tissues from the heart, liver, lung, kidney, adrenal glands, ovary glands, bone marrow (sternum), and the thyroid and parathyroid glands. For the control and the 3.0 percent dosage level, tissues from these plus at least 11 other body organs are examined.

b/In all cases many tissues were usable; however, one or more of the required tissues were missing.

APPENDIX II