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Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, it is proposed that 21 CFR part 101 be amended as follows:

PART 101—FOOD LABELING

1. The authority citation for 21 CFR part 101 is revised to read as follows:

Authority: Secs. 4, 5, 6 of the Fair Packaging and Labeling Act (15 U.S.C. 1453, 1454, 1455); secs. 201, 301, 402, 403, 409, 501, 502, 505, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 331, 342, 343, 348, 351, 352, 355, 371).

2. Section 101.71 is amended by adding paragraph (c) to read as follows:

§ 101.71 Health claims: claims not authorized.

* * * * *

(c) Folic acid and neural tube defects (insert cite and date of publication in the Federal Register of the final rule).

Dated: November 4, 1991.

David A. Kessler,

Commissioner of Food and Drugs.

Louis W. Sullivan,

Secretary of Health and Human Services.

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21 CFR Part 101

[Docket No. 91N-0101]

RIN 0905-AB67

Food Labeling: Health Claims and Label Statements; Antioxidant Vitamins and Cancer

AGENCY: Food and Drug Administration, HHS.

ACTION: Proposed rule.

SUMMARY: The Food and Drug Administration (FDA) is proposing not to authorize the use on foods, including dietary supplements, of health claims relating to the association between antioxidant vitamins and cancer. FDA has reviewed the authoritative documents and scientific data in conformance with the requirements of the Nutrition Labeling and Education Act (the 1990 amendments) and concluded that there is not significant scientific agreement to support the use of health claims relating to antioxidant vitamins and cancer on labels and labeling. Although scientific evidence is suggestive of an effect of beta-carotene on cancer risk, studies available to date have been based on consumption of fruit and vegetables high in beta-carotene and not beta-carotene itself. Clinical trials are currently underway to clarify

List of Subjects in 21 CFR Part 101

Food labeling, Reporting and recordkeeping requirements.

this relationship. Although scientific evidence is suggestive of an effect of vitamin C on cancer risk, data on the relationship are not consistent and have mostly been obtained in studies of consumption of foods containing high levels of vitamin C. Such studies do not permit a conclusion on the specific role of the nutrient. Evidence for an effect of vitamin E on cancer risk are limited and inconclusive.

DATES: Written comments by February 25, 1992. The agency is proposing that any final rule that may issue based upon this proposal become effective 6 months following its publication in accordance with the provisions of the Nutrition and Labeling Act of 1990.

ADDRESSES: Written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1-23, 12420 Parklawn Dr., Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: John N. Hathcock, Center for Food Safety and Applied Nutrition (HFF-268), Food and Drug Administration, 200 C Street SW., Washington, DC 20204, 202-245-1193.

SUPPLEMENTARY INFORMATION:

I. Background

A. Nutrition Labeling and Education Act of 1990 (the 1990 Amendments)

On November 8, 1990, the President signed into law the 1990 amendments (Pub. L. 101-535), which amend the Federal Food, Drug, and Cosmetic Act (the act). The 1990 amendments, in part, authorize the Secretary of Health and Human Services (the Secretary) to issue regulations authorizing nutrient content and health claims on the label or labeling of foods. With respect to health claims, the new provisions provide that a product is misbranded if it bears a claim that characterizes the relationship of a nutrient to a disease or health-related condition, unless the claim is made in accordance with the procedures and standards established under the act (21 U.S.C. 343(r)(1)(B)).

Published elsewhere in this **Federal Register** is a proposed rulemaking to establish general requirements pertaining to the use on food labels and in labeling of health claims that characterize the relationship of nutrients, including vitamins and minerals, herbs or other nutritional substances (referred to generally as "substances") to a disease or health-related condition (proposed in "General Requirements for Health Claims"). In the proposal on general requirements for health claims, FDA following the provisions of the 1990 amendments has

tentatively determined that such claims would only be justified for substances in dietary supplements as well as conventional foods if the agency determines based on the totality of the publicly available scientific evidence (including evidence from well designed studies conducted in a manner which is consistent with generally recognized scientific procedures and principles) there is significant scientific agreement, among experts qualified by scientific training and experience to evaluate such claims, that the claim is supported by such evidence.

The 1990 amendments also require (section 3 (b)(1)(A)(ii), (b)(1)(A)(vi), and (b)(1)(A)(x)) that within 12 months of their enactment, the Secretary shall issue proposed regulations to implement section 403(r) of the act, and such regulations shall determine, among other things, whether claims respecting 10 topic areas, including antioxidant vitamins and cancer, meet the requirements of the act. In this document, the agency will consider whether a claim on food or food products, including conventional foods and dietary supplements, on the relationship between antioxidant vitamins and cancer, would be justified under the standard proposed in "Food Labeling: General Requirements for Health Claims for Food: Proposed Rule."

B. Antioxidant Vitamins and Cancer—Public Health Aspects

Section 3(b)(1)(A)(x) of the 1990 amendments requires that FDA determine whether claims for effects of dietary supplements, including the antioxidant vitamins in relation to cancer, meet the standard that FDA adopts under section 403(r)(5)(D) of the act for determining the validity of health claims. Because the standard that FDA is proposing under that section is the same as the standard that is established in section 403(r)(3)(B)(i) of the act for conventional foods, FDA is extending consideration of this topic area to all sources of antioxidant vitamins.

To comply with the provisions of the 1990 amendments, FDA requested, in the **Federal Register** of March 28, 1991 (56 FR 12932), scientific data and information on 10 specific topic areas, including the topic of antioxidant vitamins and cancer. The agency has considered data and information obtained from these submissions in its evaluation of the scientific basis for a health claim for antioxidant vitamins and cancer.

1. Cancer

Cancer is a disease of major public health importance in the United States.

All forms of cancer taken together are ranked as the second most common leading cause of death in the United States and account for one in five deaths (Ref. 1). Deaths from cancer numbered more than 475,000 in 1987. The overall economic cost of cancer, including the direct health care costs and losses from morbidity and mortality, was estimated to be \$7.25 billion (Ref. 2). The social impact of cancer can be measured in part by the potential years of life lost by death before age 65. Potential years of life lost were 18 million years for cancer compared to 15 million years for heart disease (Ref. 3).

Risk of occurrence differs markedly for various types of cancer. In 1990, the leading types of cancer in men in the United States were lung (35 percent of all cancer deaths), colorectal (11 percent), and prostate cancer (11 percent). For women, the leading types were lung (21 percent), breast (18 percent), and colorectal cancer (13 percent) (Ref. 3).

Factors that may influence the risk of cancer include not only diet but also life style, environment, and genetics. Antioxidant vitamins may have important, possibly protective, influences on the risk of certain cancers, but other dietary factors such as intakes of energy (calories), type and amount of fat, and other food components may also make important contributions to the relationship between diet and cancer (Ref. 4a).

2. Antioxidant Vitamins

The antioxidant substances that FDA has chosen for consideration are vitamin C, vitamin E, and beta-carotene. Vitamins C and E were chosen because they are vitamins that function as antioxidants (Ref. 4). FDA chose beta-carotene because it is an antioxidant, and because it is a provitamin and an important source of dietary vitamin A activity (Ref. 4). FDA did not choose vitamin A (retinol) and retinoic acid because their biological functions are not through an antioxidant role, and because vitamin A cannot function in a fashion similar to that of beta-carotene (carotenoids) and vitamins C and E (Ref. 5b).

a. Beta-carotene. Beta-carotene, a yellow-orange plant pigment, is only one of a large family of carotenes, some of which serve as vitamin A precursors and some of which do not (Refs. 6 and 7). Of all of the carotenoids, beta-carotene has the greatest provitamin A activity.

In addition to serving as a source of vitamin A activity, the antioxidant function of beta-carotene is important.

As a common constituent of the human diet, beta-carotene is a component of the body's primary defenses against the harmful substance known as free radicals and reactive oxygen molecules (Refs. 8, 9, and 11). Beta-carotene and similar carotenoids trap, deactivate, and destroy these molecules and prevent the damage they can cause. A way in which beta-carotene might possibly alter the risk of cancer is through its effect on the immune system. Beta-carotene and canthaxanthin have been shown to enhance the immune response in rats (Ref. 12). Beta-carotene has been demonstrated to produce changes in immune cellular marker expression *in vivo* in humans (Ref. 13) in which there was an increase in the percentage of cells expressing natural killer cell characteristics and activities, changes often related to increased resistance to cancer in experimental animals.

All of the carotenoid pigments similar in chemical structure to beta-carotene have antioxidant activity and the capacity for trapping and deactivating free radicals and reactive oxygen molecules. Of the carotenoids that predominate in human plasma (Ref. 131), i.e., lycopene, beta-cryptoxanthin, zeaxanthin, lutein, and alpha- and beta-carotene, all have this activity and capacity. Carotenoids are prevalent in yellow and greenish-yellow foods, such as carrots, squash, broccoli, sweet potatoes, tomatoes, and kale; these foods are some of the best sources of carotenoids. The fact that, collectively, carotenoids contribute significantly to the daily vitamin A intake and function as antioxidants makes these compounds important components of the daily diet.

b. *Vitamin C*. Vitamin C (*L*-ascorbic acid) is a watersoluble antioxidant vitamin that cannot be synthesized by humans. Dehydroascorbic acid, an oxidized form of ascorbic acid, may be present in the diet, and also has vitamin C activity. Dietary deficiency of vitamin C, which is extremely rare in the United States, leads to scurvy.

Vitamin C functions in a variety of enzymatic reactions that require molecular oxygen. For example, vitamin C is involved in enzyme reactions required for formation of proteins in connective tissue. Vitamin C also serves as an effective free-radical scavenger to protect cells from damage by oxidants. It is in this capacity that vitamin C may provide protection against adverse effects of potential carcinogens. Vitamin C plays roles in maintaining the integrity of intracellular matrices, enhances the immune system, and is necessary for several types of biochemical reactions (Ref. 6).

The richest food sources of vitamin C are green peppers, broccoli, citrus fruits, melons, tomatoes, raw cabbage, and green leafy vegetables such as spinach, turnip, and mustard and collard greens. The amount of vitamin C in the United States food supply has increased significantly since the beginning of the century; this has been the result, in part, of the greater availability of citrus fruits and dark green vegetables and to fortification of some foods with vitamin C. About 73 percent of the vitamin C intake in the United States is derived from fruits and vegetables (Nationwide Food Consumption Survey of 1977 and 1978).

The Recommended Dietary Allowance (RDA) for vitamin C for adults is 60 milligrams per day (Ref. 14). The major contributors of vitamin C to the United States diet are orange juice, grapefruit and grapefruit juice, fortified fruit drinks, oranges, tangerines, and potatoes (Ref. 4a).

Estimated average intakes of vitamin C are well above the RDA for men, women, and children (182, 133, and 186 percent, respectively, of the RDA's) (Ref. 4a). Between 35 and 40 percent of the adult U.S. population are estimated to take nutrient supplements, and approximately 90 percent of these take a supplement that includes vitamin C.

A 1988 National Health Interview Survey (Ref. 15) of vitamin and mineral supplement use in the United States found that men and women (about 31 percent of all adults) consumed vitamin C more than any other nutrient, and the median intake of vitamin C was 200 percent of the RDA due to this supplement intake. Current dietary data indicate that mean intake of vitamin C is well in excess of the RDA's for all population groups, and the additional supplement use provides a large segment of the population with intakes several fold the RDA levels (Ref. 2).

c. *Vitamin E*. Vitamin E has received attention as an antioxidant vitamin that may reduce the risk of cancer. The vitamin represents a family of compounds, tocopherols and tocotrienols. These compounds exist in various forms and have different biological activity. The most active form of vitamin E is alpha-tocopherol followed by beta-tocopherol, gamma-tocopherol, and alpha-tocotrienol. The basic biological function of vitamin E in animal tissues is as an antioxidant where it acts as a defense against potentially harmful reactions with oxygen (Ref. 2). Determination of the requirements for vitamin E is complicated by variations in susceptibility of dietary and tissue fatty

acids to peroxidation (reaction with an oxygen molecule to form a fatty acid peroxide), and difficulty in demonstrating changes in vitamin E status in the general healthy population (Ref. 16). The richest food sources of vitamin E are wheat germ, vegetable oils (corn, cottonseed, safflower, soybean and sunflower oils), and products made from vegetable oils. Other good sources are nuts, seeds, whole grains, and wheat germ.

The National Research Council (NRC) in the 1989 edition of "Recommended Dietary Allowances" (Ref. 14) stated that an adequate level of vitamin E implies that the ratio of tocopherol to polyunsaturated fatty acid in the tissues protects lipids from peroxidation, permits normal biological functions, and allows for individual variations of lipids in tissues. According to the NRC, these criteria of adequacy appear to be met by amounts of vitamin E and polyunsaturated fatty acids in balanced diets consumed by healthy individuals. Therefore, the RDA's are primarily based on customary intakes from the U.S. food supply.

The RDA's for vitamin E have been set by the NAS (Ref. 14). The RDA is 10 mg of alpha-tocopherol equivalents for males 11 years of age and older and 8 mg of alpha-tocopherol equivalents for females 11 years and older. The allowance of alpha-tocopherol equivalents for infants and children 1 to 10 years of age is 6 to 7 mg and the allowance for infants less than 1 year of age is 3 to 4 mg. One alpha-tocopherol equivalent is defined as 1 mg d-alpha-tocopherol. The adequacy of the RDA's for vitamin E will vary, if the polyunsaturated fatty acid content of the diet increases greatly over intakes in current balanced diets in the United States.

d. *Interactions among antioxidant vitamins*. The antioxidant vitamins are interactive in that they complement each other during situations of biological stress. Vitamin C, most of which is located in the aqueous portion of the cell, spares vitamin E until the vitamin C reserve is depleted (Ref. 17). Vitamin E is located in the lipid portions of all membranes, and it deactivates free radicals. Sparing action has also been observed between beta-carotene, which is also found in the lipid portions of the cell, and vitamin E in that beta-carotene exhibits deactivation of free radicals similar to that of vitamin E. Beta-carotene, vitamin C, and vitamin E all inhibit damage by oxidative chemicals, including carcinogens (Ref. 7). More specifically, beta-carotene traps reactive oxygen molecules, vitamin E and beta-

carotene remove free radicals, and vitamin C inhibits oxidative reactions and also removes free radicals.

The antioxidant vitamins may also interact negatively in that high doses of one can counteract the uptake, transport and storage of another. For example, high intakes of beta-carotene administered to humans for 6 months or more can deplete plasma levels of vitamin E (Ref. 18b). In another report (Ref. 26), high intakes of beta-carotene for over 4 years did not alter vitamin E levels in humans. However, high intakes of vitamin A in rats were shown to deplete plasma levels of vitamin E (Ref. 19).

Because of the complementary and antagonistic interactions among the antioxidant vitamins, high intakes of one without commensurate increases in the others may not support optimum status and functions for these nutrients (Refs. 4a and 21). Foods may, therefore, provide a better balance of these nutrients than do supplements, and foods supply other substances such as fiber, minerals, and many nonnutrients that may be important in reducing the risk of some cancers (Refs. 4a and 17).

3. Basis for Associations Between Antioxidant Vitamins and Cancer

a. *Mechanisms of carcinogenesis and relationship to antioxidants.* The complex process of carcinogenesis is often described as occurring in distinct phases (Refs. 4 and 23) including: (a) Initiation, (b) promotion, (c) progression, and (d) metastasis. Initiation of the carcinogenic process results from chemical or physical damage to cellular genetic material (deoxyribonucleic acid (DNA)). Cell replication then results in fixation of the damage as a mutation. Promotion involves stimulation of the growth of initiated cells. Progression is the malignant transformation of the initiated cell mass into an invasive form that is not subject to the normal limits or controls of cellular replication. Metastasis is the spread or dissemination of cells from the original tumor to new sites in the body.

Most dietary carcinogens occur as procarcinogens, i.e., as substances that require activation to a reactive form that is termed the proximate carcinogen (Refs. 22 and 23). The ultimate carcinogen, an extremely reactive and unstable species, is produced by rearrangement of the proximate carcinogen. The ultimate carcinogen is the form that is directly involved in the reaction with nucleic acids or other substances with similar chemical reactivity (nucleophiles).

This general pattern of carcinogen activation, chemical reaction of the

carcinogen molecule with nucleic acids, mutagenesis, and carcinogenesis is firmly established through experiments with animals and with cultures of human and animal cells (Refs. 22 and 24).

A major effect of vitamin C that could be the basis of protection against cancer is its ability to inhibit nitrosamine formation (Ref. 25). Nitrosamines (N-nitroso amines and N-nitroso amides) are types of carcinogens which occur in foods and are produced within the body by reaction of nitrite with other dietary or endogenous amines and amides (Refs. 25 and 26). Some nitrite occurs in food, but more is produced from reduction of nitrate by bacteria in the mouth and small intestine (Ref. 26). Nitrate occurs in food (Ref. 26), and some is produced in the body from L-arginine (Ref. 27). The nitrosation reactions occur rapidly in the acid environment of the stomach and upper duodenum (Ref. 25). Most nitrosamines tested in experimental animals are carcinogenic, and some are very potent carcinogens affecting multiple sites (Ref. 26).

b. *Associations between antioxidant vitamins and risk of cancer Beta-carotene.* Epidemiological studies in the 1970's and early 1980's showed an inverse association between vitamin A intake and lung cancer and cancers at various sites (Ref. 28). A reexamination of the early vitamin A intake data in relation to cancer, however, revealed that the sources of the vitamin A were fruits and vegetables which contain beta-carotene and other carotenoids. As stated above, beta-carotene is a precursor of vitamin A and is not vitamin A itself. Therefore, the focus of research on the relationship between vitamin A and cancer shifted to the carotene content of the foods consumed.

This hypothesis of the relationship between ingestion of beta-carotene and decreased cancer risk has persisted. Results of animal studies indicate that beta-carotene is effective in preventing cancers induced by certain chemical carcinogens. This information however, is helpful only with regard to discerning mechanisms involved in cancer development, because the type and amount of carcinogen used in the animal experiment are not typical for humans. The strongest evidence to support the hypothesis that beta-carotene decreases the risk of cancer is from epidemiological studies involving fruit and vegetable consumption, and not from studies of beta-carotene itself in humans. When completed, clinical trials currently underway should provide more direct evidence on whether beta-carotene alters the risk of cancer in humans.

c. *Vitamin C.* A hypothesized relationship between ingestion of vitamin C and risk of cancer has developed from several types of studies. For example, human studies have shown a protective association between consumption of foods that contain vitamin C and cancers of several sites (Ref. 29). Studies in experimental animals have shown that animals fed both vitamin C and precursors of carcinogenic nitrosamines develop fewer tumors than animals fed the precursors alone (Ref. 30). Vitamin C has been shown to reduce bladder tumors in animals induced by one carcinogen (Ref. 31), but not by another (Ref. 32). Biochemical studies have shown that vitamin C prevents the oxidation of specific chemicals to active carcinogenic forms (Ref. 31), and vitamin C blocks the formation of carcinogenic nitrosamines from nitrates and nitrites in the digestive tract (Refs. 30 and 33b). The combination of evidence from epidemiological studies and evidence from several types of studies with animals which involved administration of carcinogens and carcinogen precursors provides a strong basis on which to postulate that vitamin C reduces the risk of cancer in humans.

d. *Vitamin E.* The hypothesis that vitamin E may reduce the risk of some types of cancer is based on two kinds of scientific findings. Animal studies have demonstrated an inhibitory effect of vitamin E on cancers induced by ultraviolet light and certain chemicals. These studies date back to the earliest days of vitamin E chemistry (Ref. 34). More recently, the implications of reactive oxygen molecules in cancer development provide a theoretical basis for the involvement of vitamin E (a strong antioxidant) in the development of cancer, because carcinogens are activated by oxidative processes and oxidation of cell components may contribute to cancer development (Refs. 4a and 22).

C. Regulatory History of Antioxidant Vitamins

1. Nutrition Labeling

FDA published a proposal to amend the food labeling regulations to codify and clarify the agency's policy on the appropriate use of health claims on food labeling in the **Federal Register** of August 4, 1987 (52 FR 28843). On August 8, 1989, FDA published in the **Federal Register** (54 FR 32610) an advance notice of proposed rulemaking requesting public comments on (among other areas) how to reasonably permit the use of claims on food labels that link food

components to reducing the risk of chronic disease. In the **Federal Register** of February 13, 1990 (55 FR 5176), FDA withdrew the August 4, 1987 proposal (52 FR 28843) and repropoed to amend the food labeling regulations to provide for the use of health claims on food labeling. The purpose of the reproposal was, in part, to establish procedures for permitting valid and reliable consumer information on food labels about the value that ingestion of dietary components may have in either lowering the risk or forestalling the premature onset of particular chronic disease conditions. The topic of antioxidant vitamins and cancer was not specifically mentioned in any of these documents.

Comments from a consumer advocacy group and several industries suggested that topics other than the six specified in the repropoed rule making were worthy of consideration and stated that the role of antioxidant vitamins and beta-carotene in preventing diverse diseases, especially cancer, should be considered. One comment suggested that health claims might be appropriate for the relationship between cancer and fruits and vegetables and the antioxidant vitamins. Another comment argued that a health claim for beta-carotene rich foods would be consistent with the dietary recommendations of organizations such as the National Cancer Institute. Several comments asserted that a substantial body of evidence exists showing an inverse association between foods high in beta-carotene and a reduced risk of certain forms of cancer.

A trade association for the dietary supplement industry commented that, in its opinion, the evidence is "overwhelming" that consumption of fruits and vegetables high in antioxidant vitamins reduces the risk of certain forms of cancer, and that the scientific evidence suggests that vitamins C and E and beta-carotene are responsible for this effect. A pharmaceutical company commented that it felt antioxidant vitamins, particularly vitamin C, are effective in vivo antioxidants and are involved with other diseases such as heart disease, cataracts, rheumatoid arthritis, and Parkinson's disease. A major food manufacturer commented on the relationship of beta-carotene and cancer, citing currently completed epidemiological evidence and ongoing clinical trials.

No comments were received from consumers or from professional organizations of physicians or other health care workers.

These comments were superseded to by section 3(b)(1)(A)(x) of the 1990 amendments, which directed FDA to

consider the relationship between dietary supplements of antioxidant vitamins and cancer, among other topics. These topics are considered in this and other documents published in this issue of the **Federal Register**.

2. Regulatory Status

Substances that are added to food may be categorized as generally recognized as safe (GRAS) ingredients, food additives, or subject to a sanction or approval granted by FDA or the U.S. Department of Agriculture (USDA) prior to September 6, 1958. Substances may be GRAS in accord with the general principles stated in 21 CFR 170.30, listed as GRAS in 21 CFR part 182, or affirmed as GRAS in 21 CFR part 184. The status of the most common uses in foods of carotenoids, vitamin C compounds, and tocopherol compounds is summarized below.

The following carotenoid compounds and sources rich in these carotenoid compounds are approved color additives for food use exempt from certification: beta-carotene (21 CFR 73.95), betaapo-8'-carotenal (21 CFR 73.90), and carrot oil (21 CFR 73.300). Beta-carotene is affirmed as GRAS for use as a nutrient supplement (21 CFR 184.1245) and listed for use in dietary supplements § 182.5245. Carrot oil, essential oil or extractive, is listed for use as a flavoring substance § 182.20.

Ascorbic acid (vitamin C) is listed as a GRAS ingredient for use as a chemical preservative (antioxidant) (21 CFR 182.3013), in dietary supplements (21 CFR 182.5013), and as a nutrient (21 CFR 182.8013). Ascorbyl palmitate, ascorbyl stearate, calcium ascorbate, and sodium ascorbate are listed as chemical preservatives (21 CFR 182.3149, 166.110, 182.3189, and 182.3731, respectively). Ferrous ascorbate is affirmed as GRAS for use as a nutrient supplement (21 CFR 184.1307a), although, at usual levels of intake, ferrous ascorbate contributes little vitamin C activity. A nicotinamide-ascorbic acid complex is listed as a special dietary and nutritional food additive in 21 CFR § 172.315.

Tocopherols are listed as GRAS for use as dietary supplements (21 CFR 182.5890), as nutrients (21 CFR 182.8890), and as chemical preservatives (antioxidants, in 21 CFR 182.3890, and inhibitors of nitrosamine formation (21 CFR 184.1890)). Alpha-tocopherol acetate is listed as GRAS for use in dietary supplements (21 CFR 182.5892) and as a nutrient (21 CFR 182.8892).

D. Evidence Considered in Reaching the Decision

The agency reviewed all relevant scientific evidence on associations

between ingestion of antioxidant vitamins (vitamins C and E) and beta-carotene and reduced risk of cancer. The scientific evidence and conclusions that the agency reviewed included those in the "Surgeon General's Report on Nutrition and Health" (Surgeon General's Report) (Ref. 4a), "Nutrition and Your Health: Dietary Guidelines for Americans" (Ref. 35), and the U.S. Department of Health and Human Services (DHHS) "Healthy People 2000: National Health Promotion and Disease Prevention Objectives" (Ref. 3). The agency also reviewed other authoritative documents, NRC's "Diet and Health" (Ref. 2), and "Recommended Dietary Allowances" (Ref. 14), and the Life Sciences Research Office's "Vitamin A and Cancer" (Ref. 36), "Vitamin C and Cancer" (Ref. 37) and "Vitamin E and Cancer" (Ref. 38).

The agency updated the evidence reached in these documents by reviewing all human studies in the literature subsequent to these documents and all new review articles. The updated literature search began with 1987. The agency also considered the results of animal studies to the extent that they clarified human studies or suggested possible mechanisms of action.

To assure that its review of relevant evidence was complete, FDA requested, in the **Federal Register** of March 28, 1991 (56 FR 12932), scientific data and information on the 10 specific topic areas identified in section 3(b)(1)(A)(vi) and (b)(1)(A)(x) of the 1990 amendments. The topic of antioxidant vitamins and cancer was among the 10 subjects on which the agency requested scientific data and information. The agency reviewed all of the documents it received, including letters, press releases, scientific articles, review articles, and recommendations. Where appropriate, data submitted in scientific articles were considered in the review of the scientific literature (section II.)

E. Comments Received in Response to FDA's Request for Scientific Data and Information

FDA received 17 comments in response to the March 28, 1991 **Federal Register** notice (56 FR 12932) concerning antioxidant vitamins and cancer health claims. Of the comments received, nine were from industry, three from professional organizations, two from trade associations, one from an individual consumer, one from a state government, and one from the Government of Canada.

Over 435 references were received, including recent review articles on

antioxidant vitamins and cancer. Some of the responses included references only, while others contained references and comments, or just comments. The comments generally cited data or opinions from specific references. The majority of the comments supported a health claim relative to the antioxidant vitamins and cancer. In general, comments from manufacturers and trade associations, as well as from a few research organizations, argued that the evidence for beneficial effects of antioxidant vitamins in relation to cancer is strong, or even compelling, that these substances are safe, and therefore, the only reasonable course of action is to allow health claims in this area.

The regulatory status of health claims for antioxidant vitamins and cancer in Canada was described in comments from the Director General, Food Directorate, Health and Welfare, Canada. The official position of Canada on the relationship of diet and nutrients to disease and the metabolic effects of nutrients is stated in the volume "Nutrition Recommendations, the Report of the Scientific Review Committee—1990." In sections related to Water-Soluble Vitamins and Fat-Soluble Vitamins, this report concluded that, for vitamin C and the fat-soluble vitamins, evidence for protection against cancer lacks specificity and is inconclusive. The Government of Canada pointed out that health claims would be illegal under Canadian law and thus advised against such claims.

All of the nine responses received from industry included literature references on antioxidant vitamins and cancer. One chemical company provided data to demonstrate the efficacy and safety of antioxidant vitamins, especially vitamins C and E, and beta-carotene. A food manufacturer suggested that FDA also consider the interactions between antioxidant vitamins and selenium. The comment also suggested that antioxidants may be depleted by oxidative stress, thereby enhancing risk of a variety of diseases including heart disease, breast and cervical cancer. A diet systems company provided a tabular summary concluding that antioxidant vitamins (but not any one specifically) are protective against cancers of the breast and colon. Another manufacturer argued that animal research and human epidemiologic and blood level studies support a protective effect for vitamin E, vitamin C, and beta-carotene, but that continued research is warranted.

A pharmaceutical manufacturer commented that: (1) Free radical

damage is widely accepted as a major theory of carcinogenesis and that the antioxidant vitamins are effective inhibitors of this process; (2) many persons are not consuming RDA levels of the vitamins; (3) epidemiologic evidence is supportive of beneficial effects by the antioxidant vitamins, and the National Cancer Institute and the USDA have recommended increased intakes of certain foods on this basis; (4) there is little or no evidence that consumers will replace conventional medical care with dietary "treatments" for cancer or other disease; and (5) supplementation is beneficial and essentially risk-free. A pharmaceutical and a supplement manufacturer argued that they recommend a benefit/risk approach instead of consideration of only what has been established as an "incontrovertible scientific fact." These two pharmaceutical manufacturers also expressed extreme concern and dissatisfaction with the rulemaking process.

FDA notes in response to these comments that the "benefit/risk" or "essentially risk-free" rationale does not qualify as a basis for a health claim. The 1990 amendments provide that a health claim is justified only if the agency determines, based on the totality of publicly available scientific evidence relating the nutrient and the disease, that there is significant agreement among qualified experts that the claim is supported by such evidence. Therefore, the agency rejects the concepts of "benefit/risk" or "essentially risk-free" as basis for a health claim because of their inconsistency with requirements of the 1990 amendments.

A private research foundation commented that evidence supports the conclusion that increased intakes of micronutrients including the antioxidant vitamins lowers the risk of cancer, especially in the stomach and esophagus. The comments stated, however, that the emphasis should be on avoiding intake of carcinogens, rather than on consumption of anticarcinogens. On the other hand, another professional organization argued that there should be no doubt concerning the antioxidant efficacy of certain vitamins.

A trade association commented that the NRC's book "Diet and Health" (Committee on Diet and Health, 1989) virtually ignored this important topic of antioxidant vitamins and cancer, even though data and conclusions of important benefits have been published by respectable scientists. A trade association for the dietary supplement industry submitted abstracts on

selenium and cancer. It also cited evidence that vitamin C is effective, both in animals and in humans, against a wide variety of cancer types/sites, through a variety of mechanisms, and with exposure of animals to a variety of carcinogens.

A state government agency provided only general comments on the nutrient health relationships it believes must be established to justify any health claim. This agency recommended that scientific agreement should be considered the cornerstone of any health claim. This state government agency and a professional organization, however, urged that FDA be extremely cautious in making its decision.

The agency has considered each of these comments in its development of this proposal.

F. Criteria for Evaluation of Scientific Evidence

FDA has evaluated the data from human studies against general criteria for good experimental design, execution, and analysis. The criteria used in evaluating epidemiological studies included:

- (1) Reliability and accuracy of the methods used in food intake analysis and measurement of disease endpoints;
- (2) Choice of control subjects (e.g., hospital-based versus population-based);
- (3) Representativeness of subjects;
- (4) Control of confounding factors, particularly fat, which has an inverse correlation with, and fiber intake which has a positive correlation with, fruit and vegetable intake, in data analysis;
- (5) Potential for misclassification of individuals with regard to dietary exposure or disease end points;
- (6) Presence of recall bias and interviewer bias; and
- (7) Degree of compliance and how compliance was assessed.

FDA evaluated the weaknesses and strengths of individual studies (see "Assessment" column of tables following each antioxidant vitamin). The agency then assessed the strength of the overall combined evidence (e.g., epidemiologic studies and animal studies), taking into account the strength of the association, the consistency of findings, specificity of the association, evidence for a biological mechanism, and presence or absence of a dose-response relationship. FDA's conclusions reflect the strength, consistency, and preponderance of data.

II. Review of Scientific Evidence: Beta-Carotene

A. Federal Government Documents and Statements

In 1988, "The Surgeon General's Report on Nutrition and Health" (Ref. 4a) considered the role of carotenoids in cancer. The report concluded that some epidemiologic studies provide suggestive evidence that frequent consumption of vegetables and fruits, particularly dark green and deep yellow vegetables which contain carotenoids, including the beta-carotene precursor of vitamin A, may lower risk for cancers of the lung and bladder as well as some cancers of the alimentary tract. The report pointed out, however, that these studies have not ruled out the possibility of protection from some other active component of fruits and vegetables. Until the results of chemoprevention clinical intervention trials examining these relationships become available, the report stated that it could conclude only that an increase in consumption of fruits and vegetables might benefit persons who now consume below average amounts of these foods. No risks from consumption of beta carotene or carotenoids were identified.

USDA and DHHS in "Nutrition and Your Health: Dietary Guidelines for Americans" (Ref. 35) recommended the consumption of generous portions of vegetables and fruit, but did not make a specific reference to the role of increased consumption of these foods and the lowered risk of cancer.

B. Other Authoritative Documents and Statements

The NAS 1989 report on "Diet and Health" (Ref. 2) found that there is strong evidence that a low intake of carotenoids, which are present in green and yellow vegetables, is related to an increased risk of developing lung cancer. The report stated that the mechanism for the link between frequent consumption of vegetables and fruits, especially green and yellow vegetables and citrus fruits, and decreased susceptibility to cancers of the lung, stomach, and large intestine is not well understood because the responsible agents in these foods and the mechanisms for their protective effect have not been fully determined.

The 10th edition of the "Recommended Dietary Allowances" (Ref. 14) contains a discussion of carotenoids with regard to the recommended allowances for vitamin A. The report stated that most carotenoids, unlike vitamin A, trap free radicals and remove reactive oxygen molecules which can cause changes in cells. The report further stated that because only

about 10 percent of carotenoids in nature show provitamin A activity, any anticancer effects that carotenoids possess might be related more to their antioxidant or other properties than to their conversion into vitamin A. The RDA report suggested that, a generous intake of carotenoid-rich foods may be of benefit.

The Life Sciences Research Office (Ref. 36) reviewed the recent scientific literature on "Vitamin A (including beta-carotene) and Cancer" and concluded that: (1) The data relating vitamin A to cancer are inconclusive, (2) the strongest evidence supports a possible protective role of fruits and vegetables in reducing the rates of cancer of various sites, particularly cancers of the lungs, colon/rectum and breast, and (3) the beta-carotene content of these foods may, in part, be exerting some of the effects.

C. Review of the Scientific Literature

The agency reviewed available scientific evidence on beta-carotene and carotenoids and cancer provided by epidemiological studies (prospective and retrospective) and clinical studies published since the publication of "The Surgeon General's Report on Nutrition and Health" (Ref. 4a). The data that FDA reviewed were evaluated according to specific criteria. Studies that involved healthy matched controls (e.g., age, sex, and ethnic origin) that were controlled for confounders, such as smoking, and that used validated dietary assessment instruments, appropriate storage conditions for test samples, and adequate sample size were given the greatest weight by FDA. When the possibility could not be excluded that the observed association was mainly a result of the disease altering indications of beta-carotene, or resulted from an effect of another substance in the foodstuff influencing the disease, the study was given less weight.

1. Primary Human Studies

Epidemiological studies of a retrospective nature that have assessed consumption of fruits and vegetables in relation to cancer mortality are shown in Table 1. Most of these case-control retrospective studies show an inverse association between intakes of fruits and vegetables rich in carotenoids and the risk of lung cancer. However, some studies also show that intakes of these foods are associated with a lowered risk of colorectal and stomach cancers.

LeMarchand and coworkers (Ref. 39) reported that higher intakes of foods containing beta-carotene are associated with lower risk of lung cancer in a multi-ethnic population of men and women in Hawaii. These researchers also reported

stronger associations of lower lung cancer risks with consumption of all vegetables, dark green and cruciferous vegetables, and tomatoes than with consumption of beta-carotene.

Bond and coworkers (Ref. 40) reported similar findings of a lower lung cancer risk with higher intakes of foods with a high carotenoid index in a population of chemical company employees in Texas. Recently, upon reevaluation of data relating dietary intake to prostate cancer, these researchers (Ref. 41) reported that beta-carotene intake is not associated with prostate cancer risk. Prostate cancer risk, however, was reported to be inversely related to intake of foods containing beta-carotene in men over 70 years of age in two studies conducted in Japan (Refs. 42 and 43).

Adjusted risk of ovarian cancer was reduced with high intakes of beta-carotene in the study of Slattery et al. (Ref. 44). In a study conducted in England, Coggon et al. (Ref. 45) reported that intakes of fruits and salad vegetables were inversely associated with risk of stomach cancer. Kune et al. (Ref. 46) and LaVecchia et al. (Ref. 47) reported that risks of colorectal cancer were inversely associated with higher intakes of vegetables containing beta-carotene or those with a higher carotenoid index and vitamin C. In both of these studies, however, as with other studies showing a significant inverse relationship of higher intakes of fruits and vegetables and cancer risks, complementary effects of the antioxidants or effects of other components of the foods cannot be ruled out as the cause of the observed association.

In a unique type of case-control retrospective study, Smith and Waller (Ref. 50) included immediate family members of cancer patients and immediate family members of control hospitalized subjects in assessing the association between serum beta-carotene and cancer. Lower serum levels of beta-carotene were observed in patients with cancer of the lung, stomach, esophagus, small intestine, cervix and uterus. Lower serum levels of beta-carotene were also found in relatives of the cancer patients compared to serum levels in control subjects and their relatives. The inclusion of immediate family members as part of the study design corrects for effects from the illness. Additionally, the matching of subjects as to smoking status, age, sex, length of sample storage as well as the use of relatives, make the findings in this study consistent with those of prospective studies.

Prospective studies shown in Table 2 assessed dietary intake of foods rich in carotenoids and the subsequent development of cancer. There was an inverse association between intake of carotenoid-rich fruits and vegetables and risks of breast, lung, and other epithelial cancers in three studies (Refs. 51 through 53). However, Paganini-Hill et al. (Ref. 54) reported no association between intake of beta-carotene-rich foods and cancer at various sites.

In prospective studies (Table 3) in which serum or plasma carotenoid levels were measured in subjects who later developed cancer, Wald et al. (Refs. 55 through 57) and Connett et al. (Ref. 58) reported an inverse association between blood beta-carotene levels and lung and stomach cancers. In a 12-year followup prospective study, overall cancer mortality was inversely associated with plasma carotene adjusted for cholesterol levels, and bronchus and stomach cancers were inversely associated with total plasma carotene (Refs. 59 and 60).

Premalignancy studies, or those in which neoplastic changes in a tissue do not affect appetite and food intake or carotenoid metabolism in blood, offer the advantage of early diagnosis and assessment of dietary associations. An example of premalignancy studies are case-control studies of cervical dysplasia and in situ carcinogenesis in which the stage of cancer development is diagnosed by microscopic examination of cells. Beta-carotene exposure was assessed as intake of deep green and yellow vegetables, dietary beta-carotene, or plasma beta-carotene levels. Beta-carotene exposure from either of the above dietary sources was inversely correlated with the incidence of premalignancy in all but one of the studies reported (Table 4) (Refs. 62 through 66). In a small group of cases and controls, De Vet et al. (Ref. 67) found no association between beta-carotene supplements, consumed at 10 mg per day for 1 month, and changes in cervical dysplasia. The authors suggested that the supplementation period may have been too short to provide conclusive results.

A number of chemoprevention intervention trials are underway that are aimed at preventing or reducing the recurrence of malignancies (Table 5). These trials will be ongoing for some time because of the long latency period for cancer. Subjects are given beta-carotene in capsule or tablet form as a supplement to the beta-carotene and other carotenoids consumed through their diets. The information gathered from these clinical intervention trials

should provide important information regarding the efficacy of the carotenoids either alone or in combination with other antioxidants in altering the cancer incidence.

One intervention trial of relatively short duration examined the effects of beta-carotene with and without vitamin A supplementation on oral leukoplakia (a condition of white, rough, sometimes fissured patches on the mucous membranes of the oral cavity that cannot be rubbed off, and which occur most often in smokers) in persons who frequently chewed betel (Ref. 68). After 3 months there was a reduction in symptoms in both beta-carotene groups, and after 6 months the investigators observed a significant regression of this condition.

A trial of longer duration, which examined the role of beta-carotene in preventing new occurrences of nonmelanoma basal cell and squamous cell carcinomas of the skin, has been completed (Ref. 20). Five hundred eighty-two subjects were supplemented with 50 mg beta-carotene per day. Median plasma concentrations after 1 year of supplementation increased 10-fold, with the largest increase occurring in nonsmokers, women and leaner subjects (Ref. 69). No effects on new nonmelanoma basal cell and squamous cell carcinomas of the skin from the supplementation of beta-carotene were observed. It has been hypothesized (Ref. 71) that one reason for the lack of effect observed may be that the amount of beta-carotene administered in the trial (50 mg per day) may be less than that suggested by other studies (e.g., 30 to 300 mg per day) to potentially reduce the risk of the recurrence of this type of cancer. A number of other explanations for the lack of effect are also possible. Other clinical trials employ beta-carotene alone or in combination with vitamins A, C, or E (for summary, see Table 5).

2. Supporting Data From Animal Studies

Experimental cancer in animals occurs by administering a chemical compound, exposure to irradiation or ultraviolet light, or a combination of the two. The efficacy of beta-carotene or other carotenoids reducing the incidence of tumors has been demonstrated at various sites in different animal models. These tumor sites include salivary gland, skin, mammary tissue, colon, and stomach (Ref. 72a). Moreover, feeding a naturally occurring source of beta-carotene, algae *Dunaliella bardawil*, resulted inarked inhibition of spontaneous mammary tumors (Ref. 73).

3. Safety Issues

No risks from consumption of beta-carotene intake per se or carotenoids were identified in any of the above reports. Carotenoids, even when ingested in very large amounts for weeks to years, are not known to be toxic (Ref. 135). An important reason for their lack of toxicity is their relatively limited conversion to vitamin A in the intestine, liver, and other organs. Carotenoids taken in large doses for several weeks are absorbed well enough to color the adipose tissue stores, including the subcutaneous fat. Thus, the skin, especially the palms of the hands and the soles of the feet, appears yellow.

D. Conclusions

Evidence relating beta-carotene intake per se to reduced risk of cancer in humans is, at this time, inconclusive. There is strong evidence that high intakes of fruits and vegetables rich in carotenoids are associated with a reduced risk of developing cancer. However, whether the components of fruits and vegetables responsible for reducing the apparent effect are beta-carotene and other carotenoids or some other compound remains unknown. The positive effects of beta-carotene administration in lowering the frequency and severity of experimental cancer in animals suggest that effects are the result of antioxidant properties. The conclusions cannot be directly applied to humans partly because the type and amount of carcinogen exposure in the experimental conditions was not similar to human exposure.

The most promising prospects of clarifying the possible role for carotenoids in human cancer risk rests with the clinical intervention trials that are currently in progress. In these trials, the administration of purified beta-carotene supplements to individuals at increased risk for developing cancer can provide more information regarding the direct role of beta-carotene. However, based on all of the scientific evidence published since 1987 on the role of carotenoids in human cancer risk, the agency finds only that the conclusion of the Surgeon General's Report (Ref. 4a) remains valid: the Surgeon General's report concluded that some epidemiologic studies provide suggestive evidence that frequent consumption of vegetables and fruits, particularly dark green and deep yellow vegetables which contain carotenoids, including beta-carotene, may lower risk for cancers of the lung and bladder, as well as some cancers of the alimentary tract.

Moreover, the recommendation in the "U.S. Dietary Guidelines" to consume generous portions of fruits and vegetables remains appropriate advice.

E. Tentative Decision To Deny a Health Claim Relating to Ingestion of Carotenoids to Risk of Cancer

Evidence of a direct relationship between beta-carotene intake and lowered risk of developing cancer at various sites in humans is inconclusive. The primary source of beta-carotene in the U.S. diet is green and yellow fruits and vegetables. From epidemiological data, there is strong evidence that consumption of fruits and vegetables has an associative relationship to lowered risk of cancer at various sites in humans. However, whether the protective effects of fruits and vegetables against cancer risk is the result of beta-carotene content or some other component remains unknown. Experimentally induced cancer at various sites in animals has been shown to be delayed and decreased in frequency with beta-carotene administration. This effect in animals is through the antioxidant potential of the beta-carotene. Clinical intervention trials for cancer that are in progress may, in a few years, provide additional information of effect of beta-carotene per se, rather than foods containing this substance, on cancer.

FDA has tentatively determined, based on all of the publicly available scientific evidence regarding an association between beta-carotene and cancer, that there is not significant scientific agreement, among experts qualified by scientific training and expertise to evaluate such claims, that a claim associating beta-carotene to the risk of cancer is supported by such evidence.

The agency recognizes that the evidence is strong that consumption of fruits and vegetables, good sources of beta-carotene, is associated with lowered risk of cancer at a number of sites. However, the agency believes that the data are not sufficiently convincing that beta-carotene per se is responsible for this association. Further, even if the evidence for a cause-and-effect relationship of beta-carotene intake with lowered risk of cancer was assumed to be adequate, the agency finds the data to be insufficient to determine the quantity of beta-carotene needed to produce the effect. If such data were available, it would be necessary for the agency to determine whether the food supply already provides enough beta-carotene to produce that effect.

In summary, the agency requests submission of data which directly bear on: (1) Whether beta-carotene per se, rather than some other component of food, decreases the risk of cancer in humans, and (2) the range of beta-carotene intake which produces this effect.

III. Review of the Scientific Evidence: Vitamin C

A. Federal Government Documents

"The Surgeon General's Report on Nutrition and Health" (Ref. 4a) reviewed human studies relating to associations between vitamin C and cancers of specific sites. These included studies of foods that contain vitamin C and cancers of the esophagus, stomach, and cervix. The report also reviewed: (1) Studies that showed that colonic polyps regressed or decreased in area with vitamin C therapy; and (2) studies that reported variable effects (positive and negative) of supplements of vitamin C and vitamin E on formation of fecal mutagens (Ref. 4a). The report also noted that most studies demonstrating beneficial effects of vitamin C did not quantify its actual intake (Ref. 4a). The report concluded that no wholly consistent picture of the role of vitamin C in human cancer has been defined.

The Surgeon General's report also observed that, despite limitations in the data, the American Cancer Society guidelines recommend consuming foods rich in vitamins A and C, and that the National Cancer Institute suggests eating a variety of fruits and vegetables to ensure an adequate supply of vitamin C (Ref. 4a). There is no adequate evidence that larger intakes of vitamin C provide any additional benefits (Ref. 4a).

The 1990 "Nutrition and Your Health: Dietary Guidelines for Americans" (Ref. 35) recommends consumption of diets with "plenty" of vegetables and fruits. Such diets contain generous levels of the antioxidant vitamins, which were recommended because fruits, vegetables and whole grain cereals are likely to reduce the fat content of the diet, a change associated with decreased risk of cancer. Any possible direct association of antioxidant vitamins with lowered risk of cancer was not discussed.

The DHHS "Healthy People 2000" (Ref. 3) recommends increased consumption of complex carbohydrates and fiber-containing foods to 5 of more daily servings of vegetables (including legumes) and fruits and 6 or more daily servings of grain products. Vegetables, fruits, and grains are good sources of complex carbohydrates and fiber as

well as of several vitamins and minerals. The report noted that dietary patterns with higher intakes of vegetables, fruits, and grains are associated with a variety of health benefits including a decreased risk for some types of cancer (Refs. 2 and 4a).

B. Other Authoritative Documents

The NAS in its 1989 Report "Diet and Health" (Ref. 2) reviewed and summarized the role of dietary factors as related to risk of various types of cancer. The NAS reviewed epidemiologic data and evidence from animal studies and studies on mechanisms of carcinogenesis.

The report reviewed associations among dietary factors and stomach cancer. It described major dietary associations between gastric cancer and consumption of dried, salted, or smoked fish or pickled vegetables (foods high in salt and nitrates). It also indicated that a second major dietary association observed with stomach cancer is the protective effect of fresh fruits, vegetables, and vitamins, particularly vitamin C. The report noted in summary that stomach cancer is associated with diets containing large amounts of salt-preserved foods and low levels of fresh fruits and vegetables. The report found, however, that evidence was inconclusive concerning the significant decline in stomach cancer mortality in the United States over the last half-century relating to dietary shifts away from consumption of high salt-preserved foods and toward increased consumption of fruits and vegetables (Ref. 2).

In summary, the report stated that although the contribution of diet to total incidence and mortality from cancer in the United States cannot be determined with certainty, it is reasonable that approximately one-third of all cancer mortality may be related to diet. Data on the carcinogenicity of most components of human diets are quite limited, however, and the exact mechanism of carcinogenesis in humans have not yet been established for any diet-related cancer (Ref. 2).

The NAS in its "Recommended Dietary Allowances" (Ref. 14) noted that vitamin C may prevent the formation of carcinogenic nitrosamines by reducing nitrites. The NAS concluded that ingestion of fruits and vegetables rich in vitamin C has been associated with reduced incidence of some cancers, but that there is no evidence that vitamin C is responsible for such effects.

The Life Sciences Research Office of the Federation of American Societies for Experimental Biology, reviewed the

relationship between ingestion of vitamin C or foods rich in vitamin C and cancer (Ref. 37). The report concluded that epidemiologic studies have provided evidence for a role of nutrients in the reduction of the risk of specific cancers. The report found that one of the most consistent epidemiologic findings has been an association between high intakes of vitamin C-rich foods and a reduction in risk of stomach cancer (Ref. 29). The report noted that intake of citrus fruits has been associated with a significantly reduced risk of oral cancer, and that risk of esophageal cancer was reduced with increased intakes of vitamin C-rich fruits and juices. Intake of vitamin C-rich foods, the report concluded, appears to have no relationship to cancers of the colon, prostate, or ovary. The report found that results with respect to pancreatic cancer are equivocal, and results with respect to vitamin C and breast cancer are inconsistent (Refs. 2 and 29).

C. Review of the Scientific Literature

The agency reviewed the publicly available scientific evidence on vitamin C and cancer provided by epidemiologic and clinical studies in accord with the standard described in the general document on health claims on foods (published elsewhere in this **Federal Register**). Studies that included healthy matched (e.g., age, sex, and race) controls, that controlled for confounders such as smoking, and that used validated dietary assessment instruments and adequate study size were given the highest weight. When the possibility could not be excluded that the observed association was mainly a result of the disease altering indicators of vitamin C status, or resulted from the effect of another substance in the food, the study was given less weight.

1. Evidence for an Association Between Ingestion of Vitamin C or Foods High in Vitamin C and Reduced Risk of Cancer

Research studies published since 1987 include more than 30 case-control studies, 1 prospective study, and 2 randomized clinical intervention trials. Because associations between dietary patterns and cancer appear to be site-related, the recent studies are grouped by site and summarized as follows: Table 6, lung; Table 7, colon and rectum; Table 8, breast; Table 9, prostate; Table 10, pancreas; Table 11, stomach; Table 12, head and neck; Table 13, cervix or ovary. Table 14 summarizes results of a study of patients with bladder cancer and a second study of cancer at various sites. Details of the studies including type and location, description of

subjects, methods, and results are presented in these Tables.

a. *Human studies*—(i) *Lung cancer* (Table 6). Adjusted risk analysis (for age and smoking) showed no difference in vitamin C intakes between 450 patients with lung cancer and 902 control subjects (Ref. 74). Dietary information in this study was obtained from a food frequency questionnaire.

In contrast, an inverse association between vitamin C intake and specific types of lung cancer was reported by Fontham et al., (Ref. 75) in a case-control study involving 1,253 patients with lung cancer and 1,274 controls matched for race, sex, and age. Dietary information was obtained from a food frequency questionnaire. No descriptive data were reported for nutrient intakes, and no comparisons were made with standards (e.g., RDA's).

A protective effect of high consumption of leafy green vegetables, carrots, tofu, fresh fruit, and fresh fish against specific types of lung cancer (adenocarcinoma and large cell cancer) was reported by Koo et al., (Ref. 76). Dietary data was obtained from a food frequency questionnaire. Consumption of fresh fruits was found to offer protection against squamous cell tumors of the lung. The data obtained in this study were analyzed by foods without specific analysis for single nutrients.

(ii) *Cancers of the colon and rectum* (Table 7). Food frequency questionnaires were used to obtain information on dietary patterns and risk of colorectal cancer. Freudenheim et al. (Ref. 77) reported that decreased risk of rectal cancer was associated with increased intake of carotenoids, vitamin C, and dietary fiber from vegetables. Graham et al. (Ref. 78) reported that there was a significantly reduced risk of colon cancer associated with high intakes of tomatoes, peppers, carrots, onions, and celery. LaVecchia et al. (Ref. 47) reported that the risk of both colon cancer and rectal cancer was inversely related to intake of green vegetables, tomatoes, melon, and coffee. There were also inverse relationships between risk and indices of carotenoid and vitamin C intake. Tuyns et al. (Ref. 79) found no association between risk for either colon cancer or rectal cancer and vitamin C intake (determined from food frequency questionnaires) in a case-control study in Belgium. West et al. (Ref. 80) also reported no association between risk of colon cancer and intake of vitamin C (determined from a food frequency questionnaire) in a case-control study of colon cancer patients and matched controls in Utah.

Several of these studies identified other dietary risk factors for colon and rectal cancers including increased risk with increasing intakes of calories and fat (Refs. 77 and 78) and consumption of rice and pasta.

A recent randomized clinical trial has been reported that describes a test of the effect of a vitamin C supplement on recurrence of polyps in the colon or rectum (Ref. 81). Subjects in this trial were randomized to receive 400 mg of vitamin E and C (96 subjects) or lactose (placebo; 89 subjects) for a 2-year period. Examination of subjects after 2 years revealed polyps in 41.4 percent of the vitamin-treated group and in 50.7 percent of the placebo group. The relative risk of polyps in the treatment group was not significantly different from that in the placebo group.

In a second intervention trial, De Cosse et al. (Ref. 82) studied the effects on rectal polyps of vitamin C plus vitamin E with and without grain fiber supplements. Fifty-eight patients with familial adenomatous polyposis who had total colectomy and ileorectal anastomosis 1 year before the study were randomized into groups receiving 2.2 grams (g) of fiber and placebo (low fiber), 2.2 g fiber plus 4 g vitamin C and 400 mg vitamin E per day (low fiber plus vitamins C and E), or 22.5 g fiber plus both vitamins per day (high fiber plus vitamins C and E). All groups also received a supplement containing vitamins C and A and several other vitamins and minerals. The results did not show any protective effect of the vitamin C and vitamin E supplement on the occurrence of rectal polyps.

(iii) *Breast cancer* (Table 8). The authors of a case-control study of women with breast cancer and hospital controls in Athens, Greece reported that there were no differences between cases and controls in intakes of vitamin C (determined from food frequency questionnaires) (Ref. 83). This study found no association between intake of vitamin C and risk of breast cancer.

In a case-control study in Italy, Toniolo et al. reported that there was no difference in vitamin C intake in 250 women with breast cancer and 499 women from the general population. Vitamin C intakes were determined from a modified food frequency questionnaire (Ref. 84).

Howe et al. performed a meta-analysis that included all case control studies relating diet and breast cancer that were completed by 1986. Dietary data were available from 9 of 12 studies. Estimates of intakes were made for the other three studies based upon responses to food frequency

questionnaires. A statistically significant inverse association between vitamin C and breast cancer was reported (Ref. 85). Beta-carotene, fiber, and carotenoids (other markers for consumption of fruits and vegetables) also showed an inverse relationship with risk of breast cancer (Ref. 85).

(iv) *Prostate cancer (Table 9)*. Kolonel et al. (Ref. 86) found no associations between risk of prostate cancer and total of food sources of vitamin C in a case-control study involving 452 cases of prostatic cancer and 899 age-matched controls. Dietary information was obtained from a food frequency questionnaire. Increased risk was found to be associated with intake of saturated fat and zinc (Ref. 86).

In two case control studies, Ohno (Ref. 43) and Oishi (Ref. 42) reported that intake of vitamin C from foods was not significantly associated with risk of prostate cancer.

(v) *Pancreatic cancer (Table 10)*. LaVecchia et al. (Ref. 48) reported a statistically significant decreased risk of pancreatic cancer with increased intake of fresh fruits. A similar inverse relationship between intake of fish and oil and risk of pancreatic cancer was also reported in this study. This case-control study, carried out in Italy, involved 247 patients with pancreatic cancer and 1,089 age and sex-matched hospital-based controls with acute nondigestive, nonneoplastic disease. Subjects were interviewed to obtain data on socioeconomic status, tobacco and alcohol use, coffee consumption, medical history, and dietary intake of 14 "indicator" foods. No assessment of intake of individual nutrients was made in this study.

Mills et al. (Ref. 87) investigated dietary habits and risk of pancreatic cancer in about 34,000 non-Hispanic Seventh Day Adventists in California. All subjects completed a life-style questionnaire. A significant protective relationship between consumption of vegetable protein products, beans, lentils, or peas, and dried fruits and fatal pancreatic cancer was reported (Ref. 87). No relationship was found between risk and intake of other fresh fruit, canned or frozen fruit, fresh citrus fruit, green salads, or cooked green vegetables (Ref. 87). No data on individual nutrients were provided in this study.

Falk et al. (Ref. 88) reported a significant inverse relationship between consumption of fresh fruit and fruit juice and pancreatic cancer. The case-control study involved 363 cases of pancreatic cancer and 1,234 hospital-based controls matched on hospital of admittance, race, sex, and age. A food frequency

questionnaire was used to obtain dietary information. No descriptive nutrient data was reported, and there were no comparisons of dietary intakes to reference standards (e.g., RDA's).

(vi) *Stomach cancer (Table 11)*. You et al. (Ref. 89) reported the results of a case-control study in China involving 564 subjects with gastric cancer and 1,131 population-based control subjects. An undefined number of years had elapsed between the "reference period" and the interviews which served to collect data about demographics, medical histories, occupations, smoking histories, and diet. There was a decline in risk of gastric cancer with increased consumption of beta-carotene, vitamin C, and calcium which was associated with high intakes of fresh fruits and vegetables (Ref. 89).

Kono et al. (Ref. 90) reported an inverse relationship between intake of fruits and risk of gastric cancer in a case-control study involving 139 cases of newly diagnosed gastric cancer, 2,574 hospital-based control subjects, and 278 randomly-selected community control subjects in Japan. Gastric cancer patients and hospital-based control subjects were interviewed before diagnosis. A questionnaire was used to obtain information on dietary habits and on consumption of specific food items. The data showed a protective effect against stomach cancer associated with increased frequency of consumption of fruits, mandarin oranges, and green tea (Ref. 90). Attribution of effects to vitamin C could not be made because of the design of this study. Dietary components other than vitamin C, or in addition to vitamin C, could have been responsible for the protective effects.

Buiatti et al. (Ref. 91) studied 1,016 cases of gastric cancer and 1,159 control subjects matched for age and sex. Data regarding demographics, socio-economic status, occupational histories, smoking, medical histories, and diet were obtained from an interview. Intakes of specific nutrients were calculated from responses to a food frequency questionnaire. Buiatti et al. (Ref. 91) reported that decreased risk of gastric cancer was associated with increased consumption of citrus fruits, other fresh fruits, and raw vegetables. Results of further evaluation of the data (Ref. 92) suggested that the protective effects of fresh fruits, fresh vegetables, and olive oil may be associated with vitamins C and E present in these foods. Estimates of the intakes of nitrates and nitrites were calculated for several geographic areas studied, and results indicated an increasing risk of stomach cancer with increasing consumption of nitrates and nitrites. Risk decreased with increasing

intakes of vitamin C and vitamin E (Ref. 92).

Chyou et al. (Ref. 93) initiated a case-cohort study of about 8,000 Hawaiian men of Japanese ancestry during 1965 to 1968. Stomach cancer was diagnosed in 111 men during the following 18 years. Dietary data obtained from these subjects and from 361 cancer-free men revealed that consumption of all types of vegetables was protective against stomach cancer (Ref. 93). Increased intake of fruits was also protective against stomach cancer, but this trend was weakened when cigarette smoking was taken into account (Ref. 93).

Burr et al. (Ref. 94) carried out a cross-sectional study in two towns in England and Wales that had differing death rates from stomach cancer. Burr et al. (Ref. 94) reported that plasma ascorbate levels and fruit intakes were significantly higher in individuals in the low-risk town than in individuals in the high-risk town. No direct relationship between plasma ascorbate levels and the presence of severe atrophic gastritis was found. No dietary data were presented in this study, and the nature of a food frequency questionnaire was not described. There were significant socio-economic differences between the towns, and subjects were not matched for smoking, health histories, or demographics. There were also significant differences in incidence of gastric surgery and severe atrophic gastritis between towns.

Stahelin et al. (1989) (Ref. 61) reported on a prospective cohort study of 2,975 men, 17 of whom subsequently developed stomach cancer. The authors reported that after adjusting for smoking, plasma vitamin C was significantly lower in cases of stomach cancer than in control men. This study relied on a point sample analysis and provided no dietary intake data.

(vii) *Cancers of the head and neck (Table 12)*. Franco et al. (Ref. 95) investigated risk factors for oral cancer in a case-control study in Brazil. Dietary information and health and demographic characteristics were obtained from interviews with 232 patients with oral cancer and 464 hospitalized non-cancer subjects. The strongest risk factors identified in this study were use of tobacco and alcohol. A decrease in risk was observed with more frequent consumption of citrus fruits. It was not possible to calculate intakes of vitamin C in this study.

Results of a population-based case-control study on association of dietary factors with oral cancers were reported by McLaughlin et al. (Ref. 96). Frequency of consumption of food items was

obtained by questionnaire from 871 patients with oral cancer and 979 control subjects. Protective effects were reported with increased consumption of citrus fruits. The study was not designed to specifically address a possible role of vitamin C in reduction in risk of oral cancer.

(viii) *Cancers of the cervix and ovary* (Table 13). Brock, et al. (Ref. 62) reported that when considered together, vitamin C, fruit juices, and plasma beta-carotene showed a significant protective effect against cervical cancer. Dietary information was obtained from a food frequency questionnaire. Cases in this study were not matched on sexual habits, smoking, or use of oral contraceptives. Plasma levels of ascorbic acid were not determined.

Shu et al. (Ref. 97), Slattery et al. (Ref. 44), and Ziegler et al. (Ref. 98) reported no effects of dietary vitamin C on risk of ovarian cancer or cervical cancer. Dietary information in these studies was obtained from a food frequency questionnaire. Significantly increased risk of ovarian cancer was associated with intake of total and saturated fat (Ref. 97). Verreault et al. (Ref. 63) found a decreased risk of cervical cancer associated with high intakes of vitamin C. After adjustment of data for other known risk factors, increased intakes of dark green or yellow vegetables and fruit juices were associated with significantly reduced risk (Ref. 63).

(ix) *Other studies* (Table 14). Results of a case-control study of bladder cancer patients and hospital controls in Italy showed that risk of bladder cancer was not related to intake of vitamin C (Ref. 49).

b. *Studies on vitamin C in relation to carcinogen-forming reactions.* Ascorbic acid is an effective antioxidant in human plasma (Ref. 99). It is also secreted into gastric juice in concentrations that often exceed those in plasma (Ref. 100).

Patients with chronic gastritis have lower concentrations of vitamin C in gastric juice than do those without chronic gastritis, and those with lower gastric juice vitamin C levels are more likely to develop stomach cancer (Ref. 101). Concentrations of vitamin C in both gastric juice and in plasma are lower in patients with chronic atrophic gastritis who also have intestinal metaplasia than in chronic atrophic gastritis patients without intestinal metaplasia (Ref. 101). The patients with intestinal metaplasia also had higher plasma nitrite levels than those without the disorder.

Biochemical and experimental animal evidence is compelling that vitamin C can inhibit nitrosation reactions, and

thereby act as an effective anticarcinogen under experimental conditions (Ref. 25). Most nitrosamines, including diethylnitrosamine, are animal carcinogens that are mutagenic through reactions with DNA (Ref. 102).

Carcinogenic nitrosamines can be formed in vivo (Ref. 103). In vivo synthesis of nitroso compounds may be the greatest source of exposure for the general population (Ref. 2). Ascorbic acid in gastric juice may be an effective inhibitor of gastric nitrosation reactions.

N-nitrosoproline is one of the few apparently noncarcinogenic N-nitrosamines. As such, it is considered a good model compound for studying nitrosation reactions in humans. A method to quantify in vivo nitrosation in humans using sequential oral doses of nitrate and proline and measuring excretion of N-nitrosoproline has been reported (Ref. 104). This technique has been used to show that large doses of ascorbic acid can inhibit formation of nitrosoproline (Refs. 132 and 133). Recent studies in humans by Leaf et al. (Ref. 27) showed that in vivo nitrosation is very complex and may involve many factors in addition to nitrate exposure. These findings indicate that ascorbic acid is capable of inhibiting in vivo synthesis of putative human carcinogens. It is not clear from these studies what dosage of vitamin C is necessary to achieve biologically significant inhibition of nitrosamine synthesis at various intakes of nitrite and nitrate, or whether the food supply already supplies that amount of vitamin C. It also is not clear what degree of such inhibition would be necessary to generate a meaningful decrease in cancer risk.

The relevance of ascorbic acid inhibition of nitrosation and possibly other reactions to human cancer risk may be more directly addressed by studies of dietary relationships to fecal genotoxicity (Ref. 105). The basic assumption is that at least a large fraction of total cancer is produced by genotoxic (i.e., mutagenic) mechanisms, an assumption that is well documented (Refs. 4a, 22, 24). Consumption of supplements containing ascorbic acid decreases fecal mutagenicity (Ref. 106), but this effect may not be completely attributable to inhibition of nitrosation reactions. Ascorbic acid inhibits nitrosation reactions but also decreases stool concentrations of mutagenic products, fecapentenes, which are derived from lipids (Ref. 105).

c. *Animal studies.* Data from research links vitamin C to lowered risk of cancer in animals under a variety of experimental conditions (Refs. 107 and 108). In animals and in in vitro systems,

both vitamin C and vitamin E can inhibit the formation of carcinogenic nitrosamines. Most nitrosamines are carcinogenic in one or more assay systems, but they are most accurately described as procarcinogens, because biological activation is required to convert them to the reactive ultimate carcinogen.

Because of the antioxidant nature of vitamin C, it is reasonable to hypothesize that this vitamin may prevent activation of procarcinogens to the ultimate carcinogen, or, if activation occurs, vitamin C may react with and deactivate the ultimate carcinogen. Animal studies that examined the effect of vitamin C on nitrosamine synthesis and subsequent carcinogenesis as a result of exposure to the nitrosamine precursors, nitrite and a monoamine, provide strong evidence that vitamin C can lower risk of cancer through this mechanism (Refs. 25 and 134). Other studies with animals that examined the effect of vitamin C on other types of chemically-induced cancers have not produced consistent results (Ref. 108).

2. Safety Issues

The Surgeon General's report stated that amounts of vitamin C in excess of the RDA's may cause rare adverse effects including gastrointestinal disturbances, iron overload in susceptible individuals, altered metabolism of certain drugs, precipitation of calcium oxalate kidney stones, altered absorption (both positive and negative) of several minerals, and interference with clinical laboratory tests (Ref. 4a).

3. Conclusions

The majority of the studies summarized above on the association between vitamin C and cancer are epidemiologic studies. These studies depended on dietary data gathered with the use of food frequency questionnaires. In some studies, retrospective dietary information was collected to provide insight into associations between nutrient intakes and cancer at specific sites. However, in most studies, collection of direct data on actual intakes of specific nutrients was not possible.

In a number of the studies, protective effects against cancer at specific sites were observed with increased frequency of consumption of such foods as vegetables, green leafy vegetables, fresh fruits, citrus fruits, and fruit juices. In most studies, it was not possible to determine whether a protective effect was due to the presence of vitamin C, beta-carotene, other nutrients, or

combined effects of both vitamins and other dietary factors such as fiber. In addition, levels of ascorbic acid were rarely measured in the studies reviewed.

The evidence for associations between consumption of foods high in vitamin C and reduced risk of cancer appears to differ markedly by site. Consumption of vitamin C-rich foods appears to be most frequently associated with lower risk of cancer of different parts of the gastrointestinal tract. Intake of vitamin C-rich foods does not appear to be associated with risk of breast cancer. Recent data provide mixed or negative results with regard to vitamin C-rich foods and cancer of the colon/rectum, pancreas, lung, prostate, and cervix/ovary.

Overall, the recent human studies provide evidence that consumption of certain foods notably many fruits and vegetables (which contain higher levels of vitamin C and other nutrients) may reduce the risk of certain cancers, notably those of the stomach and other parts of the gastrointestinal tract. These studies do not clearly demonstrate that the effects are the result of the vitamin C per se.

D. Tentative Decision To Deny Health Claims Relating Ingestion of Vitamin C to Reduced Risk of Cancer

The agency is proposing not to authorize the use on foods, including conventional foods and dietary supplements, of claims relating to associations between ingestion of vitamin C and reduced risk of cancer.

There is strong epidemiologic evidence that consumption of certain foods, notably many fruits and vegetables (which tend to contain higher levels of vitamin C), reduce the risk of cancers at some sites, notably the stomach and other parts of the gastrointestinal tract. It is not possible to determine from the currently available data whether the reduced risks of cancers at specific sites are caused by the vitamin C content of foods or by other components that are also present.

The agency's tentative conclusion is consistent with information and conclusions found in Federal Government and other authoritative documents. The Surgeon General's report on "Nutrition and Health" (Ref. 4a) concluded in 1988 that no wholly consistent picture of the role of vitamin C in human cancer had been defined. The NAS "Diet and Health" report (Ref. 2) concluded that diets high in plant foods (fruits, vegetables, legumes, and whole-grain cereals) are associated with a lower incidence of coronary heart disease and cancers of the lung, colon,

and stomach. The NAS report observed that such diets are low in total fat and rich in complex carbohydrates and certain vitamins and minerals. The NAS report concluded that epidemiologic studies suggest that vitamin C-containing foods such as citrus fruits and vegetables may offer protection against stomach cancer, and that animal studies indicate that vitamin C itself can protect against nitrosamine-induced stomach cancer. Evidence linking vitamin C, or foods containing vitamin C, to cancer at other sites is more limited and less consistent.

The scientific data that have become publicly available since the publication of authoritative documents of the Federal Government and others provide no substantive evidence that would alter the conclusions found in these reports.

FDA has tentatively determined, based on the totality of publicly available scientific evidence regarding an association between vitamin C and cancer, that there is not significant scientific agreement, among experts qualified by scientific training and expertise to evaluate such claims, that the claim associating vitamin C to the risk of cancer is supported by such evidence. Therefore, the agency is proposing to deny the use on foods of claims relating to associations between ingestion of vitamin C and reduction in risk of cancer.

The agency recognizes that the evidence is strong that consumption of fruits and vegetables, good sources of vitamin C, is associated with lowered risk of cancer at a number of sites, especially of the stomach. However, the agency believes that the data are not sufficiently convincing that vitamin C itself is responsible for this epidemiological association, even though the inhibition of nitrosation reactions in human subjects by vitamin C is established. Further, even if the evidence for a cause-and-effect relationship of vitamin C intake with lowered risk of stomach cancer was assumed to be adequate, the agency finds the data to be insufficient to determine the quantity of vitamin C needed to produce the effect. If such data were available, it would be necessary for the agency to determine whether the food supply already provides enough vitamin C to produce that effect.

In summary, the agency requests submission of data which directly bear on: (1) Whether vitamin C itself, rather than some other component of food, decreases the risk of cancer in humans, and (2) the range of intake in which vitamin C produces this effect.

IV. Review of Scientific Evidence: Vitamin E

A. Federal Government Documents

In 1988, the "Surgeon General's Report on Nutrition and Health" (Ref. 4a) summarized the evidence on the role of vitamin E and cancer. The Surgeon General's Report stated that in human studies, no relationship had been found between vitamin E levels and the risk of cancer when the incidence rates of all cancer sites were combined. In addition, the report proposed that because vitamin E is an antioxidant, the protective role tentatively assigned to both the carotenoids and vitamin C may be hypothesized to apply, but that present data are too limited to draw conclusions.

B. Other Authoritative Documents

The 1989 NAS report on "Diet and Health" (Ref. 2) concluded that some investigators have postulated that vitamin E may block the initiation or promotion of cancer, but the committee judged the evidence to be too limited to draw conclusions.

The Life Sciences Research Office (Ref. 38) in a detailed review of recent observational and intervention studies on vitamin E and cancer concluded that available information still is not sufficient to support definite conclusions concerning vitamin E intake and the risk of human cancer. The report stated that more studies, especially well designed intervention trials and observational studies, are needed.

C. Review of Scientific Literature

1. Introduction

The agency reviewed the available scientific evidence on vitamin E and cancer provided by epidemiological studies (prospective and retrospective) and clinical studies. The data reviewed were evaluated according to specific criteria. Studies that involved healthy matched controls (e.g., age and sex), that were controlled for confounders, such as smoking, used validated dietary assessment instruments, appropriate storage conditions for test samples, and adequate sample size were given the most weight. When the possibility could not be excluded that the observed association was mainly a result of the disease altering indicators of vitamin E status, or resulted from an effect of another substance in the food, the study was given less weight.

2. Primary Studies in Humans

Prospective studies are considered first in this section, followed by retrospective studies and clinical trials.

The prospective studies were generally conducted in case-control designs (Table 15). In most of the prospective studies, plasma/serum vitamin E and subsequent cancer development was studied.

The prospective studies generally concentrated on all sites of cancer, hormone-related cancers, and cancers of the lung and gastrointestinal tract (Table 15). Studies that concentrated on all cancer sites generally observed an inverse association between serum vitamin E and subsequent cancer, (Refs. 109 through 113). In a 12-year cancer mortality followup of one of the studies, no significant association was found between low plasma vitamin E levels and all cancer sites combined (Ref. 61). Constock et al. (Ref. 114) reported a significant dose response trend between serum vitamin E and lung cancer. However, other workers, reported no significant association of serum vitamin E and the subsequent development of lung cancer (Ref. 57 and 109).

Two studies using two different population groups observed an association between vitamin E and gastrointestinal cancers (Refs. 109 and 113). A 12-year followup study (Refs. 60 and 61) conducted using one of the same population groups did not confirm the association between vitamin E and gastrointestinal cancer. In another prospective study (Ref. 115) reported an apparent association between low serum levels of vitamin E and reduced risk of pancreatic cancer. However, the difference was not statistically significant. The number of cases was small, and the storage time for the serum was very long prior to analysis, making the reliability of these data doubtful. Schober et al. (Ref. 116) studied the same population group and found no association between vitamin E and colon cancer.

No relationship was found between vitamin E levels and the subsequent development of breast cancer (Refs. 110, 113, 114, and 117). In an earlier study, Wald et al. (Ref. 55) reported a significant inverse association between vitamin E and breast cancer. The number of breast cancer cases in these studies was generally very small.

The maximum followup time in these prospective studies generally ranged from 7 to 8 years (Refs. 109 and 117) to 9 to 13 years (Refs. 60, 61, and 114). In all the studies that FDA examined, except the Basel study (Refs. 60, 61, and 109), vitamin E determinations were based on stored frozen serum/plasma samples collected in baseline examination and thawed for analyses at the end of the followup. It has been reported that the length and storage conditions of the test

samples may decrease the levels of vitamin E in serum/plasma, and therefore make the data unreliable (Ref. 57).

Retrospective studies on vitamin E and cancer are shown in (Table 16). Breast cancer has been the subject of several case-control studies (Refs. 84, 118, and 119). With one exception (Ref. 119), the studies generally found no significant association between breast cancer and vitamin E levels in the serum or vitamin E intake. Gerber (Ref. 119) found that plasma vitamin E was significantly higher in breast cancer cases than hospital controls. However, the vitamin E intake data did not support this observation.

Another study observed lower serum vitamin E levels in lung cancer patients (Ref. 120). Adjustment of the vitamin E levels for serum cholesterol reduced the difference between lung cancer cases and controls. The experimental groups used in this study were not matched for smoking history.

Two studies conducted in other countries reported a significant inverse association of vitamin E with either gastric or digestive cancers (Refs. 92 and 121). One author (Ref. 92) suggested that the observed decrease in serum vitamin E in digestive cancer cases may be a consequence of nutritional inadequacies. Verreault (Ref. 63) examined the association of vitamin E using invasive cervical cancer cases and population controls. High intakes of vitamin E were associated with lower cancer risk. Another study reported (Ref. 122) a significant trend of lower mean serum vitamin E levels in association with cervical intraepithelial neoplasia. The sample size was small and the authors pointed out the need to examine interactive factors such as sexual behavior and smoking.

Two studies (Refs. 123 and 124) observed no significant association between vitamin E and specific cancer sites (rectal and larynx). Stryker (Ref. 125) observed that vitamin E intake, but not serum vitamin E, displayed a significant trend of decreasing risk of malignant melanoma. The vitamin E intake in the studies cited were based on retrospective dietary data, and the accuracy of the data is therefore difficult to assess. In addition, the evidence based on dietary intake data is often confounded by other nutrients that may also be associated with the risk of cancer.

Intervention trials (clinical) involving vitamin E and cancer are limited. One recent clinical trial examined the effect of vitamins C and E in the reduction of the risk of recurrence of colorectal polyps (Ref. 81). Another study (Ref. 82)

reported a chemoprevention trial on large bowel neoplasia using either supplements of ascorbic acid plus vitamin E alone or with a grain fiber supplement. The combined use of vitamins prevented an analysis of the independent effect of vitamin E or interactions with vitamin C.

3. Supporting Data From Animal Studies

Animal studies on the effect of vitamin E (usually alpha-tocopherol) on carcinogenesis have yielded conflicting results. Several recent reports (Refs. 5b, 34, and 126) have reviewed the experimental evidence on the effects of vitamin E in cancer prevention. Most studies have dealt mainly with chemically induced cancers. Birt (Ref. 126) summarized the results of 14 animal studies involving the use of 3 different species of animals and 4 preformed carcinogens. Some of the studies observed inhibition of two-stage skin, oral and forestomach tumorigenesis. Other studies showed enhancement, no effect, or inhibition of rat mammary gland and rat and mouse carcinogenesis.

Mergens and Bhagavan (Ref. 34) and Merrill et al. (Ref. 5b) summarized animal studies on the inhibition of carcinogenesis by alpha-tocopherol. These reviews show that results from animal studies were frequently positive, however, many studies show no effect or are negative. Animal studies have shown that, under certain conditions, vitamin E has been shown to inhibit the formation of carcinogenic nitrosamines (Refs. 5b and 34).

4. Safety Issues

The NRC, in "Diet and Health" (Ref. 2), summarized two studies (Refs. 127 and 129) and a review article on the safety of oral intake of vitamin E. The studies suggested that large doses of vitamin E are relatively nontoxic. The review article (Ref. 130) cited by the Council extensively reviewed overall safety issues of vitamin E. The authors reported that in human studies conducted with double blind protocols and in large population studies, oral vitamin E supplementation resulted in few side effects even at doses as high as 3,200 mg per day. They concluded that most of the reported side effects: breast soreness, altered creatinine excretion, emotional disorders, fatigue, gastrointestinal stress, thrombophlebitis, and decreased thyroid levels, have come from uncontrolled studies or case reports (Ref. 130). However, in persons with vitamin K deficiency produced either by malabsorption or anticoagulant therapy, vitamin E

supplementation can exacerbate the coagulation defect.

In the 1989 edition of "Recommended Dietary Allowances," the NRC (Ref. 14) stated that compared with other fat-soluble vitamins, vitamin E is relatively nontoxic when taken by mouth.

D. Conclusions

Some recent prospective studies provide suggestive evidence of an association of lower plasma/serum vitamin E levels with increased risk of cancer (Ref. 113). However, the different population groups studied were limited and results from some followup studies were inconsistent. Clarification of the role of vitamin E in the reduction of cancer risk would be greatly enhanced by studies that also examine the quantitative dietary intake of vitamin E. Many of the studies were based on relatively small numbers of cancer cases, used test samples that had been stored for long periods of time prior to analysis, or relied on retrospective food frequency interviews. These factors may contribute to unreliable interpretations of the results.

While some of the recent retrospective studies have provided suggestive evidence that low vitamin E intake or low plasma/serum levels of vitamin E may be associated with the increased risk of some cancer, other studies have not. Many of the studies have study design flaws (e.g., inappropriate controls, small sample size). Too few clinical trials have been conducted on the association of vitamin E status and the risk of cancer to clearly implicate vitamin E.

E. Tentative Decision To Deny a Health Claim Relating to Ingestion of Vitamin E to the Risk of Cancer

FDA considered conclusions reached by Federal Government documents, and other authoritative documents, a review of the topic by Life Sciences Research Office of the Federation of American Societies for Experimental Biology, and all comments received in response to a Federal Register notice of a request for scientific data and information. The agency also reviewed recent publicly available scientific data on the association between vitamin E and cancer.

In 1988, the *Surgeon General's Report on Nutrition and Health* (Ref. 4a) documented and reviewed available literature on the possible relationship of vitamin E and cancer. The Surgeon General did not find the evidence strong enough to justify a recommendation to the general public on vitamin E and cancer. However, the report proposed that the protective role tentatively

assigned to both carotenoids and vitamin C may apply to vitamin E. The NRC Committee on Diet and Health (Ref. 2) also reviewed current literature on the topic. The committee's report concluded that the scientific evidence was insufficient to draw conclusions about the relationship between vitamin E and cancer.

Several followup studies and new studies have appeared in the literature since the above reports (see Tables 1 and 2). Recent epidemiological studies exploring the association of blood levels of vitamin E as well as dietary vitamin E intake with the risk of cancer are contradictory. Some studies support an inverse association of blood levels of vitamin E/vitamin E intakes with the risk of certain cancers, while others do not.

FDA has tentatively determined, based on the totality of publicly available scientific evidence regarding an association between vitamin E and cancer, that there is not significant scientific agreement among experts qualified by scientific training and expertise to evaluate such claims that the claim associating vitamin E to the risk of cancer is supported by such evidence.

The agency recognizes that consumption of food sources of vitamin E is frequently, but not consistently, associated with lowered risk of cancer at a number of sites. However, the agency believes that the data do not sufficiently demonstrate that vitamin E itself is responsible for this association, or permit identification of what other factors may produce or prevent the effect. Further, even if the evidence for a cause-and-effect relationship of vitamin E intake with lowered risk of cancer was assumed to be adequate, the agency finds the data to be insufficient to determine the quantity of vitamin E needed to produce the effect. If such data were available, the agency would have to determine whether the food supply already provides that amount of vitamin E.

In summary, the agency requests submission of data which directly bear on: (1) Whether vitamin E itself, rather than some other component of food, decreases the risk of cancer in humans, (2) the range of intake in which vitamin E produces this effect, and (3) factors which may limit any vitamin E effect.

V. Environmental Impact

The agency has determined under 21 CFR 25.24(a)(1) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment

nor an environmental impact statement is required.

VI. Effective Date

FDA is proposing to make these regulations effective 6 months after the publication of a final rule based on this proposal.

VII. Comments

Interested persons may, on or before February 25, 1992 submit to the Dockets Management Branch (address above) written comments regarding this proposal. Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in the brackets in the heading of this document. Received comments may be seen in the office between 9 a.m. and 4 p.m., Monday through Friday.

VIII. Economic Impact

The food labeling reform initiative, taken as a whole, will have associated costs in excess of the \$100 million threshold that defines a major rule. Therefore, in accordance with Executive Order 12291 and the Regulatory Flexibility Act (Pub. L. 96-354), FDA has developed one comprehensive regulatory impact analysis (RIA) that presents the costs and benefits of all of the food labeling provisions taken together. The RIA is published elsewhere in this issue of the *Federal Register*. The agency requests comments on the RIA.

IX. 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.

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List of Subjects in 21 CFR Part 101

Food Labeling, Reporting and recordkeeping requirements.

PART 101—FOOD LABELING

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, it is proposed that 21 CFR part 101 be amended as follows:

1. The authority citation for 21 CFR part 101 is revised to read as follows:

Authority: Secs. 4, 5, 6 of the Fair Packaging and Labeling Act (15 U.S.C. 1453, 1454, 1455); secs 201, 301, 402, 403, 409, 501, 502, 505, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 331, 342, 342, 348, 351, 352, 355, 371).

2. Section 101.71 is amended by adding new paragraph (d) to read as follows:

§ 101.71 Health claims: claims not authorized.

(d) Antioxidant vitamins and cancer (insert cite and date of publication in the Federal Register of the final rule).

Dated: November 4, 1991.

David A. Kessler.

Commissioner of Food and Drugs.

Louis W. Sullivan,

Secretary of Health and Human Services.

Note: The following tables will not appear in the annual Code of Federal Regulations.

TABLE 1.—ANTIOXIDANT VITAMINS AND CANCER: RETROSPECTIVE STUDIES OF DIETARY AND SERUM CAROTENOIDS AND CANCER

Authors and year	Source exposure	Study population	Cancer site	Cases/controls	Results	Assessment of study
LeMerchand, et al., 1991 (Ref. 41).	Beta-carotene, reevaluation of dietary data.	Oahu, Hawaii, multiethnic men.	Prostate	452/839	Reexamined data eliminating papaya and found no association of beta-carotene with prostate cancer risk in men 70 years.	Positive association between papaya and prostate cancer was observed in men > 70.
LeMerchand, et al., 1989 (Ref. 39).	Beta-carotene, other provitamin A carotenes, vegetables and fruits.	Oahu, Hawaii, multiethnic men and women.	Lung	230/597	Higher intakes of foods containing beta-carotene associated with lower lung cancer risks.	Stronger inverse association occurred with all vegetables, dark green and cruciferous vegetables, and tomatoes than with beta-carotene.
Bond, et al., 1987 (Ref. 40).	Dietary carotenoids	Chemical company employees, Texas, men.	Lung	308/616	Higher intakes of foods with high carotenoid index were associated with lower lung cancer risks.	The reported reliance on surrogate sources of dietary information and the relatively long retrospective period are flaws which detract from the results.
Kune, et al., 1987 (Ref. 46).	Vegetables	Case-control in Melbourne, Australia, men and women.	Colon, rectum	715	Higher intakes of beta-carotene, total vegetables, fiber and vitamin C were related to lowered risks.	Also some protection occurred with supplement usage. Retrospective period of long duration.
Coggon, et al., 1989 (Ref. 45).	Fruits and vegetables	Case-control, England.	Stomach	95/190	Intakes of fruit and salad vegetables inversely associated with risk.	Did not report on supplement alcohol, smoking or health history. Difficult to draw conclusions on effects due to beta-carotene.
LaVecchia, et al., 1988 (Ref. 47).	Fruits and vegetables	Case-control, Italy	Colon, rectum	339, 236, 778, controls.	Risk of both cancers inversely related to carotenoid and vitamin C indices.	Reported no information on supplement usage or community-based controls.
Smith and Waller, 1991 (Ref. 50).	Beta-carotene	Wellington, New Zealand cases, controls and their families.	Lung, stomach, esophagus, small bowel, cervix, uterus.	618/675	Plasma beta-carotene was lower in cancer patients and their relatives compared with control patients and their relatives.	A unique study of cancer and control patients and their families.
Ohno, et al., 1983 (Ref. 43).	Beta-carotene and vitamin A.	Japan case-control	Prostate benign prostatic hyperplasia	100/100	Intakes of beta-carotene and vitamin A inversely related to prostate cancer.	Report did not provide data on smoking or supplement usage. The time between the disease reported and dietary intake data is of long duration.

TABLE 2.—ANTIOXIDANT VITAMINS AND CANCER: PROSPECTIVE STUDIES OF DIETARY CAROTENOIDS AND CANCER

Authors and year	Source or exposure	Study population	Cancer site	Cases/controls	Results	Assessment of study
Paganini-Hill, et al., 1987 (2) (Ref. 54).	Carotenoids	5 yr cohort study, California retirement community, men and women.	All cancer, breast, bladder, lung, colon.	638, 123, 58, 55, 110.	No association found between beta-carotene consumption and subsequent development of cancer.	Study conducted in a retirement community where intakes of all nutrients are already very high. No specifics on supplement use was reported. The agreement between the questionnaire used in the first and second followup was reported to be only 50% reducing reliability of study.
Lee, et al., 1991 (Ref. 52).	Beta-carotene, soya, products and PUFA ¹ .	Chinese women of Singapore.	Breast	200/420.....	Inverse association with higher intakes of beta-carotene and other foods. Cannot separate effects due to beta-carotene versus those due to soya foods and poly-unsaturated fatty acids.	Authors suggested that soya products which are rich in phytoestrogens and beta-carotene may have influenced results in premenopausal women.
Harris, et al., 1991 (Ref. 51).	Beta-carotene.....	Men with cancer.....	Lung, other epithelia cancer controls.	96, 75, 97.....	Stronger inverse association with beta-carotene intake and cancers than with carotene-rich vegetables. Serum carotene correlated with dietary carotenes.	Age-matched controls were inpatients with nonmalignancy. Relatively small sample population.
Rohan, et al., 1988 (Ref. 53).	Beta-carotene foods....	Australia. Case-control women.	Breast	451/451 (community-based controls).	Risk in premenopausal women decreased with increased intake of beta-carotene foods. In postmenopausal women risk was highest in the second lowest quintile of consumption.	Reported no data on supplement use. The standardized questionnaire used in collecting food intake data did not make use of food models or photographs in estimating portion sizes.

¹ Polyunsaturated fatty acids.

TABLE 3.—ANTIOXIDANT VITAMINS AND CANCER: PROSPECTIVE STUDIES OF SERUM OR PLASMA CAROTENOIDS AND CANCER

Authors/year (#)	Source/exposure	Study population	Cancer site	Cases/controls	Results	Assessment of study
Wald, et al., 1988 (Ref. 56).	Beta-carotene.....	BUPA ¹ Medical Center, London, United Kingdom, men.	All cancer, lung, colorectal, CNS, bladder, stomach.	271/533, 50/99, 30/59, 17/34, 15/29, 13/26.	Reduced serum beta-carotene levels in all, lung, and stomach cancers and nonsignificant reduced levels in colorectal, CNS, ² and bladder cancers.	No information reported on median storage times between sample collection and cancer diagnosis or death.
Connett, et al., 1989 (Ref. 58).	Total carotenes and beta-carotene.	22 MRFIT ³ centers, men.	All cancer, lung, gastro-intestinal, colon.	158/311, 66/131, 28/56, 41/28.	Higher serum levels were related to lower rates of lung cancer and trends towards relationship to reduced rates of gastrointestinal cancer.	One limitation is that this study used a single 24-hour dietary recall for collecting dietary information, therefore, the trend toward lower intake of beta-carotene in lung cancer cases may have assumed greater significance had more extensive dietary data been collected.
Stahelin, et al., 1991 (Ref. 60).	Beta-carotene.....	Chemical Company employees, Basel, Switzerland, men.	Bronchus, stomach, colorectal.	68, 20, 17.....	Overall cancer mortality was associated with low plasma carotene, but specifically that from bronchus and stomach cancer.	Well-designed study with good controls. However, no dietary data or supplement usage reported.

TABLE 3.—ANTIOXIDANT VITAMINS AND CANCER: PROSPECTIVE STUDIES OF SERUM OR PLASMA CAROTENOIDS AND CANCER—Continued

Authors/year (#)	Source/exposure	Study population	Cancer site	Cases/controls	Results	Assessment of study
Burney, et al., 1989 (Ref. 115).	Beta-carotene, lycopene.	Washington County, Maryland men and women.	Pancreas	22/44	Lower serum lycopene, not beta-carotene was associated with higher mortality from pancreatic cancer.	Storage of serum samples was of long duration and there was no report of dietary intake or supplement usage.
Knekt, et al., 1990 (Ref. 142).	Beta-carotene	Finland, cohort	All cancer sites	766/1419	Inverse relationship between mean serum beta-carotene levels and smoking and cancer risk after adjustment for smoking.	Blood storage time was of long duration. No dietary intake or supplement usage data were reported.

¹ British United Provident Association.² Central nervous system.³ Multiple risk factor intervention trial.

TABLE 4.—ANTIOXIDANT VITAMINS AND CANCER: PREMALIGNANCY AND BETA-CAROTENE

Authors and year	Source/exposure	Study population	Cancer site	Cases/controls	Results	Assessment of study
VanEenwyk, et al., 1991 (Ref. 65).	Dietary and Serum Beta-carotene.	Women	Cervical Intra-epithelial neoplasia (CIN).	102/102	Higher intakes and serum levels of lycopene were associated with lower frequency of CIN.	Combined dietary and serum data with biopsy data plus the control of confounding variables makes this a convincing study.
De Vet, et al., 1991 (Ref. 67).	Supplemental beta-carotene 10 mg per day.	Women	Cervical dysplasia	137/141	Inconclusive	Duration of supplementation too short and sample size was too small to allow conclusions to be drawn.
Paian, et al., 1991 (Ref. 66).	Plasma beta-carotene.	Cross-sectional study, women.	Uterine cervix dysplasia.	116	Reduced plasma beta-carotene and vitamin E associated with increased cervix dysplasia and cancer.	Cross-sectional study without proper control groups. However, study considered for smoking behavior as a confounding variable.
Brock, et al., 1988 (Ref. 62).	Dietary and plasma beta-carotene.	Patients and community-based matched controls.	Cervical cancer <i>in situ</i> .	117/195	Higher dietary and plasma beta-carotene were inversely associated with cancer.	Matched cases with controls for smoking and other confounding variables. Good overall design.
Verreault, et al., 1989 (Ref. 63).	Beta-carotene and dark green and yellow vegetables.	Case-control, Washington.	Cervical cancer	199/227	Higher intakes of carotenes related to lower risks of squamous cell cancers.	Limitations are the long duration between interview and reference period and long retrospective period.
Zasu, et al., 1991 (Ref. 64).	Plasma beta-carotene.	Women patients with diagnosed cervix abnormality.	Cervical dysplasia	75	Reduced levels of beta-carotene in smokers and nonsmokers with cervix dysplasia.	No report as to whether or not reduced plasma beta-carotene is due to the disease state.

TABLE 5.—ANTIOXIDANT VITAMINS AND CANCER: CHEMOPREVENTION INTERVENTION TRIALS WITH BETA-CAROTENE

Study site/investigator	Target site/organ	Target/risk group	Supplementary agent	Results	Assessment of study
Greenberg, et al., 1990, Dartmouth, UCLA, ¹ U Minn, ² UC-San Francisco ³ . (Ref. 20).	Skin	Biopsy proved nonmelanoma basal-cell or squamous-cell carcinoma.	Beta-carotene, 50 mg per day.	No difference between cases and control groups in new cases of nonmelanoma skin cancer.	Well-designed double-blind intervention trial of 5-year duration.
Buring and Hennekens, 1990, Harvard School of Public Health (Ref. 40).	All cancer sites	American Physicians	Beta-carotene, aspirin	Continuing	N/A.
Stich et al., 1988 (Ref. 68).	Oral leukoplakias and micronucleated cells.	Betel nut/tobacco chewers.	Beta-carotene, 130 mg/week; Vitamin A, 100,000 IU ⁴ /week.	Inhibition of micronucleated cells after 3 months. Regression of leukoplakias after 6 months.	Authors suggested considerable variation in tissue levels of beta-carotene may affect study outcome.

¹ University of California, Los Angeles.² University of Minnesota.³ University of California at Los Angeles.⁴ International units.

TABLE 6.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND LUNG CANCER

Study	Type and location	Subjects	Methods	Results	Comments
Fontham, et al., 1988 (Ref. 75)	Case-control, Los Angeles, California	1,253 cases, 1,274 hospital controls matched for race, sex, age	All subjects interviewed via questionnaire regarding tobacco use, diet, etc. Food frequency questionnaire used with reference period before appearance of symptoms.	An inverse association was found between intake of vitamin C and specific types of lung cancer. A similar but less strong association was found for carotene.	Control group had 21.6% nonsmokers versus 4.3% in the case group. No biochemical indices measured. No population-based control group. No comparisons were made to nutrient intake standards.
Koo, et al., 1988 (Ref. 76)	Case-control, Hong Kong	88 cases, 137 district-matched controls. All subjects were women with no known history of smoking.	All subjects interviewed regarding demographics, etc. Diet information obtained from food frequency questionnaire	Reduced risk of adenocarcinoma and large cell cancer associated with consumption of leafy green vegetables, carrots, tofu, fresh fruit, fresh fish. Reduced risk of squamous cell tumors associated with consumption of fresh fruits.	No biochemical measurements. No analysis of diet information for specific nutrients.
Le Marchand, et al., 1989 (Ref. 39)	Case-control, Hawaii	432 cases, males and females, 865 community controls matched for age and sex.	Structured interview provided data on smoking, alcohol use, diet, etc. Food frequency questionnaire given to all subjects.	Inverse association found between vitamin C from foods and supplements and risk of lung cancer. A dose-dependent negative association was found between dietary beta-carotene and risk of lung cancer.	No biochemical measurements. No controls for ethnic differences in intake.
Le Gardeur, et al., 1990 (Ref. 120)	Case-control, Los Angeles, California	59 cases, 59 hospital controls, 31 community controls.	Cases and hospital controls interviewed regarding smoking, history, diet, etc. Community controls not interviewed. Blood samples collected from all.	Mean serum levels of carotenoids, vitamin E were lower for lung cancer cases than for hospital controls. Hospital controls had lower levels of vitamins C and E than community controls.	No diet results reported. No diet data collected for community controls. Hospital control group not appropriate because of nature of illnesses.

TABLE 7.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND CANCERS OF THE COLON AND RECTUM

Study	Type and location	Subjects	Methods	Results	Comments
Graham, et al., 1988 (Ref. 78)	Case-control, New York, N.Y.	428 cases of colon cancer, 428 controls matched for sex, age, neighborhood.	Interview consisted of food frequency questionnaire, smoking and alcohol use, health history, etc.	Increasing intake of tomatoes, peppers, carrots, onions, celery associated with decreased risk of colon cancer. Risk was positively associated with increasing intake of total fat and total calories.	No biochemical measurements. No reference period for food frequency questionnaire.
La Vecchia, et al., 1988 (Ref. 47)	Case-control, Italy	339 cases of colon cancer, 236 cases of rectal cancer, 778 hospital controls (acute nonneoplastic, nondigestive disorders).	Dietary information obtained from food frequency questionnaire. Data also collected on demographics, smoking, alcohol use, etc.	Intake of green vegetables, tomatoes, melon, coffee inversely related to risk of colon and rectal cancers. Inverse relationship between indices of carotenoid and vitamin C intake and risk.	Variability in reference period among groups. No biochemical measurements. No population-based controls.
Neugut, et al., 1988 (Ref. 136)	Cross-sectional, New York, N.Y.	105 cases of polyps, 56 cases of colon cancer, 83 controls without neoplastic disease. All subjects were women who had undergone colonoscopy.	Questionnaire used to obtain information on demographics, health history. Subjects asked about use of vitamin supplements.	Significant differences in socio-economic factors among the groups.	No dietary information. No information on duration of supplement use. No biochemical measurements.

TABLE 7.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND CANCERS OF THE COLON AND RECTUM—Continued

Study	Type and location	Subjects	Methods	Results	Comments
West, et al., 1989 (Ref. 80).	Case-control, Utah.....	231 cases of colon cancer, 391 controls matched by age, sex, county of residence.	Interviews provided information on demographics, health history. Food frequency questionnaire used to obtain dietary information.	Protective effects of beta-carotene and cruciferous vegetables in males and of fiber in females. No effects of vitamin C.....	No biochemical measurements. Relationship of reference period for dietary data and time of diagnosis not identified.
Freudenheim, et al., 1990 (Ref. 77).	Case-control, New York, N.Y.	422 cases of rectal cancer, 422 controls matched for sex, race, age.	Interview used to obtain information on food frequency, smoking, alcohol use, health history, etc.	Increasing intake of carotenoids, vitamin C, and dietary fiber from vegetables associated with decreased risk of rectal cancer.	No biochemical measurements. Reliance on retrospective food frequency information.
McKeown-Eyssen, et al., 1988 (Ref. 81).	Randomized double-blind intervention trial, Toronto, Canada.	185 cases with at least one colon or rectal polyp.	96 subjects received vitamins E plus C, and 89 received placebo over a 2 year period. Food frequency questionnaire used to obtain dietary data.	137 participants completed the trial. There was no significant difference in the number of polyps found in vitamin-treated or placebo groups.	No biochemical measurements Small sample size Study design did not allow differences in effect of individual vitamins to be distinguished
De Cosse, et al., 1989 (Ref. 82).	Randomized double-blind intervention trial study. Participants from several states, United States.	58 patients with familial adenomatous polyposis who had total colectomy and ileorectal anastomosis 1 year prior to study.	Vitamin group received 2.2 g fiber and 4 g vitamin C and 400 mg vitamin E daily. Fiber group received both vitamins plus 22.5 g fiber daily. Control group received placebo and 2.2 g fiber daily. All groups received supplement of vitamins C and A, and several other vitamins and minerals daily.	There were no significant effects of the vitamins.	No biochemical measurements. Analysis of independent effects of vitamins was not possible because of design of the study.

TABLE 8.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND BREAST CANCER

Study	Type and location	Subjects	Methods	Results	Comments
Katsouyanni, et al., 1988 (Ref. 83).	Case-control, Athens, Greece.	120 cases, 120 hospital controls.	Dietary histories collected with a 120 item food frequency questionnaire. Nutrient intakes adjusted for calories.	No association between intake of vitamin C and risk of breast cancer. No differences in intakes of vitamin C between cases and controls.	No biochemical measurements. Supplement use not documented. 25% of controls had osteoarthritis, which affects vitamin C metabolism.
Toniolo, et al., 1989 (Ref. 84).	Case-control, Italy.....	250 cases, 499 controls from general population stratified by age, geographical area.	Dietary information collected by use of a modified food frequency questionnaire.	No differences in vitamin C intake between groups. Reduced risk of breast cancer was associated with decreased intakes of saturated fat and animal fat.	No biochemical measurements. Retrospective diet data not necessarily indicative of diets prior to diagnosis.
Howe, et al., 1990 (Ref. 85).	Case-control, meta-analysis of all studies of diet and breast cancer completed by 1986.	4,437 cases, 4,341 population-based controls, 1,754 hospital controls.	Data on vitamin C intake available from 9 to 12 studies. Estimates of intake were made for other three studies using food frequency answers.	Vitamin C intake had a statistically significant inverse association with risk of breast cancer. Consumption of B-carotene, fiber [markers of fruit and vegetable consumption] were also inversely related to risk.	Lack of control of independent variables that could be related to outcome. Reliability of dietary data questioned for some of studies used.

TABLE 9.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND CANCER OF THE PROSTATE

Study	Type and location	Subjects	Methods	Results	Comments
Kolonel, et al., 1988 (Ref. 86)	Case-control, Hawaii	452 cases, 899 age-matched controls	Home interview used to collect data on dietary, medical, occupational history, etc. Food frequency questionnaire used to determine dietary intakes.	No associations were found between intake of vitamin C from food sources and risk of prostate cancer.	Total vitamin C intake ranged from 2,500 to 3,000 mg/week in all groups.
Ohno, et al., 1988, and Oishi, et al., 1988 (Ref. 43)	Case-control, Japan	100 cases of prostatic cancer, 100 controls with benign prostatic hyperplasia, 100 hospital controls without neoplasia or hyperplasia. All subjects matched for hospital, age, date of admission.	Interviews used to collect data on occupation, medical history, etc. Dietary information obtained from food frequency questionnaire	Intake of vitamin C from foods was not associated with risk of prostate cancer	No biochemical measurements. No community-based control group

TABLE 10.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND CANCER OF THE PANCREAS

Study	Type and location	Subjects	Methods	Results	Comments
Falk, et al., 1988 (Ref. 88)	Case-control, Los Angeles	363 cases, 1,234 hospital controls matched on hospital. Race, sex, age	Interview used to obtain data on smoking, medical history, etc. Food frequency questionnaire used to obtain dietary information.	Significant inverse relationship between consumption of fruit (fresh or juice) and risk of pancreatic cancer.	No biochemical measures. Use of surrogates for more than 50% of cases. 13% of controls were not available.
Farrow, et al., 1990 (Ref. 137)	Case-control, Washington	148 cases, 188 randomly selected controls.	Data for all subjects collected from surrogates. Food intake was determined from food frequency questionnaire.	No association between intakes of vitamin C and risk of pancreatic cancer.	Questionable validity/reliability of data acquisition
La Vecchia, et al., 1990 (Ref. 48)	Case-control, Italy	247 cases, 1,089 hospital-based controls.	Interview used to obtain data on tobacco, alcohol, coffee intake, etc. Dietary intake of 14 "reference foods" determined.	Statistically significant decrease in risk of pancreatic cancer with increased intake of fresh fruits	No biochemical measurements. No community based control. No matching for confounding variables.

TABLE 11.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND STOMACH CANCER

Study	Type and location	Subjects	Methods	Results	Comments
Burr, et al., 1987 (Ref. 94)	Cross-sectional, England, Wales	267 and 246 subjects selected randomly from pools of men aged 65 to 74 years in two towns with differing death rates from stomach cancer.	Standardized questionnaire. Blood samples for ascorbate and pepsinogen.	Intake of fruit and plasma ascorbate levels were significantly higher in men from town with lower rates of stomach cancer.	No information on nature of food frequency questionnaire. No diet data provided. No matching of subjects by health history, smoking, demographics, etc.
You, et al., 1988 (Ref. 89)	Case-control, China	564 cases of stomach cancer, 1,131 population-based controls.	Dietary data obtained from a food frequency questionnaire. Reference periods were about 1980 and about 1965.	Increased consumption of vitamin C, carotene, and calcium (associated with high intakes of fruits, vegetables) associated with decreased risk of stomach cancer.	Data represented 1980 interviews. No biochemical measurements. No information on mean intakes of nutrients.
Bianchi, et al., 1989, 1990 (Refs. 91 and 92)	Case-control, Italy	1,016 cases of gastric cancer, 1,159 community controls matched for age, sex.	Interview used to obtain information about demographics, medical history, diet, etc.	Significantly reduced risk associated with increased intake of raw vegetables, fresh fruit, citrus fruit. Risk decreased in proportion to intake of vitamin C, beta-carotene, alpha-tocopherol and vegetable fat.	No biochemical data. Reliance on retrospective data. No comparison with known standards of intake.

TABLE 11.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND STOMACH CANCER—Continued

Study	Type and location	Subjects	Methods	Results	Comments
Chyou, et al., 1990 (Ref. 93).	Case-control, Hawaii.....	111 cases of gastric cancer, 361 controls. All subjects were American men of Japanese ancestry interviewed from 1965 to 1968.	Food frequency questionnaire provided information on food items consumed.	Statistically significant inverse relationship between risk of gastric cancer and intake of all vegetables.	No biochemical measurements. No assessments of individual nutrients.
Kono, et al., 1988 (Ref. 90).	Case-control, Japan.....	139 cases of stomach cancer, 2,574 hospital controls, 278 randomly selected community controls.	Data collected on dietary histories, occupation, smoking, etc.	Inverse relationship between risk of stomach cancer and consumption of fruits. Decreased risk also associated with increased consumption of green tea.	No biochemical measurements. No evaluation of individual nutrients. No information on health problems of hospital-based controls.

TABLE 12.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND CANCERS OF THE HEAD AND NECK

Study	Type and location	Subjects	Methods	Results	Comments
Brown, et al., 1988 (Ref. 139).	Case-control, South Carolina.	207 males with esophageal cancer and 74 hospitalized cases and 133 deaths, 157 hospitalized noncancer controls and 265 noncancer deaths.	Patients interviewed about alcohol, tobacco use and diet. Next of kin of cancer and control subjects interviewed at home.	Leading risk factors for esophageal cancer after adjusting for smoking and tobacco use were low intakes of citrus fruits and juices and high intakes of liver.	No biochemical measurements. Reliance on retrospective dietary data and use of proxy data for mortality phase of study.
McLaughlin, et al., 1988 (Ref. 96).	Case-control, four regions of United States.	871 cases of oral and pharyngeal cancer, 979 population-based controls matched for age, sex, and race.	All subjects or next of kin provided data on alcohol and tobacco use and normal diet during adulthood.	Vitamin C was associated with decreased odds ratios and risk of oral cancer in men and women. There was a significant protective effect of vitamin C, vitamin A, and fiber derived from fruit.	No biochemical measurements. Reliance on retrospective diet data.
Franco, et al., 1989 (Ref. 95).	Case-control, Brazil.....	232 cases of oral cancer, 464 hospital, noncancer controls matched for sex, age, trimester of hospital admission.	All subjects interviewed regarding history of alcohol and tobacco use, general health, etc. Dietary information obtained from a food frequency questionnaire.	Strongest risk factors were alcohol and tobacco use. Without adjustments, significantly reduced risk associated with increased consumption of carrots, pumpkins, papaya, citrus fruits.	No biochemical measurements. No data on vitamin C specifically. Lack of population-based control group.
Li, et al., 1989 (Ref. 138).	Case-control, Linxian, China.	1,244 cases of cancer of esophagus or gastric cardia. 1,314 sex and age-matched controls from same area.	Data collected on smoking, diet history, personal health history. Questions were referenced to 2 time periods (late 1950's and late 1970's).	All subjects consumed diets low in fruits and vegetables. No association with risk of cancer at the sites examined.	Insufficient variability in intake to assess relationship to risk of cancer at sites examined.

TABLE 13.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND CANCER OF THE OVARY OR CERVIX

Study	Type and location	Subjects	Methods	Results	Comments
Brock, et al., 1988 (Ref. 62).	Case-control, Australia.....	117 cases of cervical cancer, 196 controls matched for socioeconomic status, age.	Interview and food frequency questionnaire used to obtain information on demographics, reproductive history, dietary data, etc.	Cases not matched for sexual habits, smoking, use of oral contraceptives. Adjusted risk estimate showed protective effect for vitamin C.	No biochemical measures of vitamin C status. No comparisons made with regard to dietary intake and blood levels.
Shu, et al., 1989 (Ref. 97).	Case-control, Shanghai.....	172 cases of epithelial ovarian cancer, 172 controls (age-matched).	Interview used to obtain information on demographics, reproductive history, dietary intake, etc.	No effect of intake of vitamin C on risk of ovarian cancer.	No biochemical measurements. No descriptive statistics. No comparisons to known standards of intake.

TABLE 13.—ANTIOXIDANT VITAMINS AND CANCER: VITAMIN C AND CANCER OF THE OVARY OR CERVIX—Continued

Study	Type and location	Subjects	Methods	Results	Comments
Slattery, et al., 1989 (Ref. 44).	Case-control, Utah	85 cases of ovarian cancer, 492 population-based controls matched for age.	Interview provided data on demographics, health history, dietary intakes, etc.	No effect of intake of vitamin C on risk of primary ovarian cancer.	No reference period given for dietary data. No biochemical measurements.
Verrault, et al., 1989 (Ref. 63).	Case-control, Washington.	189 cases of cervical cancer, 227 randomly selected age-matched controls.	Demographic, dietary, etc. data collected by interview.	Decreased risk of cervical cancer associated with high intakes of vitamin C.	No biochemical measurements. Retrospective period was 2.7 to 2.8 years. Food portion sizes estimated from food composition tables.
Ziegler, et al., 1990 (Ref. 98).	Case-control, cross-sectional multi-center study.	271 cases of cervical cancer, 502 controls matched by age, race, telephone exchange.	Data collected by interview. Medical history, family health, food intake data collected.	No effect of vitamin C or vitamin C-rich foods on risk of cervical cancer.	Cases confirmed during 1982 and 1983. No reference period relative to onset or symptoms of diagnosis in cases.

TABLE 14.—ANTIOXIDANT VITAMINS AND CANCER: OTHER STUDIES

Study	Type and location	Subjects	Methods	Results	Comments
La Vecchia, et al., 1989 (Ref. 49).	Case-control, Northern Italy.	163 cases of confirmed bladder cancer; 181 hospital controls.	All subjects interviewed for information about health history, alcohol, tobacco use, coffee consumption, etc. Dietary data obtained from food frequency questionnaire.	Bladder cancer cases had lower frequency of consumption of green vegetables, carrots. No reduced risk related to intake of vitamin C.	No biochemical measurements. Food frequency questionnaire had only 10 items.
Stahelin, et al., 1989 (Ref. 61).	Prospective cohort, Basel, Switzerland.	2,975 men, 102 cancer deaths (lung, 37; stomach, 17; colorectal, 9; others 39).	Samples were collected in 1971 to 1973 and analyzed immediately.	Vitamin C was significantly lower in cases of stomach cancer than in controls.	Reliance on point sample analysis. No dietary data.

TABLE 15.—ANTIOXIDANT VITAMINS AND CANCER: PROSPECTIVE STUDIES ON VITAMIN E AND CANCER

Authors/year	Source/exposure	Study population	Cancer site	Cases/controls	Results	Assessment of the study
Wald, et al., 1987 (Ref. 57).	Serum vitamin E	22,000 men, 35 to 64 years, London, England.	Lung, colorectal, stomach, bladder, CNS, skin, other sites, all sites.	9/17, 6/12, 3/6, 8/15, 5/10, 31/57, 28/55, 90/172.	Mean vitamin E level of the cases was not significantly different from the controls in cancer subjects diagnosed >1 year after blood collection. Vitamin E levels of cancer cases diagnosed <1 year after blood collection was significantly lower than controls.	No dietary use data or data on the health history of the controls. Cases per cancer site were small. The difference reported for cases diagnosed at <1 year may have been the consequence of cancer rather than the precursor.
Russell, et al., 1988 (Ref. 117).	Serum vitamin E	5,086 women, 26 to 88 years, Guernsey, England.	Breast	30/288	No significant difference between the serum vitamin E levels of cases with breast cancer and the controls.	No matching for smoking, long storage time of the samples and no dietary use data.
Gey, et al., 1987 (Ref. 109).	Plasma vitamin E	3,000 men, Basel, Switzerland.	All sites, lung, gastrointestinal survivors.	102, 37, 17, 2,707.	Significantly lower plasma vitamin E levels were associated with overall cancer and combined gastrointestinal cancer mortality.	No dietary use data. Small size was very small per site.
Stahelin, et al., 1989, 1991 (Refs. 60 and 61).	Plasma vitamin E	2,974 men Basel, Switzerland.	Bronchial, stomach, gastrointestinal, colon, survivors.	68, 20, 37, 17, 2,421.	No significant association of plasma vitamin E and the risk of cancer.	No dietary data. Results did not confirm findings reported in an earlier study using the same population group.

TABLE 15.—ANTIOXIDANT VITAMINS AND CANCER: PROSPECTIVE STUDIES ON VITAMIN E AND CANCER—Continued

Authors/year	Source/exposure	Study population	Cancer site	Cases/controls	Results	Assessment of the study
Schober, et al., 1987 (Ref. 116).	Serum vitamin E.....	25,802 adults, Washington County, Maryland.	Colon.....	72/143.....	No significant association of serum vitamin E and colon cancer.	No dietary data and long storage time of the sera samples.
Burney, et al., 1988 (Ref. 115).	Serum vitamin E.....	25,620 adults, Washington County, Maryland.	Pancreas.....	22/44.....	Low levels of serum vitamin E appear to have a protective effect on pancreatic cancer.	A chance association between vitamin E and pancreatic cancer could not be excluded. Samples were stored for a long time period and there was no information on diet history or intake. The number of cases was also very small.
Comstock, et al., 1991 (Ref. 114)	Serum vitamin E.....	25,800 adults, Washington County, Maryland.	Colon; rectum; pancreas; lung; melanoma, skin; basal cell, skin; Breast; Prostate; Bladder.	72/143, 31/68, 22/44, 99/196, 20/40, 21/42, 30/59, 103/ 103, 35/70.	The association of vitamin E with lung cancer showed a dose-response trend in a protective direction. There were no other significant associations.	Storage time of samples was very long, sample size at some cancer sites was small and no diet history or usage reported.
Knekt, et al., 1988 (Ref. 112).	Serum vitamin E.....	36,265 men and women, 15 to 99 years, Finland.	Gastrointestinal.....	150/276.....	Upper gastrointestinal tract cancers were associated with lower serum vitamin E. The risk differed significantly between tertiles among men.	Samples collected and stored a long time before analysis of vitamin E and no diet usage data were reported.
Knekt, et al., 1988 (Ref. 111).	Serum vitamin E.....	21,172 men, Finland.....	All sites, Stomach, Pancreas, Colon and rectum, Other sites.	453/841, 46/90, 17/28, 21/39, 367/723.	Significant increase in the risk for all cancers unrelated to smoking and some gastrointestinal cancers with low serum vitamin E.	The number of cases at certain sites was small, samples were stored for a long period of time and no dietary data were reported.
Knekt, et al., 1988 (Ref. 110).	Serum vitamin E.....	15,093 women, Finland.	All sites, Epithelial, Breast, Cervix, Endometrium, Ovary.	313/578, 184/ 339, 67/123, 23/44, 12/21, 16/29.	Significant inverse association between serum vitamin E for all cancer sites combined and epithelial cancer.	The number of cases at some sites was small, samples were collected and stored a long time before analysis of vitamin E and no dietary data reported.
Knekt, et al., 1991 (Ref. 113).	Serum vitamin E.....	36,265 men and women, Finland.	All sites, Gastrointestinal.	557 sets, 160 sets.	The association of serum vitamin E with cancer was strongest for all cancer sites and gastrointestinal cancer.	Same as above.

TABLE 16.—ANTIOXIDANT VITAMINS AND CANCER: RETROSPECTIVE STUDIES ON VITAMIN E AND CANCER

Authors/year	Source/exposure	Location	Cancer site	Cases/controls, type of control	Results	Assessment of the study
Basu, et al., 1980 (Ref. 118).	Serum vitamin E.....	Canada.....	Breast.....	30/30, Population...	No significant association between breast cancer and serum vitamin E.	All cases were in the advanced stage of breast cancer. No dietary or clinical data were reported. Sample size was small.
Gerber, et al., 1989 (Ref. 119).	Vitamin E intake..... Plasma Vitamin E.....	France.....	Breast.....	120/109, Hospital ..	Plasma vitamin E was significantly higher in cases than controls.. No significant association of vitamin E intake and breast cancer.	The control group was made of hospital patients and no information was given on medication use. Dietary data were retrospective.

TABLE 16.—ANTIOXIDANT VITAMINS AND CANCER: RETROSPECTIVE STUDIES ON VITAMIN E AND CANCER—Continued

Authors/year	Source/exposure	Location	Cancer site	Cases/controls, type of control	Results	Assessment of the study
Toniolo, et al., 1989 (Ref. 84).	Vitamin E intake		Breast	250/499, Population	No significant association of vitamin E intake and breast cancer.	No biochemical data were reported. Dietary data were retrospective and obtained on the average of 7.8 months after diagnosis.
LeGardeur, et al., 1990 (Ref. 120)	Serum vitamin E	New Orleans, Louisiana	Lung	59/59, Hospital	Lower serum vitamin E of cases when compared with controls. Adjustment for serum cholesterol reduced the differences.	No dietary data were reported. Hospital patients who may have been predisposed to changes in serum vitamin E were used as controls. Groups were not matched for smoking history.
Charpiot, et al., 1989 (Ref. 121)	Serum vitamin E	France	Digestive	56/50, Hospital	Serum vitamin E of cases were significantly lower than controls.	Dietary intake information was not reported. No information was provided on whether the hospitalized controls were on any medication. Data were not provided on the stage or type of digestive cancer.
Buiatti et al. 1990 (Ref. 92).	Vitamin E intake	Italy	Gastric	1,016/1,159, Population	Risk decreased with increased intakes of vitamin E and C.	No biochemical data were reported. Retrospective dietary data on vitamin E were calculated using English tables.
Verrault, et al., 1989 (Ref. 63).	Vitamin E intake	Seattle, Washington	Cervical carcinoma	189/227, Population	High vitamin E intake was associated with lower cancer risk.	No biochemical data were reported. Retrospective dietary data focused on the recall of foods use several years prior to the interview.
Cuzick, et al., 1990 (Ref. 122).	Serum vitamin E	London	Cervical intraepithelial neoplasia I, Cervical intraepithelial neoplasia II.	30/40, 40/45. Patients of general practitioners and clinics.	Significant decreasing trend of mean levels of serum vitamin E in cases.	No dietary data reported. Sample size was small. No medical history of controls was reported.
Freudenheim, et al., 1990 (Ref. 77).	Vitamin E intake	Western New York	Rectal	277/277 (men), 145/145 (women), Neighborhood.	No significant association between rectal cancer and vitamin E intake.	No biochemical data reported and retrospective food frequency interviews were used.
Stryker, et al., 1990 (Ref. 125).	Plasma vitamin E, Vitamin E intake	Boston	Malignant melanoma	204/248, Skin clinic.	Intake alone displayed a significant trend of decreasing risk with increasing intake.	Clinic patients were used as controls and no information was reported on medication or supplement use.
Drozdz, et al., 1989 (Ref. 124).	Serum vitamin E	Poland	Larynx	22/16, Hospital	No significant association of serum vitamin E and cancer of the larynx.	Hospital controls were used who may have been predisposed to changes in serum vitamin E. No diet or supplement information were reported. Sample size was very small.