DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 356

[Docket No. 81N–033P]

RIN 0910–AA01

Oral Health Care Drug Products for Over-the-Counter Human Use; Antigingivitis/Antiplaque Drug Products; Establishment of a Monograph

AGENCY: Food and Drug Administration, HHS.

ACTION: Advance notice of proposed rulemaking.

SUMMARY: The Food and Drug Administration (FDA) is issuing an advance notice of proposed rulemaking that would establish conditions under which over-the-counter (OTC) drug products for the reduction or prevention of dental plaque and gingivitis are generally recognized as safe and effective and not misbranded. This notice is based on the recommendations of the Dental Plaque Subcommittee of the Nonprescription Drugs Advisory Committee (NDAC) and is part of FDA's ongoing review of OTC drug products.

DATES: Submit written or electronic comments by August 27, 2003. Submit reply comments by October 27, 2003.

ADDRESSES: Submit written and reply comments to the Dockets Management Branch (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Submit electronic comments to *http://www.fda.gov/dockets/ecomments.*

FOR FURTHER INFORMATION CONTACT: Robert L. Sherman, Center for Drug Evaluation and Research (HFD–560), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301–827–2222.

SUPPLEMENTARY INFORMATION: In accordance with part 330 (21 CFR part 330), FDA received on December 3, 1998, a report on OTC antigingivitis/antiplaque drug products from the Dental Plaque Subcommittee (the Subcommittee). FDA regulations (§ 330.10(a)(6)) provide that the agency issue in the **Federal Register** a proposed rule containing: (1) The monograph recommended by the Subcommittee, which establishes conditions under which OTC antigingivitis/antiplaque drug products are generally recognized as safe and effective and not misbranded; (2) a statement of the conditions excluded from the monograph because the Subcommittee determined that they would result in the drugs not being generally recognized as safe and effective or would result in misbranding; (3) a statement of the conditions excluded from the monograph because the Subcommittee determined that the available data are insufficient to classify these conditions under either (1) or (2) of this paragraph; and (4) the conclusions and recommendations of the Subcommittee.

The unaltered conclusions and recommendations of the Subcommittee are issued to stimulate discussion, evaluation, and comment on the full sweep of the Subcommittee's deliberations. The report has been prepared independently of FDA, and the agency has not yet fully evaluated the report. The Subcommittee's findings appear in this document to obtain public comment before the agency reaches any decision on the Subcommittee's recommendations. This document represents the best scientific judgment of the Subcommittee, but does not necessarily reflect the agency's position on any particular matter contained in it.

The Subcommittee was asked for its general recommendations on combination products in which antigingivitis/antiplaque ingredients are combined with other oral health care ingredients. The Subcommittee recommended the following as rational oral health care combination products: (1) An antigingivitis/antiplaque active ingredient combined with an anticaries active ingredient, (2) an antigingivitis/antiplaque active ingredient combined with a tooth desensitizer active ingredient, and (3) an antigingivitis/antiplaque active ingredient combined with an anticaries active ingredient and a tooth desensitizer active ingredient.

However, the agency is not aware of any marketing history of such combination products eligible for the OTC drug review, nor were such combinations submitted to the Subcommittee. Therefore, the agency is dissenting from these recommendations at this time. Data are needed to establish the safety and effectiveness of these combination products. Accordingly, none of the combination products described above may be marketed OTC at this time under this advance notice of proposed rulemaking. The agency invites supporting data and information demonstrating that these combination products can be generally recognized as safe and effective for OTC use.

Based on proposals from industry, the Subcommittee also made general recommendations on testing requirements for final product formulations to be considered effective. The agency is seeking specific information from interested parties on testing protocols, effectiveness criteria, and statistical methods employed to analyze the data from these tests.

The agency notes that the Subcommittee concluded that an active ingredient could be either an antigingivitis agent or an antigingivitis/antiplaque agent. While an ingredient may also be effective in reducing plaque, the Subcommittee stated that the therapeutic endpoint for both antigingivitis and antigingivitis/antiplaque active ingredients is a significant reduction in gingivitis, which can be measured using gingival index scores (see section II.C of this document).

The Subcommittee concluded that there is an association between plaque and gingivitis. The Subcommittee agreed, however, that the exact relationship between plaque and gingivitis cannot be quantified. Because the data submitted to support the effectiveness of stannous fluoride in reducing plaque were inconclusive, the Subcommittee proposed an ''antigingivitis'' statement of identity for this ingredient. However, the Subcommittee's proposed indication for this ingredient includes a reference to plaque reduction.

Although it did not require that antigingivitis ingredients also be effective in reducing plaque, the Subcommittee agreed that ingredients that work primarily by means other than plaque reduction would be inappropriate for use in OTC antigingivitis drug products because these products may mask the symptoms of a more serious condition and cause consumers to delay seeking the advice of a dentist. Because the Subcommittee believed that none of the submitted active ingredients acted other than by reducing plaque, this issue was not further discussed.

Therefore, the agency is seeking comment on the basis for allowing an antigingivitis active ingredient that has not demonstrated effectiveness in reducing plaque to bear labeling statements relating to plaque reduction. More importantly, because of the safety concern that antigingivitis ingredients that work by a mechanism other than plaque reduction (e.g., anti-inflammatory) may give consumers a false sense of security by masking symptoms of a more serious disease, the agency is also seeking comment on whether products that are solely antigingivitis agents, i.e., products that do not significantly reduce plaque,

constitute appropriate OTC drug products.

After reviewing all comments submitted in response to this document, FDA will issue in the **Federal Register** a tentative final monograph (TFM) for OTC drug products for the reduction or prevention of dental plaque and gingivitis. Under the OTC drug review procedures, the agency's position and proposal are first stated in the TFM, which has the status of a proposed rule. Final agency action occurs in the final monograph, which has the status of a final rule.

In accordance with § 330.10(a)(2), the Subcommittee and FDA have held as confidential all information concerning OTC drug products for the reduction or prevention of dental plaque and gingivitis submitted for consideration by the Subcommittee. All submitted information will be put on public display in the Dockets Management Branch (*see* **ADDRESSES**) after June 30, 2003, except to the extent that persons submitting it demonstrate that it falls within the confidentially provisions of 18 U.S.C. 1905, 5 U.S.C. 552(b), or section 301(j) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 331(j)). Requests for confidentiality should be submitted to Robert L. Sherman, Center for Drug Evaluation and Research (*see* **FOR FURTHER INFORMATION CONTACT**).

The agency advises that the conditions under which the drug products that are subject to this monograph would be generally recognized as safe and effective and not misbranded (monograph conditions) will be effective 12 months after the date of publication of the final monograph in the **Federal Register**. On or after that date, no OTC drug products that are subject to the monograph and that contain nonmonograph conditions, i.e., conditions that would cause the drug to be not generally recognized as safe and effective or to be misbranded, may be initially introduced or initially delivered for introduction into interstate commerce unless they are the subject of an approved new drug application (NDA) or abbreviated new drug application (ANDA). Further, any OTC drug products subject to this monograph that are repackaged or relabeled after the effective date of the monograph must be in compliance with the monograph regardless of the date the product was initially introduced or initially delivered for introduction into interstate commerce unless they are the subject of an NDA or ANDA. Manufacturers are urged to comply voluntarily with the monograph at the earliest possible date.

A proposed review of the safety, effectiveness, and labeling of all OTC drugs by independent advisory review panels was announced in the **Federal Register** of January 5, 1972 (37 FR 85). The final regulations providing for this OTC drug review under § 330.10 were published and made effective in the **Federal Register** of May 11, 1972 (37 FR 9464). In accordance with these regulations, a request for data and information on all active ingredients used in OTC drug products bearing antiplaque and antiplaque-related claims was issued in the **Federal Register** of September 19, 1990 (55 FR 38560). These claims included the reduction or prevention of plaque, tartar, calculus, film, sticky deposits, bacterial buildup, gingivitis, diseased, inflamed, or swollen gums, pyorrhea, Vincent's disease, periodontal disease, and tooth-destroying acids.

The Commissioner of Food and Drugs appointed the following members of the Dental Products Panel (the Panel) to review the information submitted and to prepare a report under § 330.10(a)(1) and (a)(5) on the safety, effectiveness, and labeling of those products:

Paul B. Robertson, Chairperson
Charles N. Bertolami (resigned March 24, 1997)
William H. Bowen (term ended October 31, 1995)
Carlos E. del Rio (resigned December 14, 1994)
Julianne Glowacki (term ended October 31, 1994)
Deborah Greenspan
Richard D. Norman
Burton Rosan
Christine D. Wu

The Subcommittee, comprised of two members from the Panel plus five nonvoting consultants to the Panel, was subsequently formed to evaluate the submitted data and report its findings on the safety and effectiveness of ingredients for the reduction or prevention of dental plaque and gingivitis. Each of the following was a voting member of the Subcommittee:

William H. Bowen, Chairperson (term ended April 1995)
Robert J. Genco, Chairperson (from April 1995 to December 3, 1998)
Ralph D'Agostino
Max A. Listgarten
Shelia M. McGuire
Eugene D. Savitt
Stanley R. Saxe
Jorgen Slots (resigned April 12, 1995)
Christine D. Wu

Several nonvoting liaison representatives served on the Subcommittee. P. Jean Frazier, served as the consumer liaison until June 6, 1996, followed by Susan Cohen, until May 1997, and Donald S. Altman, on May 27, 1998. Frederick A. Curro, served as industry liaison (drug) until October 31, 1995, followed by Lewis P. Cancro. Gerald N. McEwen, Jr., served as industry liaison (cosmetic) until October 31, 1996.

On August 27, 1997, oversight of the Subcommittee was transferred from the Panel in the Center for Devices and Radiologic Health (CDRH) to the Nonprescription Drugs Advisory Committee in the Center for Drug Evaluation and Research (CDER).

The following FDA employees assisted the Subcommittee:

Carolyn Tollendi served as CDRH Executive Secretary to the Panel until June 7, 1996. Kennerly K. Chapman served as CDER Executive Secretary to the Subcommittee until December 17, 1996, followed by Andrea Neal until May 9, 1997, followed by Rhonda Stover (interim) until May 1998, followed by Kathleen Reedy. Jeanne L. Rippere served as CDER liaison to the Subcommittee until June 7, 1996, followed by Robert L. Sherman. Stephanie A. Mason served as special assistant to the Subcommittee until June 7, 1996.

The Panel and the Subcommittee were first convened on August 2 and 3, 1993, for a joint organizational meeting. Working meetings of the Subcommittee were held on December 16 and 17, 1993; June 28 and 29, October 11, and December 5, 6, and 7, 1994; April 10, 11, and 12, August 14 and 15, and December 4 and 5, 1995; June 6 and 7, and December 16 and 17, 1996; October 29 and 30, 1997; May 27, 28, and 29, October 22, and December 2 and 3, 1998. Joint meetings of the Panel and the Subcommittee were held on August 2 and 3, 1993, and December 6, 1994. Minutes of most Subcommittee meetings are on public display in the Dockets Management Branch (see **ADDRESSES**).

The following individuals appeared before the Panel and/or the Subcommittee at their own or at the Panel's or Subcommittee's request to discuss drug products for the reduction or prevention of plaque and gingivitis: Gariela Adam-Rodwell, Sam Amer, Daniel M. Bagley, John E. Bailey, Michael L. Barnett, Robert D. Bartizek, Kenneth Baumgartner, William J. Blot, Nancy L. Buc, Gregory A. Burkhart, Lewis P. Cancro, James R. Cheever, Philip Cole, W. Greg Collier, Mark M. Crisanti, Catherine C. Davis, Phillip Derfler, John M. DeSesso, Harvey L. Dickstein, Jerry A. Douglass, Matthew J. Doyle, W. Gary Flamm, William E. Gilbertson, Brian F. Gillespie, David M. Graham, Robert Heller, Jane E. Henney,

Ira D. Hill, Peter B. Hutt, Frederick N. Hyman, Eugene Kamper, Linda M. Katz, Bruce Kohut, Surinder Kumar, Anthony C. Lanzaiaco, Mark S. Leusch, Debbie L. Lumpkins, Milton V. Marshall, Stephanie A. Mason, Stephen F. McClanahan, Stephen H. McNamara, Jerome A. Merski, David Morrisson, Kevin P. Mulry, Anne J. Mustafa, Paul J. Okarma, C. Lee Peeler, Julie H. Rhee, David I. Richardson, Jeanne L. Rippere, Norman A. See, James M. Serafino, Samuel Shapiro, Robert L. Sherman, Chakwan Siew, Gregory Singleton, James Skiles, Thomas J. Slaga, R. William Soller, Steven D. Stellman, George K. Stookey, Howard Strassler, Stanley Tarka, Jr., John M. Treacy, Jack Vincent, Frank A. Volpe, Michael Weintraub, Clifford W. Whall, Jr., Donald J. White, Robert White, Charles Wiggins, David Williams, Gary M. Williams, Deborah Winn, Roy Witkin, and Patrice Wright. No person who so requested was denied an opportunity to appear before the Panel or Subcommittee.

The Subcommittee has thoroughly reviewed the literature and data submissions, listened to additional testimony from interested persons, and considered all pertinent data and information submitted through December 3, 1998, in arriving at its conclusions and recommendations. The Subcommittee wishes to thank the American Dental Association's (ADA) Council on Scientific Affairs for its assistance in providing data, information, and testimony during the course of the Subcommittee's deliberations. The ADA also provided its ''Guidelines for Acceptance of Chemotherapeutic Products for the Control of Supragingival Plaque and Gingivitis'' to the Subcommittee for consideration in making its recommendations on the requirements for safe and effective OTC antigingivitis/antiplaque ingredients.

In accordance with the OTC drug review regulations in § 330.10, the Subcommittee reviewed OTC drug products for the reduction or prevention of dental plaque and gingivitis with respect to the following three categories:

Category I—Conditions under which OTC drugs for the reduction or prevention of dental plaque and gingivitis are generally recognized as safe and effective and are not misbranded.

Category II—Conditions under which OTC drugs for the reduction or prevention of dental plaque and gingivitis are not generally recognized as safe and effective or are misbranded.

Category III—Conditions for which the available data are insufficient to permit final classification at this time.

I. Submission of Data and Information

Under the notices published in the **Federal Register** of September 19, 1990 (55 FR 38650), and March 8, 1991 (56 FR 9915), the following firms made submissions regarding OTC drug products that the Panel/Subcommittee determined contained active ingredients or labeling associated with claims relating to the reduction or prevention of dental plaque and gingivitis.

A. Submissions by Firms

TABLE 1.—FIRMS AND SUBMITTED PRODUCTS

Firm	Submitted Products
American Xyrofin (Morgan, Lewis & Bockius) Washington, DC 20036	Xylitol All Natural Toothpaste, Xytol 32 Dental Cream.
Amer Co., Montecito, CA 93150	Insadol Toothpaste, Pyoralene Toothpaste.
Angus Chemical Co., Northbrook, IL 60062	Hexetidine solution.
Chesebrough Pond's USA Co., Greenwich, CT 06836	CloseUp Antiplaque Toothpaste, Mentadent P Toothpaste.
Church & Dwight Co., Inc., Princeton, NJ 08543	Arm & Hammer Dental Tooth Powder, Dentifrice, and Gel.
CIBA-GEIGY Corp., Greensboro, NC 27419	Irgasan DP, Irgacare MP.
Clinical Product Research, Inc., Shreveport, LA 71109	Prozyme Toothpaste, Anti-Plaquer Oral Rinse, Anti-Plaquer Toothpaste.
Colgate-Palmolive Co., Piscataway, NJ 08855	Colgate Tartar Control Toothpaste, Gelkam Oral Care Rinse, Dentaguard Toothpaste.
E. Merck, Frankfurter, Germany	Thera-Med, Cholordont M.
E. B. Michaels Research Associates, Inc., Milford, CT 06460	Therasol Brush & Rinse Antiplaque Oral Hygiene Solution, Therasol Brush & Rinse Liquid Dentifrice Oral Irrigant.
Leaf, Inc., (Hyman, Phelps & McNamara) Washington DC 20005	Xylitol.
Lion Corp. (America), Memphis, TN 38138	Check-Up Gingival Toothpaste.
Madaus Medtech, Inc., (ACC Consulting Group, Inc.) Washington DC 20036	Parodontax Toothpaste.
Pfizer Inc, New York, NY 10017	Plax Pre-Brushing Dental Rinse.
Pierre Fabre, S.A., 81106 Castres Cedex, France	Eligydium Toothpaste, Eludil Mouthwash.
Prevention Laboratories (formerly 7-L Corp.), Harrisburg, IL 62947	Prevention Mouth Rinse.
Procter & Gamble Co., Cincinnati, OH 45242	Crest Gum Care Toothpaste.

TABLE 1.—FIRMS AND SUBMITTED PRODUCTS—Continued

Firm	Submitted Products
SmithKline Beecham Consumer Brands (Marion Merrell Dow, Inc.), Parsippany, NJ 07054	Cepacol Gold and Mint Mouthwashes, Gly-oxide Liquid.
Vipont Pharmaceuticals, Fort Collins, CO 80522	Viadent Toothpaste and Oral Rinses.
Warner-Lambert Co., Morris Plains, NJ 07950	Listerine Antiseptic Mouthwash.
WhiteHill Oral Technologies, Inc., Hazlet, NJ 07730	Omni-Med Brush-On Tooth Medication, Perio-Med Spray, Take-5 Plaque Fighter Brushless Dentifrice, Smokers Take-5 Plaque and Stain Fighter.
Witkins, Roy T., Westport, CT 06880	Perimed Oral Hygiene Rinse.

In categorizing ingredients as ''active'' and ''inactive,'' the advisory review panels relied upon their expertise and understanding of these terms. FDA has defined ''active ingredient'' in its current good manufacturing practice regulations in § 210.3(b)(7) (21 CFR 210.3(b)(7)) as:

[Any] component that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or other animals. The term includes those components that may undergo chemical change in the manufacture of the drug product and be present in the drug product in a modified form intended to furnish the specified activity or effect.

An ''inactive ingredient'' is defined in § 210.3(b)(8) as ''any component other than an active ingredient.''

B. Active Ingredients Submitted For Review

Labeled Ingredients Contained in Marketed Products Submitted to the Subcommittee:

Alkyl dimethyl amine oxide
Alkyl dimethyl glycine
Aloe vera
Bromchlorophene
Carbamide peroxide
Cetylpyridinium chloride
Chlorhexidine digluconate
Dicalcium phosphate dihydrate
Eucalyptol
Hexetidine
Hydrogen peroxide
Menthol
Methyl salicylate
Peppermint oil
Polydimethylsiloxane
Poloxamer
Povidone iodine
Sage oil
Sanguinaria extract
Sodium bicarbonate
Sodium citrate
Sodium lauryl sulfate
Soluble pyrophosphate
Stannous fluoride
Stannous pyrophosphate
Thymol
Triclosan
Unsaponifiable fraction of corn oil
Xylitol
Zinc chloride
Zinc citrate

Some of these ingredients (bromchlorophene, chlorhexidine digluconate, hexetidine, soluble pyrophosphate, triclosan, unsaponifiable fraction of corn oil) were not marketed for a material time and to a material extent for antigingivitis/antiplaque use in the United States. (See 21 U.S.C. 321(p)(2).) Although the Subcommittee reviewed data to support the safety and effectiveness of these ingredients, they are not eligible for inclusion in the OTC drug review as part of this advance notice of proposed rulemaking and, therefore, are not discussed in this document. In addition, although xylitol was reviewed by the Subcommittee, the two firms that submitted data subsequently withdrew xylitol from consideration by the Subcommittee. Therefore, xylitol is not discussed.

The nomenclature used by the Subcommittee for the ingredients reviewed in this document was the currently accepted terminology stated in the 1996 edition of ''USAN and the USP Dictionary of Drug Names.'' Names recommended by FDA were used for any ingredients which did not have USAN names.

C. Referenced OTC Volumes

All ''OTC Volumes'' cited throughout this document refer to submissions made by interested persons under the call-for-data notices published in the **Federal Register** of September 19, 1990, and March 8, 1991. The information included in these volumes, except for those deletions made in accordance with the confidentiality provisions in § 330.10(a)(2), will be put on public display after June 30, 2003, in the Dockets Management Branch (see **ADDRESSES**).

II. General Statements and Recommendations

A. Definitions

The Subcommittee adopted the following definitions as its intended meaning of terms specifically used in this document concerning OTC drug products for the reduction or prevention of dental plaque and gingivitis. The Subcommittee was aware that some degree of variation with other definitions of the same term may exist.

• *Calculus.* The hard concretions (*i.e.,* calcified plaque) that form on teeth, prostheses, and other hard surfaces. Calculus on teeth is clinically classified into supragingival calculus, which is located on surfaces not covered by the oral mucosa, and subgingival calculus, which is located apical (at the top) to the soft tissue margin of the gingiva.

• *Dental Plaque.* Organized coherent gel-like or mucoid masses consisting of microorganisms in an organic matrix derived from saliva and extracellular bacterial products such as glucans, fructans, enzymes, toxins, and acids. Plaque also contains other cells (e.g., desquamated epithelial cells) and inorganic components such as calcium and phosphate. It adheres to the teeth and other surfaces of the oral cavity. It occurs at the orifice of the gingival crevices and in the periodontal pockets. Plaques may differ markedly in biochemical or microbial composition, and their localization.

• *Gingival Sulcus.* The shallow groove between the tooth and the marginal gingiva.

• *Gingivitis.* An inflammatory lesion of the gingiva that is most frequently caused by dental plaque. Gingivitis is characterized by tissue swelling and redness, loss of stippling (a normal state in which the surface of healthy gingiva is comprised of small lobes), glossy surface, and increased tissue

temperature. The gingiva also may bleed upon gentle provocation such as toothbrushing or may bleed spontaneously. Gingivitis is usually not painful.

• *Oral Hygiene*. Self-administered processes aimed at controlling microbial and other deposits in the oral cavity.

• *Pellicle*. A thin, colorless, translucent film derived from bacterial products and saliva, which forms rapidly on tooth surfaces after natural cleansing or prophylaxis. A few hours after deposition, oral bacteria begin to adhere to the pellicle. These processes represent the earliest stages of plaque formation.

• *Periodontitis*. A disease condition of the periodontium characterized by inflammation of the gingiva, increasing probing depth, and destruction of the periodontal ligament and the adjacent supporting alveolar bone.

• *Tartar*. A synonymous term for calculus.

B. Background and General Discussion of Terms

1. Background

The Subcommittee was charged with the evaluation of the safety and effectiveness of ingredients or combinations of ingredients for the reduction or prevention of plaque and gingivitis as claimed in the labeling of OTC drug products in light of present-day knowledge and standards used in pharmacology, pharmacodynamics, therapeutics, and toxicology.

In making its evaluation, the Subcommittee relied upon factual data found in standard textbooks and scientific articles published by independent investigators in medical, dental, and other scientific journals. Manufacturers included some of these scientific articles in their submissions to FDA to provide a scientific basis for claims made for the safety and effectiveness of their ingredients. Data supplied by manufacturers in unpublished reports of studies performed by private laboratories under contract to the manufacturer or in manufacturers' laboratories were also used by the Subcommittee in making judgments. The Subcommittee also gave due consideration to data from marketing experience and widespread clinical usage when in agreement with basic data from controlled studies and scientific facts.

2. Plaque

Plaque, also known as dental plaque and/or microbial plaque, has been examined for several decades with most of the information explained in the past 25 years. Plaque has a critical etiological role in the development of dental caries, gingivitis, and periodontal disease. It is now clear that dental plaque is a variable biologic community made up of bacteria and a bacterially synthesized matrix. While dental plaque may be combined with other materials such as food particles and sloughed epithelial cells, the combination of these components is called materia alba and is no longer considered plaque.

The precise genera and species of microorganisms in each dental plaque may differ from individual to individual, site to site in the same individual, and within a specific site over time. Plaque from sites of similar clinical health within individual subjects tends to be more similar in composition than plaque from sites in different subjects. Even though there is considerable variation within dental plaques, the composition of plaque is influenced by several factors. The composition of dental plaques is currently known to be affected by plaque age, dietary intake of sucrose and other foods, and other factors (*e.g.,* friction of mastication, oral health, and salivary flow).

Plaque composition is also affected by its location above or below the gingiva. Dental plaques are subdivided into supragingival plaque and subgingival plaque. The distinction resides in the location of dental plaque as either coronal (toward the crown) or apical (toward the root tip) to the soft tissue margin. The microbial populations may differ in plaque from the two locations.

The extracellular matrix synthesized by the bacteria is a significant component of plaque. Because the matrix provides plaque organisms with strong adhesive and cohesive properties, plaque is not easily removed. The tenacity of plaque to adhere to the surfaces of oral structures can be used to distinguish plaque from debris, in that plaque is not removed by flushing the mouth with water.

Plaques differ not only quantitatively but qualitatively in their bacterial composition. For example, microorganisms found in dental plaque include *Actinomyces* species, *Streptococcus sanguis*, *S. mutans*, and other *Streptococcus* species, *Spirochetes*, *Porphyromonas gingivalis*, *Bacteroides forsythus*, and other *Bacteroides* species, *Campylobacter recta*, *Peptostreptococcus micros*, *Eikenella corrodens*, *Actinobacillus actinomycetemcomitans*, *Eubacterium* species, *Fusobacterium* species, *Capnocytophaga* species, and *Prevotella* species. This difference in bacterial composition has a major effect on its pathogenic potential both for periodontal diseases and caries. Some dental plaques are not pathogenic or associated with disease, whereas others are etiologic factors for caries and periodontal diseases. However, the two types of plaque cannot be distinguished visually. The pathogenic potential is dependent upon the microbial composition, including the metabolic products of microbes, dietary patterns, and the intrinsic resistance of the host. It may be prudent to treat all plaques as having pathogenic potential.

3. Calculus

Calculus is a hard concretion that forms on the teeth or dental prostheses through deposition of mineral salts in dental plaques. Human calculus is essentially mineralized dental plaque, which is almost always covered on its external surface by vital, tightly adherent, nonmineralized soft plaque. There may also be loosely held materials associated with calculus such as materia alba, shed bacteria, desquamated epithelial cells, and blood cells. In germ-free animals, calcified deposits may occur in the absence of bacterial accumulation (Ref. 1). However, in humans, virtually all calculus seen clinically likely results from the deposition of calcium and phosphates within bacterial plaques. Calculus formation occurs in an orderly fashion, beginning after 1 or 2 weeks of plaque formation and resulting in full calcification of plaque after 2 to 4 weeks. The process occurs more rapidly in some persons than in others.

Calculus may form subgingivally and is often stained and tenaciously attached to the crown and/or root of the tooth. Calculus may also form supragingivally, coronal (toward the crown) to the gingival margin. Supragingival calculus is found in greater amounts on tooth surfaces adjacent to the openings of the ducts of the major salivary glands. Both subgingival and supragingival calculus are often stained; supragingival calculus can be unsightly, particularly when formed in abundance on labial (facing the lips) surfaces. Although subgingival calculus is a contributing factor in the development of gingivitis, and can also be associated with the progression of gingivitis, periodontitis, and periodontal abscesses, the exact nature of the role of supragingival calculus in gingivitis is not clear. Supragingival calculus can accumulate plaque and act as a nidus (nest) for plaque formation, which can lead to gingivitis.

Calculus facilitates the retention of dental plaque in close proximity to the periodontal tissues. It reduces the

effectiveness of overall hygiene methods to control dental plaque accumulation. Subgingival calculus interferes with the regeneration of lost periodontal attachment.

The removal of calculus is considered a basic step in the prevention and treatment of inflammatory periodontal diseases. The formation of supragingival calculus can be limited through mechanical or chemical methods. Preventing subgingival calculus formation, if possible, would not necessarily reduce gingivitis, because a surface currently free of calculus can still harbor plaque. Present methods do not allow for the predictable prevention of subgingival calculus.

4. Gingivitis

Gingivitis, an inflammation of the gingiva, affects most of the population at one time or another. The signs of gingivitis are tissue swelling and redness, loss of stippling, glossy surface, and increased tissue temperature. The gingiva may also bleed upon gentle provocation, such as toothbrushing, or may bleed spontaneously. Some signs of gingivitis, such as bleeding, can be identified by lay persons.

Gingivitis is a response to injury, often resulting in localization of tissue damage and neutralization of the effects of injurious agents. If the injurious agents cannot be adequately neutralized or eliminated, they may lead to chronic inflammation of the soft tissue and periodontitis. While most cases of periodontitis are believed to start with gingivitis, most cases of gingivitis do not progress to periodontitis. Histologically, gingivitis is characterized by inflammatory exudate or infiltrate, loss of collagen of the gingival connective tissue, and proliferation of the epithelium into the infiltrated tissue. Sometimes the epithelium lining the sulcus (crevice bounded by the tooth and free gingiva) may develop microulcerations. In gingivitis, the junctional epithelium usually is at or near the cementoenamel junction (junction of the tooth crown and root).

Gingivitis, especially when severe, may be self-diagnosable because people can recognize some of the signs of gingivitis, such as bleeding, gingival discoloration, and swelling, which gives rise to pseudopockets (pocket-like structure caused by inflammation of the gingiva without effecting the sulcus base). In the early stages of gingivitis when there is little or no pseudopocket formation, only noncalcified plaque, and little or no calculus, thorough daily oral hygiene may resolve the disease. Under these conditions, self-treatment of gingivitis is appropriate. When OTC drug products for the prevention and control of plaque-associated gingivitis are used as part of a program of good oral hygiene, including regular dental checkups, they can help consumers maintain their gingival health.

The most common form of gingivitis is termed marginal gingivitis and occurs in all individuals at some time. It is limited to the gingivae around the collar of the tooth. However, people are seldom easily able to detect sites with mild gingivitis because there may be no pain or bleeding. Plaque-associated gingivitis, an inflammation of the interdental and marginal gingiva, can be controlled or prevented by removal or inhibition of microbial plaque accumulation. Chemotherapeutic agents can enhance the benefits of traditional methods of oral cleansing by toothbrushing with a dentifrice and regular use of dental floss and other cleaning aids.

Readily available OTC drug products for the prevention and control of plaque-associated gingivitis are intended to play a significant public health role. However, the effects of these products in periodontitis have not been determined in large scale studies. OTC drug products are useful adjuncts to, but do not replace, regular professional care.

In the later stages of gingivitis with the formation of pseudopockets and calculus, it becomes more difficult for people to resolve the gingivitis. Therefore, self-treatment has limited potential for resolution of severe gingivitis, which should be treated as part of a regular professional care program. Gingivitis can progressively worsen and lead to the development of pockets that can be difficult for people to clean.

5. The Interrelationship Between Plaque and Gingivitis

Dental plaque can be causally related to gingivitis. A critical plaque mass at the gingival margin for a particular length of time can initiate change. However, the Subcommittee has no knowledge of any studies where the volume, mass, or amount of plaque can be closely equated with the extent of gingival inflammation. There is a general, positive relationship between supragingival plaque levels and levels of gingivitis. For example, with little or no supragingival plaque accumulation, most often there is gingival health, whereas heavy levels of plaque accumulation, especially at the gingival margin, are often associated with gingivitis.

Plaque forms readily on tooth surfaces in individuals with poor oral hygiene. It takes, histologically, about 3 to 4 days with no oral hygiene in periodontally healthy subjects to develop microscopic evidence of gingivitis. This evidence consists of infiltration of the gingival epithelium, especially the junctional epithelium, with inflammatory cells (including neutrophils), infiltration of the gingival connective tissue with lymphocytes, and beginning loss of collagen.

The Subcommittee does not know how long plaque must be present before gingivitis spontaneously appears. When distinguishing between experimentally induced gingivitis and spontaneous gingivitis (developing under conditions of normal oral hygiene) the following are found: (1) Most subjects over a period of 1 to 3 weeks of cessation of oral hygiene developed gingivitis measurable with clinical indices, and (2) subjects must accumulate a certain level of plaque before clinical signs of gingivitis are apparent. In addition, mature plaque with complex flora appears to be correlated with gingivitis. However, mature plaque, comprised of a complex gram-positive and gram-negative flora with motile organisms, is often associated with spontaneous gingivitis.

The Subcommittee accepts that gingivitis is associated with an accumulation of plaque along the gingival margin but is unaware of any evidence that shows that there is a close correlation between the amount of plaque and the induction of gingivitis, as can be assessed using present day methods. It should be noted that the relationship between the quantity of plaque present and the degree of gingivitis is sufficiently complex such that reductions in plaque mass alone are inadequate to conclude that a therapeutic effect on gingivitis could be expected. Therefore, gingivitis reductions must be measured directly.

6. Periodontitis

Most cases of periodontitis are believed to start with gingivitis, although not all cases of gingivitis lead to periodontitis. Periodontitis is characterized clinically by gingivitis of varying severity, loss of periodontal attachment, increased probing depth, and radiographically detectable loss of alveolar and supporting bone. In advanced disease, the teeth may become increasingly mobile. Progression of gingivitis and the relationship of gingivitis to the onset of periodontitis are not well understood. However, one approach to addressing this relationship comes from human studies in which meticulous oral hygiene leading to excellent plaque control and control of gingivitis appears to prevent the onset of

periodontitis (Ref. 2). It is not clear whether this prevention was due to reduction of supragingival plaque associated with gingivitis, or to meticulous oral hygiene, which also prevents colonization of the subgingival area by periodontal pathogens that are responsible for the onset of periodontitis. What is clear, however, is that in most instances meticulous plaque control appears to lead to reduction of gingivitis and suppression of the onset or rate of progression of periodontitis. Despite periodontal treatment, loss of periodontal attachment and loss of bone often persists. Moreover, people treated for periodontitis may suffer from recurrent gingivitis, root sensitivity, and increased susceptibility to root caries. Periodontitis appears to progress in alternating cycles of exacerbation, which are often asymptomatic and localized, followed by periods of remission. Population studies indicate that systemic conditions such as diabetes mellitus and neutrophil disorders, as well as smoking, increase the risk for developing periodontitis (Refs. 3 and 4).

Histologically, the gingiva becomes inflamed, and the sulcus is deepened to form a pocket which is lined with a pathologically altered epithelial lining, the pocket epithelium. The junctional epithelium is displaced apically. The pocket is largely filled with a subgingival microbiota that is in contact with the adjacent denuded root surface or adherent subgingival calculus deposits. The alveolar process (portion of the upper and lower jaws that forms and supports the tooth sockets) shows evidence of destruction in a ''horizontal'' or ''vertical'' pattern with concomitant loss of the connective tissue attachment to the root.

There are several variants of the disease, including adult periodontitis, early-onset periodontitis (which includes localized juvenile), periodontitis associated with systemic diseases, necrotizing ulcerative periodontitis, and refractory and recurrent periodontitis. Of these, adult periodontitis is the most common form of the disease, and it responds most predictably to scaling, root planing, and plaque control.

7. Oral Hygiene

The Subcommittee's definition of oral hygiene in this document represents the self-administered processes aimed at controlling microbial and other deposits in the oral cavity. Regular oral hygiene, by interfering with plaque accumulation and maturation, favors facultative (able to grow or live with or without oxygen) over anaerobic (growing or living in the absence of oxygen) bacteria. In the process, regular oral hygiene promotes clean dentition and fresh breath, and decreases the risk of plaque-mediated inflammatory changes in the oral cavity. Today, mechanical plaque removal with assorted devices is the primary method for maintaining good oral hygiene. Chemical plaque control (*e.g.,* antiseptic or surfactant mouthrinses) is used primarily as an adjunct to mechanical methods and may be particularly useful for the treatment of surfaces that are not readily accessible to mechanical cleansing, for postsurgical plaque control, and for oral care of handicapped persons. Antibiotics may be used as adjuncts to oral hygiene to suppress or eliminate specific segments of the bacterial population not readily accessible to mechanical cleansing.

C. Drug/Cosmetic Status

The current statutory definitions of ''drug'' and ''cosmetic'' require some consideration when applying them to products for the reduction or prevention of plaque and gingivitis. According to the act, a ''drug'' includes any article ''intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease,'' or any article ''intended to affect the structure or any function of the body * * * .'' (*See* 21 U.S.C. 321(g).) According to the act, a ''cosmetic'' includes an article or component thereof ''intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance * * *.'' (*See* 21 U.S.C. 321(i).)

Some products may not clearly fall under one definition or the other. Therefore, another consideration in classifying a product is the ''intended use'' of the product, which is largely dependent on the claims made for the product and the accompanying labeling.[1] In attempting to accurately describe a product's benefits, one of the guiding principles should be to avoid misleading the public with ambiguous claims. Unfortunately, in the case of mouthrinse products, it is easy to make claims that suggest a drug-like benefit, while staying within the guidelines for cosmetic products. Much of the controversy regarding the ''drug'' versus ''cosmetic'' issue for these products revolves around the use of the word ''dental plaque'' or its synonyms (plaque, bacterial deposits, etc.).

1. Antiplaque Products

It is the position of the ADA and the American Academy of Periodontology that the control of dental plaque is a therapeutic procedure basic to the prevention and treatment of caries and periodontal diseases, particularly the latter. The well-established association between dental plaque accumulation and gingivitis demands that effective control of gingivitis be accompanied by effective control of dental plaque. ''Nonspecific'' plaque control involves decreasing the entire microbial mass in a nonspecific manner, i.e., without any attempt at differentially removing or suppressing any particular bacterial species, although shifts in bacterial composition may occur. It is the primary therapy for preventing and controlling periodontal infections that may lead to periodontal inflammatory lesions.

''Specific'' plaque control implies the control of specific pathogens, using strategies that will preferentially suppress certain species or categories of microorganisms. This approach generally requires the use of antimicrobial agents, typically antibiotics, with a specific antimicrobial spectrum. Ideally, the microbial composition of the dental plaque should be assessed before and after treatment to insure that the antimicrobial agents used are appropriate and that the therapy has the desired effect.

The nonspecific control of dental plaque needs to be thorough in order to achieve clinically significant therapeutic benefits. While some OTC oral health care products may be able to reduce the rate of plaque formation to a statistically significant degree, the inhibitory effect on plaque is often insufficient to be considered of therapeutic benefit. It is also highly unlikely that the marginal control of bacterial deposits has a significant relationship to most, if not all, of the cosmetic claims. Outcome variables such as taste and ''feel'' are more likely to be affected by flavoring agents and products that reduce surface tension than by minor variations in plaque accumulation.

The claim that a product significantly reduces dental plaque (statistically speaking) may mislead people into thinking that the reduction is therapeutically significant. Thus, people may purchase a product with the mistaken notion that a therapeutic benefit may be derived from its use, instead of seeking effective care for

[1]The legal opinions of this scientific panel in this area may not and do not necessarily reflect FDA's position.

potential signs and symptoms of disease.

Therefore, the Subcommittee proposes that any reference to the control of dental plaque or its equivalents, with or without qualifications, should be interpreted as a drug claim. In addition, the Subcommittee proposes that an OTC drug product making any reference to the reduction or prevention of dental plaque also must demonstrate a clinically significant effect on gingivitis. Thus, antiplaque claims should not stand alone.

2. Tartar Products

The Subcommittee proposes that any reference to supragingival tartar (calculus) be interpreted as a cosmetic claim. The Subcommittee did not make any reference to subgingival tartar.

D. Labeling of Antigingivitis/Antiplaque Drug Products

Having reviewed the submitted labels of antigingivitis/antiplaque drug products, the Subcommittee recommends that labeling include the following:

1. Ingredients

Antigingivitis/antiplaque agents should contain only active ingredients plus such inactive ingredients as may be necessary for formulation. The label should state the name and quantity of each active ingredient in appropriate units as specified later in this document.

For various reasons, including allergic reactions, safety concerns, and personal preference, individuals may wish to avoid using certain inactive ingredients. It is impossible to make a free choice in this regard unless all the components of drug products are listed on the labels. Therefore, the Subcommittee strongly recommends that all inactive ingredients be listed on the label in descending order of quantity. However, the product should not imply or claim that its inactive ingredients have a therapeutic benefit. The Subcommittee recognizes that although full disclosure of flavoring and coloring ingredients is desirable, this may be impractical and confusing because of the large number of ingredients that may be involved. Thus, flavoring and coloring ingredients may be listed in accordance with present regulations for labeling such ingredients in cosmetic products (21 CFR 701.3).

2. Statement of Identity

The labeling must indicate the principal intended action of the active ingredient as well as the indication for use of the product. The Subcommittee recommends that the statement of identity for active ingredients that demonstrate an antigingivitis effect should be ''antigingivitis.'' The recommended statement of identity for active ingredients that also demonstrate an antiplaque effect should be ''antigingivitis/antiplaque.''

3. Indications

The indications for antigingivitis/antiplaque drug products should be simply and clearly stated, inform the user of the general pharmacological action of the product, and provide a reasonable expectation of results to be anticipated from use of the product. The indications should be specific and confined to the conditions for which the product is recommended. The labeling for any product that contains an active ingredient for which no claim is made would be misleading.

a. *For all antigingivitis products.* The Subcommittee's recommended indication for OTC drug products containing antigingivitis active ingredients is: ''helps (select one of the following: 'control,' 'reduce,' or 'prevent') (select one or more of the following: 'gingivitis,' 'gingivitis, an early form of gum disease,' or 'bleeding gums').''

b. *For antigingivitis products containing stannous fluoride.* The Subcommittee's recommended indication for OTC antigingivitis drug products containing stannous fluoride is the statement in paragraph a. above and/or the following: ''helps interfere with harmful effects of plaque associated with gingivitis.''

c. *For all antigingivitis/antiplaque products.* The Subcommittee's recommended indication for OTC drug products containing antigingivitis/antiplaque active ingredients is: ''helps (select one of the following: 'control,' 'reduce,' 'prevent,' or 'remove') plaque that leads to (select one or more of the following: 'gingivitis,' 'gingivitis, an early form of gum disease,' or 'bleeding gums').''

d. *For antigingivitis/antiplaque products containing the fixed combination of eucalyptol, menthol, methyl salycilate, and thymol.* The Subcommittee's recommended indication for OTC drug products containing the fixed combination of eucalyptol, menthol, methyl salycilate, and thymol is the statement in paragraph c. above and/or the following: ''helps (select one of the following: 'control,' 'inhibit,' or 'kill') plaque bacteria that contribute to the development of (select one or more of the following: 'gingivitis,' 'gingivitis, an early form of gum disease,' or 'bleeding gums').''

4. Directions for Use

The directions for use should be clear, direct, and provide sufficient information to permit safe and effective use of the product. The product labeling should include a clear statement of the smallest usually effective dose and, where applicable, maximum doses (or concentration if more appropriate) per time interval. If dosage varies by age, the directions should be broken down by age groups. The Subcommittee used directions from the supportive clinical trials as the basis for its recommended directions for use.

a. *For antigingivitis or antigingivitis/antiplaque dentifrice products.* The directions for use for antigingivitis or antigingivitis/antiplaque dentifrice drug products should be consistent with the directions required in the final monograph for OTC anticaries drug products in 21 CFR 355.50(d)(1).

b. *For antigingivitis/antiplaque oral rinse products.* ''Adults and children 12 years of age and older: Vigorously swish 20 milliliters of rinse between your teeth twice a day for 30 seconds and then spit out. Do not swallow the rinse. Children 6 years to under 12 years of age: supervise use. Children under 6 years of age: do not use.''

5. Warnings

Labeling of antigingivitis and antigingivitis/antiplaque products should include warnings against unsafe use, side effects, and adverse reactions.

a. *For all antigingivitis and antigingivitis/antiplaque products.* ''If more than used for brushing (rinsing) is accidentally swallowed, get medical help or contact a Poison Control Center right away. If gingivitis, bleeding, or redness persists for more than 2 weeks, see your dentist. See your dentist immediately if you have painful or swollen gums, pus from the gum line, loose teeth, or increasing spacing between the teeth. These may be signs or symptoms of periodontitis, a serious form of gum disease.''

b. *For antigingivitis products containing stannous fluoride.* ''Keep out of the reach of children under age 6.''

6. Additional Labeling Statements

For stannous fluoride dentifrice drug products. In addition to warning statements, the following statements should appear on the label of antigingivitis dentifrice drug products containing stannous fluoride: ''This product may produce surface staining of the teeth. Adequate tooth brushing may prevent these stains which are not

harmful or permanent and may be removed by a dentist.''

E. Combination Drug Products

1. General Combination Policy

The Subcommittee recognizes that there may be a reason for combining active ingredients in certain OTC drug products. However, such combinations must be based on a sound and logical scientific rationale. The Subcommittee applied the OTC drug review regulation in § 330.10(a)(4)(iv) in developing a combination policy for antigingivitis/antiplaque drug products. The Subcommittee believes that it is rational to combine oral health care ingredients that meet the regulatory requirements as well as the criteria adopted by the Subcommittee, together with suitable inactive ingredients, provided that: (a) Each active ingredient makes a contribution to the claimed effect, (b) the active ingredients are safe and effective and combining the ingredients does not decrease the effectiveness of any individual ingredient, (c) combining the ingredients does not decrease the safety of the combination compared to a single ingredient, (d) the inactive ingredients are safe and do not interact with or otherwise inhibit the effectiveness of the active ingredients, (e) there is a significant target population that can benefit from the use of the combination, and (f) the combination contains adequate directions for use and is labeled with adequate warnings against unsafe use.

The Subcommittee concludes that the same general principles apply when an active ingredient from a different pharmacological class reviewed by another OTC drug advisory panel is combined with an active ingredient reviewed by this Subcommittee. The rationale for such combinations should be evaluated by FDA according to the combination policy set forth in the reports of both advisory panels and in accordance with the agency's regulations.

2. Criteria for Category I Combination Products

The Subcommittee recommends that each claimed active ingredient in a combination product must make a significant contribution to the claimed effects of the product. Further, two Category I active ingredients from different pharmacological groups may be combined to treat different symptoms concurrently if each Category I active ingredient is present within its established dosage range, the combination is rational, there is a significant target population that suffers from the concurrent symptoms, and the combination is as safe and as effective as each individual active ingredient used alone.

3. Category I Combination Antigingivitis/Antiplague Drug Products

The Subcommittee considers it rational to combine antigingivitis/antiplaque agents with an anticaries agent. It is also rational to combine antigingivitis/antiplaque agents with a tooth desensitizing agent. In addition, the Subcommittee considers it rational to combine an antigingivitis/antiplaque agent with an anticaries agent and a tooth desensitizer in a single drug product. Further, the Subcommittee believes that although it has been presented with no scientific basis to recommend the combination of two or more antigingivitis ingredients, two or more antigingivitis/antiplaque ingredients, or combinations of antigingivitis and antigingivitis/antiplaque ingredients, it is theoretically reasonable to combine such ingredients, provided it is demonstrated that each ingredient contributes to the claimed effect and does not decrease the safety or effectiveness of another active ingredient.

F. Testing of Antigingivitis/Antiplaque Drug Products

The Subcommittee concludes that the single active ingredients and the fixed combination of eucalyptol, menthol, methyl salicylate, and thymol placed in Category I have been shown through clinical trials to be safe and effective for OTC use in the control of gingivitis and plaque. However, because product formulation can have a significant impact on the effectiveness of these active ingredients, the Subcommittee recommends that OTC antigingivitis/antiplaque drug products demonstrate their effectiveness through the testing described below. Based on the varying mechanisms of action of the Category I active ingredients, the Subcommittee recommends testing specific to each of the Category I active ingredients to demonstrate their effectiveness in traditional dosage forms (dentifrice, gel, paste, or rinse).

1. Changes in Traditional Dosage Forms

The Subcommittee recommends that drug products containing Category I active ingredients formulated in dosage forms other than those reviewed by the Subcommittee be required to demonstrate antigingivitis/antiplaque effectiveness by a single 6-month, randomized, controlled, clinical trial.

2. Final Formulation Testing

The following testing should be conducted on the product formulation, a standard formulation with effectiveness documented by clinical trials, and a negative control. In general, for a product to be considered effective it must demonstrate that it is statistically substantially equivalent to the standard formulation and statistically superior to the negative control as assessed by reasonable statistical analyses. For validation of the study, the standard must be statistically superior to the negative control. However, during the rulemaking process, the criteria appropriate for these tests should be provided by the product manufacturers.

a. *Cetylpyridinium Chloride Rinse*.

• Determine the in vitro antimicrobial activity of the product against representative plaque organisms commonly associated with gingivitis. Representative organisms include, but are not limited to, typed stains of: *Actinomyces viscosus*, *F. nucleatum*, *P. gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Candida* species, *S. mutans*, and gram negative enteric rods. Testing to determine a product's in vitro antimicrobial activity should include minimal inhibitory concentration (MIC) assays, or 30-second kill-time studies, as appropriate.

• Demonstrate the availability of the active ingredient using a Disk Retention Assay (DRA). A suggested method for this assay is included in a submission to the Subcommittee (Ref. 5).

• Demonstrate the biological activity of the formulation using an ex vivo Plaque Glycolysis and Regrowth Model (PGRM). A suggested protocol for this assay is included in a submission to the Subcommittee (Ref. 5).

b. *Stannous Fluoride Dentifrice*.

• An *in vitro* determination of antimicrobial activity against representative plaque organisms commonly associated with gingivitis (described in paragraph F.2.a. of this document) is recommended. Testing to determine a product's in vitro antimicrobial activity should include MIC assays, 30-second kill-time studies, or plaque biofilm assays, as appropriate.

• Demonstrate the biological activity of the formulation using ex vivo PGRM (protocol for assay, Ref. 5).

c. *Fixed Combination of Eucalyptol (0.092 percent), Menthol (0.042 percent), Methyl Salicylate (0.060 percent), and Thymol (0.064 percent) Rinse*.

• Determine the *in vitro* antimicrobial activity using 30-second kill-time studies with both standard laboratory

strains and wild-type organisms obtained from saliva sampling. Representative organisms are listed in paragraph F.2.a of this document. Conduct kill-time testing using an exposure time of 30 seconds in the presence of exogenous protein. Use an initial inoculum of 1-percent transmission.

• Demonstrate the *in vivo* activity of the formulation through a short-term experimental gingivitis study of at least 2 weeks duration. A representative protocol, comparing the test product, a clinically tested standard, and a negative control, is included in a submission to the Subcommittee (Ref. 6). The criterion for study validation is statistically significant differences in plaque and gingivitis scores between the clinically tested standard and the negative control. To establish comparability to the standard mouthrinse in this test (or another generally accepted statistical test of clinical comparability), the new mouthrinse formulation must satisfy the ''at least as good as'' statistical criteria for both plaque and gingivitis scores, i.e., at least statistically significantly comparable or equivalent to the clinically tested standard.

G. Inactive Ingredients

1. Alcohol in Oral Health Care Drug Products

Many OTC mouthrinses contain alcohol (up to 26 percent or more). Concerns were raised when published reports and other information appeared to show a possible risk of developing oropharyngeal cancers from daily use of mouthrinses containing high concentrations of alcohol. After reviewing the available data, the Subcommittee has the following comments concerning high alcohol-content mouthrinses and cancer of the buccal cavity and pharynx (oral).

a. *Oral cancer*. Based on the 1993 statistics for oral cancer in the United States (Ref. 7), the buccal cavity and pharynx are the eighth most common site of cancer, representing approximately 3 percent of all cancers reported. Approximately 30,000 people per year develop oral cancer. The ratio of men to women developing oral cancer is about 2 to 1. The 5-year survival rate for persons with oral cancer is about 33 percent for African-Americans and 50 percent for Caucasians.

Alcohol consumption and tobacco smoking/chewing account for approximately three-fourths of oral cancers in the United States (Refs. 8 through 13). Other less clearly established causal factors include poor dental conditions, oral infections, nutritional deficiencies, and possibly high alcohol-content mouthrinses (Refs. 14 through 19).

b. *Adverse reactions associated with mouthrinses*. A drug that ordinarily causes no adverse effects with short-term exposure may produce pathologic tissue changes after chronic usage. Prolonged usage of a drug and/or its metabolites combined with various compounds in the mouth may result in cumulative effects in oral tissues. Mouthrinses should be evaluated for chronic, long-term usage and resulting manifestations (Ref. 20).

Mucous membranes of the mouth can absorb mouthrinse ingredients, which may pass systemically into the bloodstream. The literature describes local adverse reactions from mouthrinse usage, ranging in severity from irritancy and sensitization to cancer (Refs. 21, 22, and 23).

Some case-control studies suggest a causal association between mouthrinse use and oral cancer risk, most recently in the largest study to date by the National Cancer Institute (Ref. 24). The cancer risk seems to be greater in females (60 percent) than in males (40 percent) and varies in proportion to dose, tending to increase with increasing duration and frequency of use and the alcohol concentration of the mouthrinse (Ref. 24). Other researchers have found no evidence of an increased cancer risk associated with mouthrinses (Refs. 25, 26, and 27).

The reported risk of oral cancer pertains to mouthrinses with alcohol-contents of 25 percent or higher. However, since these mouthrinses also contain other active ingredients, such as essential oils with lipophilic, membranotropic effects, some high alcohol-content mouthrinses may affect tissues by a variety of mechanisms.

Studies that have evaluated the potential for alcohol in mouthrinses to cause cancer have a number of shortcomings: (1) Investigations based on subject accounts without benefit of medical records or other written documentation, (2) unreliable classification of exposure to known risk factors such as alcohol and tobacco in study subjects, (3) lack of consistent dose-response relationships based on frequency and/or duration of mouthrinse use, and (4) combining cases of cancer of the buccal cavity and pharynx despite the fact that mouthrinses are in direct contact only with the mucosa of the buccal cavity.

c. *Alcohol and oral cancer*. Although consumption of alcoholic beverages is a known risk factor for oral cancer, pure alcohol does not show a direct carcinogenic action in laboratory animals or humans. The cancer associated with alcoholic beverages is probably related to contaminating carcinogens. These include urethane produced from urea reacting with ethyl alcohol during yeast fermentation of fruit juices, and n-nitrosamine compounds catalyzed from precursor nitrite and amines, amides, or other nitrosatable agents. Commercial mouthrinses contain distilled ethanol free of these contaminating carcinogens. Other findings suggest an ability of ethanol to enhance the conversion of procarcinogens to mitogens, and of ethanol's metabolite acetaldehyde to produce deoxyribonucleic acid (DNA) abnormalities in human cells.

Animal studies have indicated that ethanol may also function as a cocarcinogen, in association with other substances that are true carcinogens (Ref. 28). Alcohol may act by facilitating the penetration of carcinogens into the mucosa (Refs. 29 through 33). Weak carcinogenic nitrosamines and other compounds have been shown to have enhanced carcinogenicity in the presence of alcohol (Ref. 33). Alcohol may act directly on epithelial cells by altering intracellular metabolism and rendering cells more susceptible to carcinogens (Ref. 28).

Based on these studies, the Subcommittee recommends that further studies on the possible cancer risk associated with high alcohol-content mouthrinses be conducted. These studies should include testing various components of the mouthrinse and pertinent dietary ingredients.

d. *Abuse and misuse of mouthrinses*. Although some OTC mouthrinses contain alcohol, the potential for development of drug tolerance and addiction due to use of these products seems negligible. However, misuse of any mouthrinse product may occur if the product gives the user a false sense of security, diminishing the users's desire to seek professional advice. This problem may be particularly acute for mouthrinses that may subdue signs and symptoms of a gingivitis infection without resolving a more severe, underlying periodontitis infection. A label warning should alert the consumer to this danger.

e. *Alcohol as a facilitator*. While the Subcommittee recognizes that the combination of alcohol and tobacco is associated with a marked increase in the incidence of oral cancer as compared to exposure to tobacco alone, it concludes that the mechanism of this synergism is unknown. Animal studies (Ref. 28) have shown that alcohol has a topical

potentiating effect in the production of squamous cell carcinoma in animal cheek pouches treated with 7,12–dimethylbenz(a)-anthracene (DMBA). Decreased latency and larger tumors were observed as compared to controls.

Other animal studies (Refs. 29, 30, 32, and 33) have demonstrated similar effects. These studies were older and implied a model that is not comparable to what happens in humans. Moreover, some carcinogens are extremely species-specific, and limited information is available on direct experiments performed on the human mucosa.

If the synergistic effect of alcohol in causing an increased risk of oral cancer is attributed to a topical effect, as noted in the animal studies, then daily use of oral rinses containing a high concentration of alcohol may have a tissue altering effect. Whether this may be as significant as alcoholism in the epidemiology of oral cancer warrants continued investigation.

One of the few mechanistic evidences for a local alcohol effect has been demonstrated by permeability studies. In the presence of nicotine, alcohol had a greater relative effect on penetration of carcinogens in and across the floor of the oral mucosa (floor of the mouth, oral mucosa) (Ref. 34). Also, pharmaceutical studies have demonstrated that the oral mucosa can have a reservoir effect, so that compounds are rapidly taken up and held in the oral epithelium, extending the duration of their effect (Ref. 35). This mechanism has recently been utilized in a formulation using alcohol to increase permeability, thereby obtaining systemic delivery of proprietary drugs after only a mucosa exposure.

It is clear that further research is needed to investigate the role of alcohol as an enhancer of the penetration of carcinogens through the oral mucosa. In addition, the threshold of alcohol concentration necessary to achieve this phenomena needs to be investigated.

2. The Subcommittee's Conclusions and Recommendations Regarding Alcohol Content in Mouthrinses

On June 6, 1996, the Subcommittee, along with other scientific experts (e.g., epidemiologists and statisticians) held a workshop (Ref. 36) to further consider whether alcohol-containing mouthrinses contributed to oral cancers. Although some studies have implicated high alcohol-content mouthrinses as a possible cause of oral/pharyngeal cancer, the relationship between high alcohol-content mouthrinses and oral/pharyngeal cancer is not clear. The findings of various studies are contradictory and do not show a consistent dose-response relationship. A major difficulty in deciding cause and effect in these studies is the possibility of confounding by known risk factors, such as high alcoholic beverage consumption and tobacco use.

The Subcommittee reviewed new data consisting of a specificity analysis (Ref. 37) using data from the Winn et al. study (Ref. 24) and a preliminary analysis from an unpublished study of laryngeal, esophageal, and oral cancer (Ref. 38). In addition, the Subcommittee reviewed seven case-control studies, published between 1979 and 1991 (Refs. 12, 13, and 23 through 27), of the association between mouthrinse use and oral cancer. These studies are described below.

Weaver et al. (Ref. 23) reported the use of alcohol-containing mouthrinses among 11 subjects with oropharyngeal cancer who indicated that they did not smoke or drink alcoholic beverages. These cases became part of a case-control study regarding an association between alcohol-containing mouthrinses and oropharyngeal cancer. Although the study was unevaluable, it generated the hypothesis that led to subsequent studies.

A 1983 case-control study by Wynder et al. (Ref. 12) evaluated the relationship between mouthrinses and oropharyngeal cancer. No positive findings were reported for men. In women, the relative risk, unadjusted for smoking and alcoholic beverage consumption, was statistically significant for daily use of mouthrinses. However, there was no consistent relationship for duration or frequency of use. Further, a refined analysis using a multiple logistic model indicated no association between mouthrinse use and oropharyngeal cancer. The investigators concluded that, due to the absence of a dose-response relationship and the possibility of confounding by tobacco and alcoholic beverage use, it was not possible to attribute an association between daily mouthrinse use and oral cancer in women.

A 1983 case-control study by Blot et al. (Ref. 13) included female subjects from a previous study of snuff use. A relative risk of 1.94 was reported for women who used a mouthrinse but did not use tobacco products. However, this was not statistically significant (confidence interval = 0.8 to 4.7), and there were no consistent dose-response relationships for years of use, frequency of use, time retained in the mouth, or concentration (i.e., diluted vs. full strength). Because dose-response relationships are important in considering whether there is an association between mouthrinse use and oral cancer, the Subcommittee concludes that this study does not support a causal association between alcohol-containing mouthrinses and oropharyngeal cancer.

The Subcommittee reviewed three additional case-control studies published between 1985 and 1989 (Refs. 25, 26, and 27). One study by Kabat *et al.* (Ref. 26) is of particular interest because, although mouthrinses were not associated with increased oral cancer risk in terms of frequency or duration of use, cases were significantly more likely than controls to state that mouthrinses were used to disguise breath odors caused by alcoholic beverages or tobacco. In contrast, similar proportions of cases and controls reported using a mouthrinse to conceal food odors or for mouth infections or dental problems. The Subcommittee concludes that these findings indicate that mouthrinse use may be serving as a surrogate for underreported drinking and/or smoking.

A 1991 study by Winn et al. (Ref. 24) was the largest case-control study among the seven published studies evaluating mouthrinses (866 cases and 1,249 controls). Odds-ratios for oropharyngeal cancer risk after adjusting for tobacco and alcoholic beverage use were 1.4 (confidence interval 1.0 to 1.8) in men and 1.6 (confidence interval 1.1 to 2.3) in women. Dose-response relationships, such as duration of use, frequency of use, and age when use started, were questionable, with no trend analysis of these relationships reported. This study also showed a decreased odds-ratio for dental X-rays. There is no biologically plausible reason to expect X-rays to be protective against oral cancer, and the negative association is likely a reflection of less frequent visits for dental care by cases versus controls. However, the negative association could not be eliminated by adjustment for factors that are relevant to quality of dental care (e.g., education).

Thus, this study was capable of producing a statistically significant noncausal association that could not be eliminated by adjustment of the data. Further, regarding the odds ratio for mouthrinse use, confounding due to underreported use of tobacco and alcoholic beverages, both strong risk factors for oropharyngeal cancer, could result in an artificially elevated odds ratio. Such a false association can be produced even though the extent of underreporting is the same in both the case and control groups (Ref. 39). Information in the published literature indicates that especially drinking and sometimes smoking are underreported (Refs. 40 through 44). The

Subcommittee concludes that these studies do not support a causal relationship between the use of alcohol-containing mouthrinses and oropharyngeal cancer.

The Subcommittee reviewed unpublished new data that included a specificity analysis (Ref. 37) of the data from the Winn et al. study (Ref. 24). This analysis excluded 75 cases (38 men and 37 women) who did not have oropharyngeal cancer (*i.e.,* epithelial cell cancer of the mouth) based on evaluation of the International Classification of Diseases codes. The excluded cases consisted primarily of tumors of the minor salivary glands and sarcomas and lymphomas that happened to occur within the oral cavity. Excluding these cases left 535 and 256 cases of oropharyngeal cancer in men and women, respectively. Evaluation of smoking and alcoholic beverage use indicated that both of these risk factors were more strongly associated with the included cases than with the total number of cases (included plus excluded). Neither smoking nor alcoholic beverage use were associated with the excluded cases. This analysis indicated that the excluded cases may not have the same etiology as the included cases and, therefore, should not have been included in the original analysis conducted by Winn et al. (Ref. 24) to evaluate risk associated with mouthrinse use.

When odds ratios for mouthrinse use in women were calculated for the included cases, they were decreased relative to the odds ratios for total cases originally reported by Winn et al. (Ref. 24). This was true for a number of subanalyses, including duration of use, frequency of use, age when use began, and alcohol concentration. Higher odds ratios for mouthrinse use among the excluded cases suggested that mouthrinse use was more strongly associated with excluded cases than with included cases. However, there is no biologically plausible explanation for this finding since the excluded cases represent a variety of tumor types whose origins cannot be presently explained by topical exposure to ethanol via mouthrinse use. In addition, the data were inconsistent with a dose-response with respect to duration of use, frequency of use and age when mouthrinse use started, which suggests that this finding may be related to information bias rather than a causal association. The specificity analysis among male cases was less informative than for females and supports neither a causal hypothesis nor information bias as the explanation for the weak association with mouthrinse use (odds ratio 1.4) originally reported by Winn et al. (Ref. 24). The limited value of the specificity analysis in males is likely related to the fact that: (1) The excluded male cases represented a smaller percentage of the total male cases and (2) the odds ratio for mouthrinse use in males is smaller than it is in females. Both of these factors make it difficult to detect any shifts in odds ratios. The Subcommittee concludes that, overall, the specificity analysis of the Winn et al. study (Ref. 24) indicates that this study does not support a causal association between mouthrinse use and oropharyngeal cancer (Ref. 37).

Preliminary analyses from an unpublished case-control study of laryngeal, esophageal, and oral cancer (Ref. 38) showed that the odds ratio for mouthrinse use in males and females combined (adjusted for cigarette and alcoholic beverage use) was 1.4 (confidence interval 1.0 to 2.0). However, the analyses of frequency, duration, and age when use started showed inconsistencies that question a causal relationship. In addition, when the data were evaluated with respect to alcohol content, the highest odds ratio (unadjusted for smoking and alcoholic beverage use) was found among users of mouthrinses containing no alcohol (e.g., salt water, vinegar, baking soda in water). The Subcommittee concludes that this finding differs from the Winn et al. study (Ref. 24) results showing that odds ratios were elevated only for mouthrinses having the highest alcohol content and is inconsistent with the hypothesis of a causal association between alcohol-containing mouthrinses and oral cancer.

An unpublished review of the literature concerning possible mechanisms of alcoholic beverage consumption and oral cancer risk was submitted to the Subcommittee (Ref. 45). Although alcoholic beverage consumption is a known risk factor for oral cancer and the literature on experimental mechanistic studies (e.g., in vitro and animal studies) raises speculations concerning how the biological effects of alcohol may modulate cancer risk, the Subcommittee concludes that the relevance of these studies to mouthrinse use in humans has not been established.

Based on the studies reviewed, the Subcommittee concludes that the available data do not support a causal relationship between the use of alcohol-containing mouthrinses and oral cancer. The vote was unanimous with the Chairman abstaining. The Subcommittee acknowledges that epidemiologic research on oropharyngeal cancer will continue, and that the conclusion reached by the Subcommittee is based on the data available at the time of its deliberations. However, because some studies did report a relationship between the use of high alcohol-content mouthrinses and pharyngeal cancer, the Subcommittee agrees that further studies should be conducted to determine the relationship between high alcohol-content mouthrinses and oral/pharyngeal cancers. In addition, the Subcommittee recommends that all mouthrinses should be labeled in a readily readable manner with the alcohol concentration in percent, *e.g.,* ''Contains _ % alcohol'' on the principal display panel.

H. General Guidelines on Safety and Effectiveness

1. General Statement

The Subcommittee arrived at its conclusions and recommendations regarding the safety and effectiveness of all active ingredients after considering all pertinent data and information submitted. The Subcommittee adopted the following general ''points to consider.'' These are not intended to restrict investigators, but are recommendations for studies recognized as desirable approaches to determine the safety and effectiveness of OTC antigingivitis/antiplaque active ingredients. In some cases, other methods may be equally applicable, or newer methods may be preferable. Also, these recommended studies may not produce all information necessary to determine that an ingredient is generally recognized as safe and effective.

2. Guidelines

An OTC drug included in a monograph is described in § 330.10 as generally recognized among qualified experts as safe and effective for use and as not misbranded. Proof of the safety of an OTC drug ingredient consists of adequate tests by methods reasonably applicable to show the drug is safe under the prescribed, recommended, or suggested conditions of use. This proof shall include results of significant human experience during marketing. General recognition of safety shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data. Proof of effectiveness of an OTC drug ingredient consists of controlled clinical investigations as defined in § 314.126(b) (21 CFR 314.126b)) by qualified experts to show that the drug provides clinically significant relief of the type claimed in its labeling. The latter requirement may be waived if it is not reasonably applicable to the drug in question or

essential to the validity of the investigation and an alternative method of investigation is adequate to substantiate effectiveness. Effectiveness may be corroborated by partially controlled or uncontrolled studies, and reports of significant human experience during marketing. General recognition of effectiveness shall ordinarily be based upon published studies that may be corroborated by unpublished studies and other data.

The characteristics of adequate and well-controlled studies have been developed over a period of years and are described in § 314.126. Studies supporting the safety and effectiveness of OTC drug ingredients should provide sufficient details of study design, conduct, and analysis to allow a critical evaluation of the data in relationship to the above characteristics.

In several proposed and final monographs, the agency has stated that, in order for an active ingredient to be included in an OTC drug monograph, it is necessary that the ingredient be adequately characterized and that these standards be published in an official compendium such as the United States Pharmacopeia (USP) or the National Formulary (NF) (58 FR 28194 at 28284). Such specifications are necessary to assure the identity, strength, quality, and purity of the active ingredient. Therefore, the Subcommittee recommends that a full description of the ingredient, including its physical and chemical characteristics and stability, be provided, and that manufacturers contact and work with the USP to develop monographs for ingredients that are not currently included in that compendium. For ingredients that are currently included in an official compendium, reference to the current edition of the USP or the NF may satisfy this requirement.

a. *Safety*. The Subcommittee's determination of the safety of single ingredients and ingredient combinations is based on the following criteria: (1) The incidence and risk of adverse reactions and significant side effects when the ingredient was used according to adequate directions in the labeling, (2) the margin of safety under conditions of normal use and the potential for harm that might result from abuse or misuse under conditions of widespread OTC availability, (3) the potential for inducing untoward effects on the oral tissues, including irritation, ulceration, inflammation, erosion, and minor effects such as discoloration of the teeth, restorations, and prostheses, etc., and (4) assessment of the benefit-to-risk ratio. The Panel further states that microbial safety should be determined through clinical evaluation of changes in representative oral microbial populations (*e.g.,* the possible emergence of opportunistic organisms or potential pathogens), in order to assure that there is no adverse change in the balance of the oral microflora under conditions of expected OTC use.

i. *Toxicological studies*. A variety of toxicological data can be obtained to demonstrate that an active ingredient is safe. The Subcommittee recommends that manufacturers conduct the applicable studies discussed below and emphasizes that these recommendations do not preclude the use of alternative comparable methods that are currently available or better methods that may be developed in the future. The Subcommittee recommends that the following data be available for the active ingredient(s) intended for use on the mucous membranes of the mouth and throat.

Testing the effects of various ingredients on animal subpopulations that can reflect human subpopulations should be considered (e.g., hyposalivation studies in nonsalivating animals). Adequate, acceptable, controlled in vivo studies of acute and chronic toxicity in several species of animals should be available. Such studies may include single-dose gavage studies, repeat-dose gavage studies, oral irritation studies, pharmacokinetic/biodistribution studies, and dermal sensitization studies. Information regarding the genetic, reproductive toxicologic, and carcinogenic potential should be considered for ingredients that are going to be used daily on a long-term basis. It is not necessary to determine the LD_{50} (lethal dose for 50 percent of the test animals) of the ingredient. However, information about the minimal lethal dose would be useful.

All or some of the recommended toxicological studies may not be necessary for all active ingredients. Some circumstances that might preclude an ingredient from the above testing are: (1) It is already generally recognized as safe, (2) it is a direct food additive, (3) it has been used previously in approved dental drug products, or (4) it is the subject of an OTC drug monograph with a different but similar or related use at a similar concentration and for a similar time period. Published articles may be considered in lieu of the testing recommended above.

One of the Subcommittee's primary concerns regarding antigingivitis/antiplaque ingredients is whether or not swallowing the active ingredient presents a threat to the user. The Subcommittee recommends that gavage studies be used to address concerns about potential systemic toxicity unless applicable published or unpublished studies have been conducted using a dietary admixture mode of administration and comparable toxicokinetics can be shown between gavage and dietary modes of administration. Single administration gavage studies are typically performed using a limit-value test in the rat at a specified high dose to evaluate acute toxicity potential (Refs. 46, 47, and 48). In the absence of adequate dietary admixture studies, repeat dose gavage studies may be employed to evaluate systemic toxicity from multiple exposures. The test article is administered to rats on a number of consecutive days.

Where there is a concern that antigingivitis/antiplaque active ingredients may induce untoward effects on the oral mucosa, the dosage to be used for these studies should be justified based on the concentration of human exposure levels. An appropriate dosage range may extend, for example, from a low dose comparable to swallowing a single dose of mouthrinse or the amount remaining following expectoration of a mouthrinse to a high dose that either causes dose-limiting toxicity or is several orders of magnitude greater than the clinical exposure levels. Such studies usually use four applications per day for a period of 28 consecutive days. The oral irritation should include both a negative and a positive control group. All test articles should be applied in an identical manner. A negative control group may consist of animals that are treated with either water or saline, and the positive control is a group of animals that are treated with the solution that is known to cause a minimal degree of irritation without being inhumane to the animals (e.g., 5-percent solution of sodium lauryl sulfate).

The Subcommittee recommends that the study include abraded mucosa in order to determine whether the test ingredient delays or prevents the healing of oral lesions. The parameters to include are any gross observations of changes in the oral tissue, such as sloughing, ulceration, or bleeding. Following the sacrifice of each animal, the histopathology of oral tissues should be examined.

ii. *Studies in older adults*. The Subcommittee is concerned that older adults might be at greater risk for potential systemic toxicity from the use of antigingivitis/antiplaque active ingredients. This is of particular concern because of the continually

increasing size of the older adult population, who are retaining more natural teeth and becoming a significant population for use of antiplaque/antigingivitis products.

Publications have described differences in drug responses in the elderly. Changes in pharmacokinetics have been reviewed (Ref. 49). Absorption can theoretically be altered by noted changes in gastrointestinal function, but the majority of studies have shown no difference in rate or extent of absorption of the drug examined. Distribution of a drug within the body is affected because fat content of body weight increases and intracellular water decreases. For example, albumin concentration is reduced and drugs which bind to albumin are more free to distribute to the rest of the body. Hepatic metabolism may be altered. Reduction of blood flow to the liver will decrease clearance of some drugs. Renal excretion is affected in some older adults by loss of renal mass and functional nephrons.

Russell (Ref. 50) noted that despite numerous reports in the literature of impaired GI function with aging, most functions remain relatively intact because of the large reserve capacity of the intestine, pancreas, and liver. In a review critically analyzing available information on age-related changes in the digestive and absorptive GI physiology of lipids, data suggested lipid digestion and absorption are well-preserved in the aging. However, intercurrent illness or experimental stress may produce impairment in aging animals and humans that is not seen in younger controls (Ref. 51).

Atillasoy and Holt (Ref. 52) noted that the GI tract represents an organ system characterized by rapid proliferation. Contrary to generally held prejudices, the authors write, a state of hyperproliferation, not hypoproliferation, occurs in the epithelial cells of the stomach, small intestine, and large intestine of stable-fed, aged rodents when compared to young adult rodents.

In a gavage study (Ref. 53) Yamada et al. investigated renal ammoniagenesis in isolated nephron segments from control, acidotic senescent (exhibiting deteriorating teeth due to aging), and young adult rats. No significant difference was seen in glutamine-dependent ammonia production in the segments. However, ammonia production in glomeruli from old rats was significantly greater than in young rats.

There appear to be no available consistent findings to warrant that additional gavage studies of antigingivitis/antiplaque active ingredients in older animals will produce more meaningful findings relative to older adults than the usual gavage studies in adult animals. This is due to the great diversity which exists in the health and fitness status of the elderly population. The Subcommittee considers a comment by Ahronheim (Ref. 54) appropriate:

> Although much has been written about age-related alterations in drug disposition, there is disagreement as to the extent and inevitability of these changes. Studies focusing on aged individuals suffer from several problems. Cross-sectional studies comparing young and old subjects sometimes compare young, healthy individuals with aged subjects gathered from hospitals or nursing homes. If the aged subjects are ''healthy'' they may nonetheless have subclinical disease, which can alter outcomes in studies that seek to determine a drug's disposition and effects. However, aged subjects that are truly healthy may represent an elite minority so that the study's results may not be applicable to the general elderly population. Longitudinal studies are almost impossible to complete and data is sparse, but recent findings indicate that the geriatric population is, indeed, heterogeneous.
>
> In addition to these pitfalls, it is not known how generalizations about aging physiology, even if they are true, can be applied to drug disposition, since most drugs have not been subjected to exhaustive age-specific testing and few conclusions can be reached based on pharmacokinetic data. Even less is known about pharmacodynamic changes because the study of age-related tissue receptor density, activity, and sensitivity is in its infancy. We must therefore rely on clinical observations to a large extent when drawing conclusions about efficacy and potential toxicity of various agents in use. The Subcommittee concludes that the results of the usual gavage studies are adequate.

iii. *Irritation and delayed contact sensitization studies in humans.* Observations during adequate clinical studies are sufficient to demonstrate the irritation and sensitization potential of an ingredient or ingredient combination. However, if necessary, a number of methods embodying the use of patch testing have proven of value in determining skin irritancy and systemic sensitization. The Subcommittee recommends one of the following three methods of patch testing to address concerns of irritancy and sensitivity:

• *Draize testing.* In the Draize human skin irritancy and sensitization tests or one of its various modifications (Ref. 55), the testing should be performed on the skin of the subject's back or arm.

• *Method of Shelanski and Shelanski.* In this method (Ref. 56), the active ingredients or the formulation under study are applied at frequent intervals of 1 or 2 days to the test site for 3 or 4 weeks. After a rest period of 2 weeks, a single dose of the drug is applied as a challenge. The preliminary applications are made to detect primary skin irritants and provoke sensitization in susceptible individuals. The challenging dose detects whether or not the drug is a skin sensitizer.

• *Maximization procedure of Kligman.* This procedure (Ref. 57) or one of its modifications uses an irritant applied over a desquamated test site. Desquamation is performed by using a rubbing technique that facilitates penetration, thereby hastening and accentuating the skin-sensitizing potential of the substance. Other validated human models may be used.

iv. *Microbiologic evaluation.* The Subcommittee is concerned about the potential of antigingivitis/antiplaque ingredients with antimicrobial effects to allow emergence of opportunistic pathogens, induce resistance in oral microorganisms, or allow an oral overgrowth of inherently resistant potential pathogens. Representative microbial species and their relative proportion to the total cultivable microflora in supragingival plaque and saliva should be monitored over at least a 6-month period of continuous use of the antiplaque product to determine if a shift in the oral flora has occurred that might result in the proliferation of pathogenic microorganisms, which may include *Candida* species and other yeast, *Staphylococcus aureus* and other *Staphylococcus* species, beta-hemolytic *Streptococci*, and enteric gram-negative rods. Additionally, for those antigingivitis/antiplaque ingredients where the mechanism of action is suspected to be antimicrobial, an assessment of changes in microorganisms associated with gingival disease should be carried out. One determination should be made prior to the start of use, one at the conclusion of the study, and one at an intermediate time. In vitro minimum inhibitory concentrations should be assessed for representative species to determine the development of increased resistance after prolonged antimicrobial therapy.

b. *Effectiveness.* The Subcommittee's determination of the therapeutic effectiveness of ingredients and combinations of ingredients for antigingivitis/antiplaque use is based on published and unpublished studies containing pharmacological data considered by the Subcommittee to be scientifically valid and pertinent. Clinical criteria for proof of effectiveness of a single ingredient or combination of ingredients were determined by evaluating data from valid controlled studies and by calling on the clinical expertise of the

Subcommittee members. Proof of effectiveness of a single ingredient or combination of ingredients was determined by evaluating data from valid, well-controlled studies demonstrating a significant reduction of the symptoms or a therapeutic benefit for the stated indication in the labeling.

Although the OTC drug review is an active ingredient review, not a product review, the Subcommittee recognizes that a final product must be formulated properly, according to accepted pharmaceutical manufacturing practices. If a product is not formulated properly, active ingredients may be present in less than the minimum effective dose, may be in a form that does not exert the intended therapeutic effect(s), or may not be bioavailable. Therefore, the Subcommittee considered it important whether or not inert ingredients or other active ingredients in a formulation might alter the effect of the product's principal active ingredient. The designation of a pharmaceutical necessity as an inactive ingredient does not necessarily mean that the ingredient is pharmacologically inactive.

The Subcommittee considers its recommended ''points to consider'' acceptable current approaches for arriving at valid conclusions concerning the effectiveness of OTC antigingivitis/antiplaque drug products. These ''points to consider'' do not preclude the use of newer, more refined laboratory or clinical techniques to establish effectiveness.

c. Clinical trials. Acceptable studies should state the specific objectives of the study, a review of pertinent literature, and present the scientific rationale for the use of the ingredient. The mode, frequency, and duration of application should be thoroughly described. The indices and variables selected for measuring effectiveness, the methods of measurement, and the rationale for such choices should be characterized. The Subcommittee believes that the effectiveness of an OTC antigingivitis ingredient, antigingivitis/antiplaque ingredient, or ingredient combination should be demonstrated by evidence of a clinically significant endpoint, specifically a reduction and/or prevention of gingivitis. In general, the Subcommittee would also expect a reduction of dental plaque mass and/or plaque virulence (degree of pathogenicity as indicated by the severity of the disease produced). However, the Subcommittee also believes that an ingredient can reduce gingivitis without a demonstrated reduction of plaque. Where possible, additional evidence for the effectiveness of the agent should be provided by demonstrating a shift in the plaque flora.

i. *Design.* Studies should measure the difference between reduction or prevention of dental plaque and gingivitis resulting from the test ingredient as compared to a placebo. Examples of acceptable experimental designs include crossover, parallel, factorial, sequential, single-blind, and therapeutic equivalency studies. Preference should be given to using double-blind studies with a placebo control. The placebo is the formulation of the test agent without the active ingredient, or some other suitable placebo.

ii. *Subjects.* A sufficient number of subjects should be used to permit statistical analysis for the data obtained. The number of subjects tested should be sufficient to eliminate examiner bias and bias introduced by the placebo effect, if applicable, and to allow for anticipated dropouts and estimated variability of effect. The subjects should be of both genders and within the age groups for which the active ingredient is intended. Specific exclusionary criteria should be given.

iii. *Conduct of the study.* The study should be of sufficient duration to demonstrate effectiveness. The duration will depend upon the actual use, anticipated effect, potential sustained benefits, and any safety considerations. The Subcommittee believes that such studies should be at least 6 months in duration to provide sufficient time for an ingredient to exert an antigingivitis/antiplaque effect and for adverse events to manifest themselves. Six months will also provide time to investigate the possibility that an OTC oral ingredient used daily over an extended period of time might cause a shift in the oral flora that may result in the proliferation of pathogenic microorganisms. Scoring and oral health evaluations should be done at baseline, at completion, and at appropriate intervals during the study. Baseline demographic, medical, historical, and physical data for each subject should be obtained and recorded. Such data should include a medical history, a complete oral examination, laboratory studies, if indicated, and other pertinent data.

The treatments should be performed on a random basis. The randomization procedure should be used so that variables not otherwise controlled balance out. The number and frequency of applications of the preparation should be in accordance with the method outlined in the indication for use and directions in the labeling. The clinical investigative team should monitor subjects during the study to detect any adverse events and take appropriate action. An evaluation of dose response and possible mechanism of action would enhance any submission.

iv. *Appropriate assessments.* Appropriate assessments using validated or accepted techniques must be used.

v. *Interpretation of data.* Investigative methods should be described in sufficient detail so that experiments can be repeated by another investigator to verify and confirm results. Methods of statistical analysis should be determined before starting the study.

Positive evidence of effectiveness should be obtained from a minimum of two studies, each conducted by an independent investigative group. In addition to statistical significance, clinical importance should be addressed. Strength of effect and concern about statistically significant changes not being clinically significant reflect the importance of randomized controlled trials of longer duration to determine if individuals benefit from proposed agents and interventions. Statistical significance can be easily calculated using a nominal (categorical) scale such as gingival index scores. A large ''N'' offers scores with an approximately normal distribution so that parametric statistics can be used, as if using exact measures such as in an interval or ratio scale. The gingival index, however, is a nominal scale and the difference between 0 and 2 is not the same as the difference between 1 and 3. Slight differences exist in mean gingival index scores which are not clinically obvious and cannot be easily discerned in a subject. A product can produce a change in the response variable that is statistically significant, yet the question of clinical significance remains unanswered.

III. Classification of Active Ingredients

In addition to carefully reviewing the submitted data, the Subcommittee considered all pertinent data and information available in arriving at its conclusions and recommendations regarding the active ingredients. The following tables summarize the Subcommittee's recommended categorization of active ingredients:

TABLE 2.—CATEGORIZATION OF SINGLE ACTIVE INGREDIENTS

Active Ingredients	Safety	Efficacy
Aloe vera	III	III

TABLE 2.—CATEGORIZATION OF SINGLE ACTIVE INGREDIENTS—Continued

Active Ingredients	Safety	Efficacy
Cetylpyridinium chloride	I	I
Dicalcium phosphate dihydrate	I	III
Hydrogen peroxide	I	III
Sanguinaria extract	I	III
Sodium bicarbonate	I	III
Sodium lauryl sulfate	I	III
Stannous fluoride (for gingivitis)	I	I
Zinc citrate	I	III

TABLE 3.—CATEGORIZATION OF COMBINATIONS OF ACTIVE INGREDIENTS

Active Ingredient Combination	Safety	Efficacy
Alkyl dimethyl amine oxide and alkyl dimethyl glycine	III	III
Eucalyptol, menthol, methyl salicylate, and thymol	I	I
Hydrogen peroxide and povidone iodine	III	III
Hydrogen peroxide and sodium bicarbonate	I	III
Hydrogen peroxide, sodium citrate, sodium lauryl sulfate, and zinc chloride	I	III
Peppermint oil and sage oil	I	III
Polydimethylsiloxane and poloxamer	I	III
Stannous pyrophosphate and zinc citrate	I	III

A. Category I Conditions

The Subcommittee recommends Category I labeling for all Category I single antigingivitis/antiplaque active ingredients and combinations of active ingredients (see section II.D of this document).

1. Category I Single Active Ingredients

Cetylpyridinium chloride (rinse)
Stannous fluoride (dentifrice)

a. *Cetylpyridinium chloride (rinse).* The Subcommittee concludes that cetylpyridinium chloride at concentrations of 0.045 to 0.1 percent with at least 72 to 77 percent chemically available cetylpyridinium chloride is safe and effective for use in mouthrinse formulations as an OTC antigingivitis/antiplaque agent. Cetylpyridinium-containing mouthrinses have been used in the United States since 1940. Cetylpyridinium chloride 0.025 percent to 0.1 percent has been marketed nationally in several products. Products containing cetylpyridinium chloride have also been marketed internationally. The more than 55-year U.S. marketing history is significant with respect to the ingredient's safety.

Cetylpyridinium chloride is a quaternary nitrogenous compound l-hexa-decyl pyridinium chloride with antimicrobial activity against many microorganisms, including viruses. Its chemical and physical properties are well described in the USP (Ref. 58). It is classified as a cationic surface-active agent and contains a cetyl radical substituted for hydrogen atom on position 1. In hydrochloric acid it forms a chloride salt. The cetyl radical renders the molecule lipophilic, contributing to the lipophilic/hydrophilic balance which is necessary for the antimicrobial activity of such quaternary nitrogenous compounds. The antimicrobial activity is dependent upon the positioning of the charged molecule with bacterial cells that carry a net negative charge. This positioning allows the hydrophilic portion of the cetylpyridinium chloride to interact with the cell membrane, resulting in leakage of cellular components, disruption of cellular metabolism, inhibition of cell growth, and cell death (Refs. 59 through 62). Because the positively charged hydrophilic region is critical to antimicrobial activity, any formulation that diminishes the activity of this cationic group or that competes with this group may inactivate the product. Therefore, it is essential to establish that the cetylpyridinium chloride in products is sufficiently biologically active to justify an antigingivitis claim.

i. *Safety.* The Subcommittee believes there are sufficient safety data to permit final classification of the safety of cetylpyridinium chloride as an OTC antimicrobial agent for topical use in the oral cavity when used within the proposed dosage limits set forth below. The Subcommittee bases its conclusions on the safety of cetylpyridinium chloride mouthrinses used in animal and pharmacokinetic studies, assessment of adverse events in randomized, placebo-controlled clinical trials, and postmarket spontaneous adverse event data reported to the manufacturer and FDA.

The LD_{50} of cetylpyridinium chloride is 250 milligrams per kilogram (mg/kg) given subcutaneously, 6 mg/kg intraperitoneally, 30 mg/kg intravenously, and 200 mg/kg given orally as a pure compound (Ref. 63). The data (Ref. 64) show that the oral LD_{50} values in the rat from a mouthrinse containing 0.05 percent cetylpyridinium chloride were 34 mg/kg to 48 mg/kg of the mouthrinse alone. This lower LD_{50} with the rinse formulation as compared to cetylpyridinium chloride in solution is likely due to the other components of the mouthrinse, such as the alcohol.

Subchronic toxicity studies of cetylpyridinium chloride administered orally at dose levels ranging from 5 to 500 mg/kg showed morbidity and death at 125, 250, and 500 mg/kg. At lower doses, the only significant finding in rats and dogs was gastric irritation at doses of 50 mg/kg per day and higher (Ref. 65). These studies are similar to studies conducted prior to 1950.

Two chronic exposure safety studies of 6 months and 1 year were reported (Ref. 65). Doses administered daily by oral gavage ranged from 5 to 75 mg/kg. Significant decreases in body weight and weight gain were noted in 40- and 75-mg/kg animals of both sexes. At necropsy, GI irritation was manifested as thickening of the stomach mucosa observed at the 40- and 75-mg/kg level, and in some animals administered 15 mg/kg.

Local irritation studies (Ref. 65) included eye irritation tests and dermal exposure. Evidence of eye irritation was observed at high concentrations but no dermal lesions were observed. Local irritation using cetylpyridinium chloride mouthrinse formulations was assessed in the canine oral mucosa irritation model (Ref. 65). A cotton plug saturated with cetylpyridinium chloride mouthrinse was applied to the gingival mucosa three to five times a day for 4 days. Mouthrinse formulations containing up to 0.45 percent cetylpyridinium chloride did not induce irritation after 20 applications. Lin et al. (Ref. 66) evaluated inhalation toxicity in rats and found clinical signs of toxicity, including respiratory difficulty, eye irritation, and nasal discharge at concentrations of approximately 0.1 mg cetylpyridinium chloride/liter and above. However, these nonlethal effects were reversible.

A study of the effects of alcohol and cetylpyridinium chloride on the buccal mucosa of hamsters was reported (Ref. 67). Animals received daily applications of 0.05 percent cetylpyridinium

chloride for 21 days on the everted hamster cheek pouch. Abrasion was also carried out. No significant differences were found between the control and study animals.

Contact sensitization potential was assessed using a 25-percent concentration of cetylpyridinium chloride in petrolatum for sensitization and a 10-percent concentration for challenge. No evidence of sensitization was observed in any of the 24 participants (Ref. 65).

Pharmacokinetic studies assessing absorption, distribution, and elimination of cetylpyridinium chloride were done in rats and dogs (Ref. 65). In the rat study, approximately 85 percent of a single dose of radiolabeled cetylpyridinium chloride was detected in the feces and about 10 percent in the urine. The dog study was inconclusive, since only 56.5 percent of the radiolabeled cetylpyridinium chloride administered was recovered from the urine, feces, case rinses, organs, and carcass.

The safety data were systematically collected from several clinical trials (Refs. 68, 69, and 70). Adverse events did not differ between placebo and control except for tongue and tooth discoloration associated with cetylpyridinium chloride. In contrast, Lobene et al. (Ref. 71) found that approximately a quarter of the subjects using cetylpyridinium chloride reported a slight, transient irritation of the gingiva. In one short-term study (Ref. 72), more subjects in the cetylpyridinium chloride group were found to have aphthous ulcers than the placebo group. Gingival irritation and aphthous ulcers were not reported in other randomized controlled clinical trials of cetylpyridinium chloride-containing mouthrinses. Further studies of the mucosal irritancy potential of cetylpyridinium chloride, especially in those with hyposalivation, are warranted.

Studies (Refs. 65 and 73) showed that there are no significant changes in the balance of the human oral flora or in the overgrowth of potential pathogens such as *Candida*. It appears that cetylpyridinium chloride has activity in the range of 0.12 to 8 micrograms per milliliter (µg/mL) in vitro against *S. aureus*, *S. sanguis*, *E. corrodens*, *Neisseria*, *Veillonella parvula*, *P. gingivalis*, *F. nucleatum*, and *Candida albicans*.

Data on teratogenic and mutagenic effects are available from in vitro and in vivo animal studies (Ref. 65). However, long-term cumulative effects on metabolism and teratogenic effects are not available from controlled human studies. The FDA spontaneous adverse reaction reports and adverse events reports submitted suggest that clinical experience following long-term OTC use of the ingredient has not revealed overt toxic manifestations. Although the summarized FDA spontaneous adverse drug reaction report (Ref. 65) indicates that three deaths and six comas occurred after ingestion of cetylpyridinium chloride-containing mouthrinses, it is unclear to what extent the mouthrinses or other circumstances may have contributed to these severe adverse events. The Subcommittee notes that tooth and tongue staining, as well as oral irritation, may occur with the use of products containing cetylpyridinium chloride.

In summary, the safety of cetylpyridinium chloride has been extensively evaluated in a variety of controlled, clinical and nonclinical studies. Based on this information, in addition to adverse event data collected during more than 55 years of U.S. marketing of mouthrinses containing cetylpyridinium chloride, the Subcommittee concludes that cetylpyridinium chloride is safe when used at concentrations of 0.045 percent to 0.1 percent in mouthrinse formulations.

ii. *Effectiveness*. The Subcommittee concludes that cetylpyridinium chloride is effective as an OTC antigingivitis/antiplaque ingredient within the dosage limits proposed above.

The Subcommittee evaluated six placebo-controlled, blinded, clinical efficacy trials (Ref. 65). In five of the six studies, a 15- to 27-percent reduction in supragingival plaque was obtained with cetylpyridinium chloride in concentrations ranging from 0.05 to 0.1 percent. The reduction seems to persist for 6 months. Four 6-month trials and several shorter trials were also submitted (Refs. 70 and 73). All of the studies demonstrated a significant reduction of supragingival dental plaque with the use of 0.045 to 0.1 percent cetylpyridinium chloride mouthrinse. This is a reproducible finding in both short-term and 6-month studies based on the data submitted and in the published literature (Ref. 74).

The results of two 6-month studies (Refs. 68 and 69), a 2-month study (Ref. 75), and a 4-month study (Ref. 76) showed reductions in gingivitis (based upon gingival index) ranging from 15.7 to 41 percent. Although trends were noted, no clear-cut dose response in the antigingivitis effect was documented in any one study in that range.

Data from four other 6-month studies (Ref. 70) (three of which were carried out by different research groups) did not demonstrate a statistically significant reduction in gingivitis. In the Ciancio study (Ref. 77), there was no statistically significant reduction in gingivitis, although there was a reduction in plaque. Similarly, in the Lobene study (Ref. 78), no differences in gingival index were seen at 4, 20, or 26 weeks, although there was a statistically significant reduction in gingival index at 8 weeks. In two studies (012-035 and 012-037) by Ackerman and DeGenero (Ref. 79), a mouthrinse containing cetylpyridinium chloride showed no effect on gingivitis at 6 months. In a 6-week study by Moran (Ref. 80), cetylpyridinium chloride in a mouthrinse had no effect on plaque or gingivitis. Although most of the formulations reduced plaque, the gingivitis results in these studies are not consistent.

The Subcommittee believes that differences in the results of studies on the effectiveness of cetylpyridinium chloride mouthrinse are likely explained by the use of different formulations (Refs. 65, 70, and 81). Based on the data presented, the biological effectiveness and chemical availability of cetylpyridinium chloride in a mouthrinse appear to be greatly affected by the particular formulation. Cetylpyridinium chloride in mouthrinse formulations all at approximately 0.045 percent nominal concentrations were shown to vary markedly between 4 and 77 percent. Thus, it is clear that inactivation of cetylpyridinium chloride is likely based upon formulation. It is recommended that the bioavailability of cetylpyridinium chloride in each formulation be determined to reduce the possibility that the active ingredient is removed due to chemical reaction, complexing, micelle (a colloid particle formed by an aggregation of small molecules) formation, or other sources of deactivation. Assessment of mouthrinses containing cetylpyridinium chloride in formulations similar to those tested in the positive studies (Refs. 68, 69, 76, and 77) show that 72 to 76 percent of the cetylpyridinium chloride is available (Ref. 82). Therefore, it is reasonable to assume that formulations containing 72 to 76 percent available cetylpyridinium chloride are active in reducing gingivitis and plaque.

At the request of the Subcommittee, the manufacturer conducted additional analyses demonstrating the effectiveness of cetylpyridinium chloride on a site and subject basis, relative to other oral healthcare practices, and on the basis of odds-ratio calculations. Specifically, using a minimum 33 percent reduction in bleeding criterion, results of 4 long-term studies were pooled to estimate an

overall odds ratio for improvement relative to a placebo. After 3 months of product use, the odds ratio was 3.12 with a 95 percent confidence interval of 2.85 to 3.40. After 6 months, the odds ratio was 3.10 with a 95 percent confidence interval of 2.75 to 3.45. Based on the totality of the data, the Subcommittee concludes that cetylpyridinium chloride mouthrinse is safe and effective as an OTC antigingivitis/antiplaque agent.

b. *Stannous fluoride (dentifrice).* The Subcommittee concludes that stannous fluoride in a compatible dentifrice base at a concentration of 0.454 percent is safe and effective for OTC use as an antigingivitis active ingredient.

i. *Safety.* Stannous fluoride has been used as an OTC caries-preventive agent in toothpastes in the United States since 1954. Since 1981, it has been largely replaced by sodium fluoride or sodium monofluorophosphate. However, during this 27-year period, it is estimated that at least 70 billion doses of stannous fluoride were sold in the United States. Thus, a long market history exists to support its safety.

The toxicity of ingesting fluoride from toothpaste has been reviewed extensively (Ref. 83). Concern has been expressed over the need to supervise the use of fluoridated toothpaste by young children because of the potential risk of developing fluorosis (Ref. 84). Acute toxicity of stannous fluoride in the rat (LD_{50}) appears to range from 31 to 300 mg/kg. Thus, it appears to have an acute toxicity comparable to that of sodium fluoride (Refs. 85 and 86). Toxicity studies show that a dentifrice formulation containing stannous fluoride plus stannous chloride was comparable to other nationally marketed fluoride-containing dentifrices.

Several subchronic toxicity tests of stannous fluoride dentifrice formulations have been carried out (Ref. 85). In a study conducted over 3 months, rats received either 3.3 grams (g) dentifrice/kg/daily (= 13.2 mg of stannous fluoride/kg/daily) or 8.4 g dentifrice/kg/daily (= 33.6 mg of stannous fluoride/kg/daily) by gavage. Any observed effects were not attributed to stannous fluoride. Two additional 91-day studies were conducted in rats. Dentifrice slurries in distilled water were administered by gavage. All dentifrice groups revealed microscopic alterations in the stomach lining, such as eosinophilic gastritis, squamous epithelial hyperplasia, and squamous vacualization. No other abnormalities were observed. No tumorigenic effects have been reported from studies conducted in male or female rats or mice. Studies conducted in human volunteers who received 50 mg a day of the stannous ion as stannous chloride revealed that about 3 percent of the dose is absorbed.

Based on results from a 13-week oral toxicity study in rats on stannous chloride conducted through the National Toxicology Program (NTP), a safety factor of 5,000 exists for potential exposure to stannous salts from use of a dentifrice containing 0.454 percent stannous fluoride. The safety factor is defined as the ratio between no observed adverse effect level (NOAEL) in the NTP study and the anticipated exposure to stannous salts from twice daily use of stannous fluoride toothpaste.

The Subcommittee's analyses of clinical studies, including detailed examination of soft tissue and microbiological assays, revealed no adverse shifts among the oral microbiological populations studied, no overgrowth of opportunistic pathogens, and no development of oral microbial resistance to stannous fluoride. Significant reductions in *S. mutans* were observed among subjects exhibiting higher levels of this organism. Based on these data, the Subcommittee concludes that a 0.454 percent stannous fluoride dentifrice is safe for long-term use.

Stannous ion in stannous fluoride dentifrices has been associated with staining of tooth surfaces, which in some instances may be severe (Refs. 87 and 88). In studies CC–191, CC–238, and CC–247 (Ref. 89), 2.1 percent of subjects discontinued the trial due to self-perceived tooth staining. Oral desquamation was reported by five subjects using a stannous fluoride dentifrice. This adverse effect does not appear to be an extensive problem because persons with hyposalivation have used stannous fluoride gels without adverse effects.

Because staining is a common phenomenon with the use of stannous fluoride, the Subcommittee evaluated data concerning the extent of consumer sensitivity to dental staining and the ease with which these stains can be removed. Studies demonstrated that dental staining with 0.454 percent stannous fluoride was noticed by a minority of consumers and that staining can be removed from enamel surfaces and dental restorations during conventional prophylactic procedures. However, the Subcommittee recommends that product labeling include a restriction on use by children and a statement concerning the likelihood of tooth staining.

ii. *Effectiveness.* Stannous fluoride has been incorporated into numerous dentifrice formulations that contain a variety of abrasive substances, including hydrated silica gels, calcium pyrophosphate, and a variety of excipient agents (*see* the **Federal Register** of March 28, 1980, 45 FR 20666 at 20684 to 20688).

The careful formulation of stannous fluoride dentifrices to prevent rapid oxidation and hydrolysis, and thereby inactivation, of stannous ions is critical for clinical effectiveness of these dentifrices. Oxidation can be prevented in several ways. In one approach, water is excluded from the formulation. Another approach involves use of chelating agents such as pyrophosphate, citrate, gluconate, gantrez (a copolymer of maleic acid and methyl ether) or phytate, which form soluble stannous complexes. In addition, incorporation of another stannous compound, such as stannous pyrophosphate or stannous chloride, provides a steady-state situation in which the concentration of bioavailable stannous fluoride is relatively stable. It is essential to note that the inclusion of stannous fluoride alone in a dentifrice without stabilization is not sufficient to obtain optimum clinical effectiveness. Clearly, products containing stannous fluoride may have a defined shelf life.

Stannous fluoride has a long and well-established history as a caries-preventive agent (Ref. 90). Stannous fluoride at a 0.4-percent concentration results in a concentration of 970 parts per million (ppm) fluoride (Ref. 86). Effects of stannous fluoride on plaque formation and gingivitis have given mixed results which, in part, reflect the duration of the studies, the concentration used, and the type of subjects studied.

The Subcommittee evaluated the results of three primary trials and three supportive trials (Refs. 85 and 89) of a stabilized 0.454-percent stannous fluoride dentifrice for antiplaque and antigingivitis claims. Two of the primary 6-month trials (CC–191 and CC–238) carried out in Indiana had results that are consistent with each other (Ref. 89). The final assessments were consistent with the interim 3-month assessments. The third study (CC–247), conducted in Northfield, lasted for 7 months and had results that appeared to differ in some measures from those in Indiana (Ref. 89). The Indiana studies had reductions of 18.8 percent and 20.5 percent in gingival index, 30.5 percent and 33.4 percent in bleeding index, and a nonsignificant reduction of 2.6 percent and 3.1 percent compared with placebo in plaque. In contrast, the Northfield study (one evaluator) reported a 10.7-percent

reduction in gingivitis in the stannous fluoride group and a statistically not significant 6.6-percent increase in the bleeding index. There was a 17.8-percent reduction in a Turesky modified Quigley-Hein Plaque Index and a 1.1-percent reduction using the Silness & Loe Plaque Index system. Two graders were used in this study, and they obtained large numerical differences in their assessments at the 3-month assessment period and the final 7-month assessment. No significant shifts in the microbial flora were reported after 3 and 6 months of product use.

Three supportive double-blind and independent studies (CC–174, CC–178, and CC–205) have been reported (Ref. 91). Two studies (CC–174 and CC–178) continued for 6 months and the third study (CC–205) for 2 months. Study CC–174 demonstrated statistically significant differences in the indices from the stannous fluoride group compared with the negative control at the 1.5- and 3-month grading periods. However, all indices were not significant at the 7-month grading period.

Study CC–178 (Ref. 91) revealed no significant differences in the gingival, bleeding, and plaque indices after 2 months use in the stannous fluoride group, compared with the control. After 6 months use, there was a statistically significant difference in the gingivitis index (9.3 percent) in the stannous fluoride group. Significant differences were not detected in the bleeding and plaque indices among the two groups.

Study CC–205 (Ref. 91), which was conducted for 2 months only, revealed a significant difference (15.4 percent) in the gingivitis index of the stannous fluoride group compared with the control. There was a reported 23.9 percent difference in the bleeding index. However, the scores for both groups were exceptionally low compared with all of the study groups. Statistically significant differences in plaque scores among the groups were not detected.

In five of the six studies reported, no significant differences in plaque scores were observed at the end of the evaluation period in subjects using stannous fluoride dentifrices compared with those using a control dentifrice. In 7 of 12 exams in two of the six studies, there was a reported statistically significant reduction in bleeding scores, and in five of the six studies there was a reduction in gingivitis scores associated with the use of stannous fluoride dentifrices.

The Subcommittee evaluated additional information on the effectiveness of a 0.454 percent stannous fluoride dentifrice, including additional analyses it requested. The results of these analyses helped to establish that the study populations were appropriate for the OTC gingivitis indication recommended by the Subcommittee. Disease levels in the populations used in clinical studies supporting the stannous fluoride dentifrice were only slightly higher than disease levels established in published epidemiological studies and in surveys of oral health status conducted by the National Institute of Dental Research.

Additional data were presented concerning the clinical relevance of the observed beneficial effects of the dentifrice on gingivitis. These data included site-specific analyses demonstrating that a 0.454 percent stannous fluoride dentifrice provided uniform efficacy in reducing gingivitis across the dentition and, in particular, in regions of significant disease. This site-based analysis was further expanded to compare treatment effects (*e.g.,* causing a bleeding site to become a nonbleeding site) with benefits in preventing new disease (*e.g.,* preventing a nonbleeding site from becoming a new bleeding site) during clinical studies. These analyses revealed that, compared to placebo, the stannous fluoride dentifrice was beneficial in preventing and reducing gingivitis and gingival bleeding.

An analysis of the clinical benefits of stannous fluoride in reducing gingivitis compared to increased brushing, flossing, and frequent visits to a dentist indicated that a stannous fluoride dentifrice provides benefits comparable to the improvements observed from these established dental hygiene procedures.

Finally, odds ratio analyses were used to determine the likelihood of an individual deriving a benefit from the use of a stannous fluoride dentifrice. Based on the benefits achieved from dental hygiene and benefits seen in studies CC–191 and CC–238 (Ref. 89), a meaningful benefit for a subject was defined as at least a 33-percent reduction in bleeding. Using this definition, the results of five long-term studies (Refs. 89 and 91) were pooled to estimate an overall odds ratio for improvement relative to a sodium fluoride control. After 3 months of use, the odds ratio was 1.57 with a 95-percent confidence interval of 1.29 to 1.85.

A review of the cited literature indicates that a number of studies examined the effects of stannous fluoride in gels, mouthrinses, and dentifrices. Many of these studies were of short duration, used few subjects, or used special groups of subjects. Thus, the quality and relevance of the data are, in some instances, questionable. The results are far from uniform in showing benefits from the use of stannous fluoride.

With the exception of the studies submitted by the sponsor, there appear to be few studies involving the use of dentifrices containing stannous fluoride. Ogaard *et al.* (Ref. 92) studied the effect of a stannous fluoride dentifrice on plaque regrowth in 15 subjects for 24 hours and 21 subjects for 3 weeks using a crossover design. Stannous fluoride was compared to a sodium monofluorophosphate dentifrice and a dentifrice without fluoride. Stannous fluoride gave significantly lower regrowth values than monofluorophosphate or placebo.

In the 3-week crossover study (Ref. 92), 21 orthodontic subjects brushed twice daily for 1 minute with a stannous fluoride dentifrice or placebo paste. Less plaque was observed in the stannous fluoride group when the orthodontic brackets were 1 to 5 millimeters (mm) from the gingiva; if the brackets were closer, there was no difference in the effects of the stannous fluoride and the placebo dentifrice. No significant improvement was observed in gingival health regardless of treatment group.

Bay and Rolla (Ref. 93) conducted a double-blind, crossover study in 40 pupils aged 15 years to compare the effects of a stannous fluoride dentifrice and a placebo dentifrice without stannous fluoride. The number of times the dentifrice was used was not stated, and the gender of the pupils was not disclosed. The study continued for 4 weeks. There was reduced plaque formation in the stannous fluoride group and a small reduction in gingival index.

Svatun (Ref. 94) compared the effect of dentifrices containing: (1) 0.4 percent stannous fluoride, (2) a similar dentifrice without stannous fluoride, (3) 0.4 percent stannous fluoride plus stannous pyrophosphate, and (4) 0.8 percent chlorhexidine gel. Twelve female dental students were included and tests lasted for 4 days. The test products were placed in cap splints that covered the teeth only and held in place for 2 minutes twice daily. Subjects rinsed with sucrose (15 percent) for 1 minute every other hour to enhance plaque formation. No mechanical oral hygiene was allowed during the study. The dentifrice containing 0.4 percent stannous fluoride plus stannous pyrophosphate gave significantly lower plaque scores than the dentifrice containing 0.4 percent stannous fluoride alone, or a similar dentifrice without stannous fluoride. There was a wide

range in scores among subjects using the dentifrice containing 0.4 percent stannous fluoride plus stannous pyrophosphate.

In a second study in the same report (Ref. 94), Svatun examined the influence of polishing teeth with a stannous fluoride or sodium monofluorophosphate dentifrice on 24-hour plaque regrowth in 8 mentally retarded home care subjects. Oral hygiene was suspended for 24 hours. There was less plaque regrowth following the stannous fluoride treatment, confirming the results of previous studies showing the effectiveness of stannous fluoride as a plaque inhibiter. A cap splint pilot study comparing stannous fluoride and sodium monofluorophosphate dentifrices did not result in any improvement in the gingiva of these subjects.

Several studies have been carried out using rinses or gels containing stannous fluoride. It is doubtful whether the results from these studies are strictly applicable to dentifrices containing stannous fluoride. Nevertheless, the data are worth exploring because they may help to clarify the therapeutic potential of stannous fluoride.

Svatun (Ref. 95) compared the plaque-inhibiting effects of mouthrinses containing 0.2 and 0.3 percent stannous fluoride, 0.1 percent chlorhexidine, and distilled water randomly distributed among 12 dental hygienist students. Subjects rinsed with 10 mL for 1 minute twice a day for 4 days, with no other oral hygiene permitted. Plaque index scores were brought to 0 at the beginning of each test period. Mean plaque scores were 0.35 for 0.2-percent stannous fluoride, 0.20 for 0.3-percent stannous fluoride, 0.12 for chlorhexidine, and 1.02 for the placebo. A long-term study (Ref. 95) in another group of 5 students showed that the effect of a 0.3-percent stannous fluoride mouthrinse could be maintained for 3 weeks.

Klock *et al.* (Ref. 96) compared the effects of rinsing with stannous fluoride or sodium fluoride (200 ppm fluoride) twice daily for 2 years on oral health in adults. Thirty-seven subjects started the study; 15 withdrew during the first year and 3 withdrew during the second year. After 2 years, there were 12 in the stannous fluoride group and 7 in the sodium fluoride group, a total of 19 subjects. The authors commented: ''The population of subjects was generally unreliable.'' Plaque scores were not compared among the groups because the values were skewed at the baseline. Both groups showed a reduction in plaque at 1 year and subsequent increase after 2 years. Bleeding sites were significantly reduced after 1 year in the stannous fluoride group. This trend continued into the second year, but the results at 2 years were no longer statistically significant. The lack of statistical significance is probably due to the loss of subjects between the first and second years. Other possible factors are the inability of subjects to comply with the mouthrinsing regimen and the development of bacterial resistance to the stannous fluoride rinse. The stannous fluoride group harbored significantly fewer *S. mutans* than did the sodium fluoride group.

Several studies examining the effects of 0.4-percent stannous fluoride gels have been carried out in persons wearing prosthetic or orthodontic appliances. The validity of extrapolating data from these studies to support clinical claims for 0.4-percent stannous fluoride dentifrice is open to question even though these studies may provide information on the potential therapeutic effect of stannous fluoride.

Derkson and MacEntee (Ref. 97) examined the effects of a 0.4-percent stannous fluoride gel in 17 subjects with overdentures using a double-blind, crossover design. A nonfluoridated gel was used as a control. Each gel was applied daily for 6 months. Gingival and plaque index scores were recorded. A total of 34 teeth in 12 subjects who completed the study were available for assessment. No difference between the effects of two gels was observed in Gingival Bleeding Index scores from subjects who used the stannous fluoride gel first. Subjects who used the placebo first showed a 19-percent reduction in gingival index scores following use of stannous fluoride gel. The plaque index scores did not show any significant difference.

Tinanoff *et al.* (Ref. 98) conducted a double-blind study in 61 adults with fixed or removable dental prostheses. Subjects were given a thorough prophylaxis, including scaling and root planing, and were instructed to brush once daily for 2 weeks with a regular dentifrice. After the 2-week washout period, subjects then brushed twice daily (without rinsing) with a 0.22 percent sodium fluoride gel or 0.4 percent stannous fluoride gel. Subjects were not permitted to have a dental prophylaxis during the course of the study. At the end of 6 months, gingival index scores in the stannous fluoride group, using all teeth (including abutment teeth), were 48 percent lower than in the control group. The authors noted ''increasing change between groups over time in the percent bleeding site scores appears to be due to rise in the number of bleeding sites in the sodium fluoride group during course of the study.'' (There was no reduction in the number of bleeding sites compared with baseline.) Differences in plaque scores were statistically significant only when computed for abutment teeth. The authors noted ''higher baseline plaque index scores in the sodium fluoride group as compared to the stannous fluoride group might in some way influence other clinical or microbial indices.'' The stannous fluoride group harbored 2.5 log fewer *S. mutans* than did the sodium fluoride group.

Two relatively long-term studies of 0.4 percent stannous fluoride gel gave apparently contrasting results. However, the apparent disparity may be a reflection of the type of subjects and the hypothesis studied. Boyd, *et al.* (Ref. 87) monitored the gingival health of 81 adolescents undergoing orthodontic treatment with fixed appliances while investigating the effects of daily brush-on 0.4 percent stannous fluoride gels. One gel contained 98 percent available tin (used twice daily), and the other gel contained 2 percent available tin (used once daily and later twice daily). The control group did not use any gel. Subjects were instructed not to rinse after using the gel. Subjects continued their normal oral hygiene practices. Sites were scored at baseline and at 1, 3, 6, and 9 months after appliances were applied. There was a gradual increase in plaque accumulation from baseline to 9 months in all groups and no statistically significant difference in plaque scores among the groups. The gingival and plaque indices showed similar patterns. However, the percentage of sites with an index greater than 1 was statistically significantly less than observed in other groups. The percentage of sites with a Bleeding Tendency score greater than 1 also followed a similar pattern. Thus, use of stannous fluoride gel was associated with a smaller increase in gingival index and percent Bleeding Tendency compared with controls. However, there was no reduction in the indices compared with baseline.

In a second long-term study, Wolff *et al.* (Ref. 88) studied the effects of 0.4 percent stannous fluoride gel, 0.22 percent sodium fluoride gel, and a fluoride-free placebo gel in three groups of 281 subjects over 18 months. All subjects brushed with a sodium monofluorophosphate dentifrice twice daily. Subjects then used either a stannous fluoride, sodium fluoride, or placebo gel twice daily immediately after brushing with no rinsing for 30 minutes after using gel. Plaque, bleeding, and gingival indices were assessed after 6, 12, and 18 months.

There was no significant difference in the mean plaque index between any of the groups. The gingival index declined in all groups, with no differences detected between groups. No differences were observed among any groups at any time.

Based on the analyses of effectiveness on a site and subject basis compared to other oral health care practices and on odds-ratio calculations conducted on the submitted data, the Subcommittee concludes that, although available clinical data do not show reproducible long-term effects in reducing dental plaque mass, stannous fluoride is safe and effective in a dentifrice at an appropriately formulated concentration of 0.454 percent as an OTC antigingivitis agent.

2. Category I Combinations of Active Ingredients (*See* General Combination Policy in section II.E of this document)

Eucalyptol, menthol, methyl salicylate, and thymol. The Subcommittee concludes that a combination of essential oils consisting of eucalyptol (0.092 percent), menthol (0.042 percent), methyl salicylate (0.060 percent), and thymol (0.064 percent) in a hydroalcoholic vehicle containing 21.6 to 26.9 percent alcohol in a mouthrinse is safe and effective as an OTC antigingivitis/antiplaque agent.

a. *Safety.* Eucalyptol is a volatile oil prepared by steam distillation of the fresh leaves of *Eucalyptus globulus*. Eucalyptol is colorless, or a pale yellow volatile liquid with a characteristic aromatic, somewhat camphoraceous odor, and a spicy and cooling taste. Eucalyptol is also known as cineol, cineolcayeptol, and cajuptol. It is insoluble in water, but it is miscible with alcohol, chloroform, and ether.

The Dental Panel concluded that eucalyptol is safe as an OTC anesthetic/analgesic active ingredient for topical use on the mucous membranes of the mouth and throat when used at a concentration of 0.025 to 0.1 percent in the form of a rinse, mouthwash, gargle, or spray (47 FR 22712 at 22826, May 25, 1982). It was reviewed and found safe by the Flavor and Extract Manufacturer's Association of the United States (FEMA) (Ref. 99).

Menthol is a secondary alcohol extract from peppermint oil or made synthetically. Chemically, it is also known as hexahydrothymol and 3–paramenthanol. Menthol may be made synthetically by the hydrogenation (reduction) of thymol. The Dental Panel concluded that menthol is safe as an OTC active ingredient for topical use on the mucous membranes of the mouth and throat at a concentration of 0.04 to 2.0 percent in the form of a rinse (47 FR 22712 at 22813). Menthol was reviewed and found safe by FEMA (Ref. 100).

Methyl salicylate is the methyl ester of salicylic acid. Prior to the discovery of a method for chemical synthesis of methyl salicylate, it was produced by steam distillation from natural sources. The natural-source products are known as gaultheria oils, betula oil, sweet birch oil, teaberry oil, and wintergreen oil. Today, these names are used synonymously with methyl salicylate. Methyl salicylate is prepared synthetically by esterifying salicylic acid with methanol. The Dental Panel concluded that methyl salicylate is safe for topical use on the mucous membranes of the mouth and throat when used within the proposed dosage limit up to a 0.4-percent concentration in the form of a rinse, mouthwash, gargle, or spray, not more than three to four times daily (47 FR 22712 at 22828). Methyl salicylate was reviewed and found safe by FEMA (Ref. 101).

Thymol, also known as thyme camphor, is 5-methy-2-isopropyl-2-phenol. It may be prepared synthetically or obtained from volatile oils distilled from *Thymus vulgans* and other related plant sources. Thymol is an alkyl derivative of phenol and has bactericidal and fungicidal properties. It was reviewed and found safe by the Advisory Review Panel on OTC Dentifrice and Dental Care Drug Products (the Dental Panel) (47 FR 22712 at 22829, May 25, 1982) and by FEMA (Ref. 102).

The safety of the combination of the four ingredients has been assessed in numerous long-term clinical studies. These studies showed no clinical pathologic change or adverse reactions (Refs. 103, 104, and 105).

Because OTC drug products are readily available, the determination of the safety of single ingredients and combinations of ingredients also requires consideration of possible abuse. Exaggerated use studies have been done. In one study (Ref. 106), 47 healthy adult subjects screened for sensitivity and allergy histories rinsed with 20 mL of the combination of essential oils for 30 seconds under supervision at 5 hourly intervals each day for 5 days and repeated 18 days later for 1 day. No subject developed any oral mucosal lesions attributable to the test product. A second study (Ref. 107) of 45 adult subjects followed a similar protocol. One subject had erythema (2-centimeter lesion) and epithelial sloughing on day 5 of the irritation phase of the study. In a third exaggerated use study involving 18 xerostomic (dryness of the mouth from salivary gland dysfunction) adults, 2 subjects experienced what was described as ''utransient mucosal sloughing'' and continued the regimen. The remaining xerostomic subjects did not develop mucosal lesions (Ref. 108). These studies showed that the potential for mucosal irritation is minimal when these ingredients are used according to label directions.

Two studies evaluated possible shifts in oral microbial populations and the emergence of opportunistic organisms or potential pathogens. One study in 83 subjects (Ref. 109) showed analysis of plaque samples from active agent and control groups. There were no significant increase in presumptive oral pathogens, spirochetes, black-pigmented Bacteroides, *S. mutans*, or *C. albicans*. A second 6-month study (Ref. 110) examined plaque at 3 and 6 months. Three microbiological approaches were used: (1) Microscopic enumeration of cocci, motile and nonmotile rods, and spirochetes, (2) recovery on selective and nonselective culture media, and (3) enumeration by colony morphology on a nonselective medium. No clinically significant shifts were found in the composition of the flora.

Mutagenicity studies have been reported (Ref. 111). The fixed combination of essential oils did not show mutagenic potential in the Ames test, the Unscheduled DNA Synthesis test, and the Mouse Micronucleus test.

Much of the evidence of the safety of the combination of these ingredients comes from their extensive history of use (well over 100 years) and the low incidence of consumer complaints reported by the manufacturer. The data included an estimate of one adverse reaction report for every 38,700,000 doses of these ingredients sold, which is described as an extremely low rate. The four ingredients in this combination have had a long and safe marketing history which contributes to the Subcommittee's conclusion that the combination is safe when used according to label directions.

b. *Effectiveness.* The Subcommittee evaluated seven 6-month, randomized, controlled trials of the effectiveness of a fixed combination of eucalyptol (0.092 percent), menthol (0.042 percent), methyl salicylate (0.060 percent), and thymol (0.064 percent) in a hydroalcoholic vehicle containing 21.6 to 26.9-percent ethyl alcohol. One study was a 6-month, randomized, controlled study (Ref. 103) involving 145 students and staff at an East Coast university, aged 18 to 54 years, randomized into three groups using either the above fixed combination, a vehicle control (a 26.9-percent hydroalcoholic vehicle containing all the ingredients in the test

product except the essential oils), or a water control. Of the 145 subjects who entered the study, approximately 62 percent were male and 20 percent were smokers. Inclusion criteria were 20 natural teeth exclusive of large carious lesions, orthodontically banded, fully crowned, abutment, and third molar teeth, and a minimum score of 2.0 using a modified Loe-Silness Gingival Index plus a minimum score of 1.8 using the Turesky modification of the Quigley-Hein Plaque Index. Of 129 subjects completing the study, 45 were in the essential oils group (mean age 26.1 years), 43 were in the vehicle control group (mean age 27.9 years), and 41 were in the water control group (mean age 24.7 years).

Subjects were supervised as they rinsed twice daily from Monday to Friday with 20 mL for 30 seconds. Coded 3-ounce (oz) bottles and graduated plastic cups were distributed for twice daily unsupervised weekend use. Coded 16-oz bottles were distributed for holidays and recesses. Subjects were required to maintain a diary of unsupervised rinse use. Subjects followed their usual oral hygiene regimen, with no dental treatment, scaling, or polishing prior to the rinse regimen.

All intraoral examinations were performed by the same examiner. Gingivitis was scored using the modified Loe and Silness Gingival Index which adds an additional score between the 1 and 2 of Loe and Silness, thus having two levels of ''Mild Inflammation,'' and eliminates the bleeding component from the original criteria for ''Moderate Inflammation.'' This index was later published by Lobene (Ref. 112) and is used in five of the eight ''definitive'' studies. Results (*see* Table 4 below) showed a continuous decline in adjusted mean gingivitis scores for each of three groups from baseline through 6 months.

TABLE 4.—RESULTS OF THE LAMSTER STUDY GROUP

Group	Baseline	1 month	3 months	6 months
Essential Oils	2.62	2.08	1.57	1.20
Vehicle Control	2.67	2.20	1.94	1.66
Water Control	2.66	2.32	1.93	1.67

Mean scores for the fixed combination of essential oils were statistically significantly less than controls at 3 and 6 months and 28 percent less than either control group mean score at 6 months. Control groups of this monitored, supervised, mostly young, dental school population continued to show a decrease in mean gingival index scores over time. No bleeding assessments were made.

A second study (Ref. 104) involved mostly dental students and staff of the same university, with the same inclusion criteria. Subjects were randomized into three groups, with 44 in the essential oils group (mean age 25 years), 38 in the vehicle control (a 26.9-percent hydroalcoholic vehicle containing all the ingredients in the test product except the essential oils) group (mean age 29 years), and 45 in the water control group (mean age 27 years). Upon entering the study, all subjects had a dental prophylaxis (defined as a scaling and rubber cup polishing), followed in 3 weeks by a baseline 1 examination. Two additional prophylaxes were done for each subject 4 to 7 days apart, followed in 3 to 4 days by a baseline 2 assessment. Prior to the first rinse, another (fourth) polishing was done. Subjects were randomly assigned to either the fixed combination of essential oils, a vehicle control, or a colored water control.

Supervision of rinsing and monitoring was the same as in the first study and gingivitis was scored as before. No bleeding assessment was done. Results (*see* Table 5 below) were recorded at 1, 3, and 6 months, with all assessments performed by one examiner. No intra-examiner variability testing is noted. Eighty-five subjects completed an additional 3 months of unsupervised rinsing. Most of the subjects who did not participate for the additional 3 months of the study were recently graduated dental students who were not available for the 9-month examination. The 6–month mean gingival index score for the essential oils was 10.4 percent less than the water control and 6.5 percent less than the vehicle control, but no statistically significant differences existed between groups for any interval.

TABLE 5.—MEAN GINGIVAL INDEX SCORES FROM THE GORDON STUDY

Group	Baseline 1	Baseline 2	1 month	3 months	6 months
Mean Gingival Index Score					
Essential Oils	1.60	1.39	1.54	1.27	1.31
Water	1.60	1.38	1.55	1.38	1.46
Vehicle	1.59	1.33	1.49	1.25	1.37

Mean gingival index scores for the 127 subjects who completed 6 months of the study were as follows: 1.23 for the essential oil group, 1.42 for the vehicle control group, and 1.57 for the water control group. Results for the 85 subjects who completed 9 months showed a statistically significant difference in mean gingival index scores, as follows: 1.12 for the essential oils, 1.43 for the vehicle control, and 1.52 for the water control.

The investigators stated that the lack of difference for gingivitis observed between groups for 6 months was probably due to improvement in gingival health resulting from four prophylaxes initially, followed by continuation of usual oral hygiene.

A third study involving 115 subjects in two study groups (essential oils and

5–percent hydroalcohol) was conducted at the University of Maryland using the same protocol (Ref. 105). Of the 115 subjects, 107 completed the study; 60 percent were male, 40 percent were female; 17 percent were smokers and 83 percent were nonsmokers. Each subject received a dental prophylaxis on the day the first rinse was given. Baseline gingival index scores were recorded prior to the prophylaxis and after 7 days of treatment. Fifty-four subjects (mean age 28.5 years) were in the essential oils group and 53 subjects (mean age 27.6 years) were in the 5–percent hydroalcohol control group. The analysis (*see* Table 6 below) was based on adjusted mean gingival index scores at 3 and 6 months.

TABLE 6.—ADJUSTED MEAN GINGIVAL INDEX SCORES FROM THE DEPAOLA STUDY

Group	Baseline 1	3 months	6 months
Essential Oils	2.288	1.522	0.918
5% hydroalcohol	2.200	1.576	1.385

Results included the distribution of gingival index scores in percentage at both baselines and at 6 months. No zero scores were recorded at baselines 1 and 2, but zero scores accounted for 38 percent of all scores in the essential oil group and 19 percent of all scores in the control group at 6 months.

The fourth study (Ref. 113), conducted at the University of Maryland, included a bleeding index (Ref. 114) in addition to the established inclusion criteria, assessments, and regimen of supervised rinsing twice a day on weekdays. This study compared the fixed combination of essential oils to 0.12 percent chlorhexidine gluconate and a control solution of flavored, colored 5 percent alcohol. There were 41 subjects in the essential oils group (mean age 29.2 years), 41 subjects in the chlorhexidine gluconate group (mean age 29.2 years), and 42 subjects in the control group (mean age 28.6 years). Following baseline examination, all subjects were given a dental prophylaxis. Assessments were made at 3 and 6 months. Two examiners were used, but only one examiner recorded gingivitis, plaque, and bleeding indices. Teeth used for a plaque collection at time of assessment were eliminated from statistical analysis for gingival, bleeding, and plaque indices. The specific teeth used were not cited in this report. Adjusted mean gingival scores (*see* Table 7 below) were presented for 3 and 6 months.

TABLE 7.—ADJUSTED MEAN GINGIVAL SCORES FROM THE OVERHOLSER STUDY

Group	Baseline	3 months	6 months
Essential Oils	2.234	1.328	0.748
Chlorhexedine Gluconate	2.281	1.032	0.810
5% Hydroalcohol Control	2.221	1.409	1.166

At 6 months, both active mouthrinses were statistically significantly different than the control in gingival index scores; the mean value of the essential oils score was 35.9 percent less than the mean value of the control score.

The distribution of gingival index scores at baseline and at 6 months for scores 0, 1, 2, and 3 were also presented in percentages. No zero scores were recorded at baseline. At 6 months, the percentage of gingival units with zero scores was 26 percent for control, 46 percent for the essential oils and 43 percent for chlorhexidine gluconate. Scores 1 and 3 were comparable for the three study groups but score 2 differed, decreasing from baseline to 6 months from 74 to 17 percent for the essential oils, 70 to 23 percent for chlorhexidine gluconate, and 74 to 34 percent for the control.

Bleeding index scores (*see* Table 8 below) declined for all groups and were not statistically significantly different at 6 months.

TABLE 8.—BLEEDING INDEX SCORES FROM THE OVERHOLSER STUDY

Group	Baseline	3 months	6 months
Essential Oils	.71	.40	.29
Chlorhexedine Gluconate	.72	.28	.25
5% Hydroalcohol Control	.66	.37	.33

Mankodi (Ref. 115) conducted a similar study using the Loe-Silness Gingival Index, thus adding a bleeding component. This study compared the combination of essential oils to the same formulation with the addition of mint flavor and a 5-percent water-alcohol control. Each subject was given a prophylaxis on the day rinsing began. There were 42 subjects in the essential oils group (mean age 31.1 years), 44 subjects in the essential oils plus mint group (mean age 30.6 years), and 38 subjects in the control group (mean age 33.1 years). The percentage difference between mean gingival index scores (*see* Table 9 below) at 6 months showed a score for the essential oils (0.90) that was 22.4 percent less than the control score (1.16).

TABLE 9.—MEAN GINGIVAL INDEX SCORES FROM THE MANKODI STUDY

Group	Baseline	3 months	6 months
Mean Gingival Index Score (adjusted for 3 and 6 months)			
Essential Oils	1.19	0.93	0.87
Essential Oils plus Mint	1.22	1.00	0.91
Control	1.23	1.10	1.18

A second study by Mankodi *et al.* (Ref. 116) compared the effects of the combination of essential oils, chlorhexidine gluconate, and a 5-percent water-alcohol control. There were 34 subjects (mean age 32 years) in the essential oils group, 36 subjects (mean age 31.4 years) in the chlorhexidine gluconate group, and 38 subjects (mean age 32.2 years) in the water-alcohol control group. The protocol was similar to the earlier studies with the exception of the use of the Russell Periodontal Index ''to further describe the study population,'' and the use of the Loe and Silness Gingival Index for assessment. The results (*see* Table 10 below) showed a statistically significant difference between the essential oil and control groups at 6 months, with the mean gingival index score for the essential oils group being 14.0 percent less than the mean score for the control group.

TABLE 10.—MEAN GINGIVAL INDEX SCORES FROM THE MANKODI STUDY

Group	Baseline	3 months	6 months
Mean Gingival Index Scores			
Essential Oils	1.31	1.22	1.04
Essential Oils Plus Mint	1.35	1.04	0.99
Control	1.27	1.18	1.21

A third study by Mankodi (Ref. 117) compared the effects of the combination of essential oils, the same combination plus flavor, and a 5–percent water-alcohol control. There were 48 subjects in the essential oils group (mean age 32 years), 43 subjects in the essential oils plus mint group (mean age 32 years), and 50 subjects in the water-alcohol control group (mean age 34 years). The protocol was similar to previous studies, but supervision on weekdays was limited to one of the two daily rinses, and this study used the Lobene modification of the Loe-Silness Gingival Index. Subjects received a prophylaxis following their baseline examination. Gingivitis was scored at baseline, 3 months, and 6 months. All intraoral examinations were performed by a single qualified dental examiner. Units of statistical analysis were the respective mean index scores determined for each subject. Gingival indices were analyzed by the analysis of variance, using baseline scores as the covariant. Results of gingival index scoring (*see* Table 11 below) are adjusted means for 3 and 6 months. Mean score percent reduction from control at 6 months for the combination of essential oils plus flavor was 10.8 percent and 10.2 percent for the combination without flavor. Both active groups are statistically significantly different at 6 months.

TABLE 11.—MEAN GINGIVAL INDEX SCORES FROM THE MANKODI STUDY

Group	Baseline	3 months	6 months
Essential Oils Plus Mint	2.16	1.68	1.66
Essential Oils	2.20	1.63	1.67
Control	2.19	1.82	1.86

An eighth 6-month controlled trial (Ref. 118) used the fixed combination of essential oils and a ''flavor variant'' control. The results showed the mean gingival scores significantly lower than the control group at 6 months.

These studies demonstrated that the fixed combination of essential oils has some effectiveness in preventing inflammation of the gingiva. The initial analyses relied solely on statistical hypothesis testing (the use of p values), which does not convey important quantitative information. However, a number of concerns (strength of the effect and its statistical significance, the generalizibility of the studies to the population which can most benefit, and the unit of analysis (subject versus site)) were resolved to make a valid determination as to the strength of antigingivitis efficacy for these ingredients.

Generalizibility of randomized, controlled trials to the population who will use the product is a concern. These studies use young populations, weighted with dental students, where supervision and timing of use is present. Much of the population that will benefit from an antigingivitis agent is middle-aged and older, having fully crowned

and restored teeth, and abutment teeth, which have been omitted from scoring in these trials. These teeth are among the ones most in need of combating gingivitis.

Because it is the individual who is at risk, it is important to know if each subject has changed. Use of mean gingival index scores for each individual subject is the correct way to calculate the mean score for each trial group at various intervals. However, analysis of each site infers that all sites provide independent observations. This assumes that 100 sites in one subject provide the same outcome information as one site in each of 100 subjects. Differences between subjects are greater than variations within subjects (Ref. 119). The principle noted is ''In investigations where experimental units on different levels are employed, use the highest level unit as computational unit'' (Ref. 120). All sites within one subject are not at equal risk for gingivitis. Inflammation tends to be more overt at interdental areas than at lingual or facial sites. To quantify the findings (*i.e.,* who and how many in the study groups are affected, and by how much) and to present the findings with appropriate indicators of measurement error or uncertainty (such as confidence intervals), further analyses were completed.

Data from pooled analyses of the eight 6-month studies were presented to the Subcommittee. Results showed that mean index values for men differed between the control and essential oils regimen and were similar to differences seen in women for gingival bleeding, gingival index, and plaque index. Differences in mean values between the control and active agent were presented for subjects aged 18 to 39 years and were similar to differences seen in subjects 40 years old and older. The percent of subjects who improved in bleeding, gingival index, and plaque scores from the initial exam to 6 months was greater in the essential oil group than the control group.

Pooled data from the eight studies were used to compute the odds ratio for reduction in gingival index score. The odds ratio was 4.21 with a 95-percent confidence interval (CI) of 2.79 to 6.36 to achieve a goal of 33 percent reduction in score. The bleeding score odds ratio for all studies where bleeding was assessed was 5.12 (CI 3.29 to 7.97). Again, the target goal was a 33-percent reduction in score. For the reported plaque index score reduction of 33 percent, the pooled (eight studies) odds ratio was calculated at 10.53 (CI 7.06 to 15.71).

The Subcommittee concludes that a combination containing eucalyptol (0.092 percent), menthol (0.042 percent), methyl salicylate (0.060 percent), and thymol (0.064 percent) in a hydroalcoholic vehicle containing 21.6 to 26.9 percent alcohol in a mouthrinse meets the requirements of FDA's policy regarding fixed combinations of OTC active ingredients with the same pharmacological action. The Subcommittee concludes that each of these ingredients contributes to the antibacterial activity of the combination, and that each is safe individually and in combination.

Based on the data submitted, the Subcommittee concludes that the combination of eucalyptol (0.092 percent), menthol (0.042 percent), methyl salicylate (0.060 percent), and thymol (0.064 percent) in a hydroalcoholic vehicle containing 21.6 to 26.9 percent alcohol in a mouthrinse is safe and effective as an OTC antigingivitis/antiplaque agent.

B. Category II Conditions

None.

C. Category III Conditions

The available data are insufficient to permit final classification at this time. Data to demonstrate safety and effectiveness as an antigingivitis/antiplaque agent will be required in accordance with the guidelines set forth above (*see* general guidelines on safety and effectiveness in section II.H of this document.)

1. Category III Single Active Ingredients

Aloe vera
Dicalcium phosphate dihydrate
Hydrogen peroxide
Sanguinaria extract
Sodium bicarbonate
Sodium lauryl sulfate
Zinc citrate

a. *Aloe vera*. The Subcommittee concludes that there are insufficient data to permit final classification of the safety and effectiveness of aloe vera as an OTC antigingivitis/antiplaque ingredient. Aloe vera (known in commerce as Curacao Aloe) is a brownish black, opaque mass with a fractured surface that is uneven, waxy, and somewhat resinous (Ref. 121). Aloe vera is obtained from the parenchyma tissue in the center of the leaf by mechanical or chemical means and is highly variable in its properties. The main constituents are polysaccharides, mainly glucomannans, anthraquinone glycosides, and glycoproteins. Other constituents may include sterols, saponins, and organic acids. Aloe vera is topically applied as an emollient, to aid in wound healing, and relieve burns (including sunburn), and is used for colonic irrigation. Extracts of aloe vera have been shown to enhance phagocytosis (ingestion by a cell of particulate material, such as microorganisms) in adult bronchial asthma. It is also used as an ingredient in many cosmetic preparations (Ref. 122). Aloe vera is produced by boiling Aloe juice down and pouring the viscous residue into empty spirit cases, in which it is allowed to solidify. Aloe vera possesses a nauseating and bitter taste and a disagreeable, penetrating odor. It is almost entirely soluble in 60 percent alcohol and contains not more than 30 percent of substances insoluble in water. Solutions of aloes gradually undergo change and, after a month, may no longer react normally and may lose the bitterness natural to aloes (Ref. 123).

i. *Safety*. The safety of aloe vera is difficult to discern from the data. However, there are studies in which the toxicity of components of aloe vera are discussed, *e.g.,* the component, acemannan (Ref. 124). Also, there is evidence that application of aloe vera to wounds will delay healing (Refs. 125 and 126). The Subcommittee concludes that the data are insufficient to permit final classification of the safety of aloe vera.

ii. *Effectiveness*. The Subcommittee concludes that there are insufficient data to permit final classification of the effectiveness of aloe vera as an OTC antigingivitis/antiplaque ingredient.

Aloe vera, a plant extract, has been claimed to have antiinflammatory and antiprostaglandin effects, as well as cathartic effects (Ref. 127). There are also claims that aloe vera extract is effective against several gram-positive and gram-negative organisms as well as *C. albicans*. However, the Subcommittee finds that the studies are conflicting and that the concentrations required appear to be 20 percent to 90 percent.

The enzyme blend of protease, lipase, and amylase is described as contributing to 3 percent of the formulation reviewed. There is only a general rationale for use in periodontal disease for debridement resulting in reduction of deposits of hard and soft excretions. However, no valid scientific evaluation of this proposed activity is apparent from the submitted data or from the literature (Ref. 128). In addition, no specific testing of the formulation has been presented or was located in the literature (Ref. 128). Therefore, the Subcommittee concludes that there are insufficient data to permit final classification of the effectiveness of aloe vera as an OTC antigingivitis/antiplaque ingredient.

b. *Dicalcium phosphate dihydrate.* Dicalcium phosphate dihydrate is one of several phosphate preparations that have been used as buffers, fillers, and abrasives in OTC dentifrices and as inactive ingredients in numerous drug products. The Subcommittee concludes that dicalcium phosphate dihydrate is safe when used as a buffer, filler, or abrasive in a dentifrice, but not generally recognized as effective for OTC use as an antigingivitis agent.

i. *Safety.* The safety of dicalcium phosphate dihydrate has been established on the basis of animal experiments and consumer use as a primary component of oral care products. It is included in the list of inactive ingredients in OTC anticaries formulations (45 FR 20666 at 20670), and is also approved by FDA as an optional food additive ingredient in the manufacture of flour (21 CFR 137.105 and 137.185). Dicalcium phosphate dihydrate has a reported oral LD_{50} value of greater than 10 g/kg for rats, and a dermal LD_{50} value of greater than 7 g/kg for rabbits. It is nonirritating or slightly irritating on rabbit skin and in eye irritation tests, respectively. Rodent oral limit tests, dermal irritation tests, and human irritation tests using various dentifrice formulations containing 5 percent to 88 percent dicalcium phosphate dihydrate were submitted (Ref. 129). These studies were carried out using toothpaste containing from 5 percent to 88 percent dicalcium phosphate dihydrate. The LD_{50} in rats is greater than 16 g/kg for a toothpaste containing 60:40 weight to volume (w/v) suspension of dicalcium phosphate dihydrate. Oral tissue irritation or sensitization potential of toothpaste containing dicalcium phosphate dihydrate was also evaluated in a series of studies (Ref. 129). The tests were carried out by having the subject brush 7 days, 5 times a day to provide an exaggerated test for oral tissue irritation. In no instances were any of the dentifrices containing dicalcium phosphate dihydrate either irritating or sensitizing under conditions of the test.

No reports were available regarding the toxicity of ingested dicalcium phosphate dihydrate in humans. It is estimated that the average adult might consume 2 to 3 g of phosphorous per day and, with an extreme diet containing maximum quantities of additives and naturally occurring phosphorous, could consume 6 to 7 g per day. Ingestion of an entire medium-size tube of toothpaste would increase the phosphorous consumption by several g, an amount unlikely to be significantly toxic. The saline cathartic effect of large doses of phosphate-containing materials would tend to limit their absorption to nontoxic levels. The Subcommittee concludes that, in general, dicalcium phosphate dihydrate can be regarded as safe.

ii. *Effectiveness.* Studies of the short-term use of dicalcium phosphate dihydrate-containing dentifrices in man have shown reduction of supragingival plaque to be greater than toothbrushing with water (Ref. 129). These studies do not implicate dicalcium phosphate dihydrate as an active ingredient but rather might be explained by the abrasive effect of dicalcium phosphate dihydrate in assisting plaque removal by toothbrushing. Gingivitis reduction is also seen in such experiments, but this could also be related to the abrasive effects of dicalcium phosphate dihydrate and removing plaque. The Subcommittee believes there is no evidence for chemical interference with plaque formation or plaque removal and no evidence of dicalcium phosphate dihydrate as an antigingivitis agent. The Subcommittee concludes that, based on the available data, it would be inappropriate to claim that the plaque reduction associated with the use of this abrasive qualifies it as an antigingivitis/antiplaque agent.

c. *Hydrogen peroxide.* The Subcommittee concludes that hydrogen peroxide is safe at concentrations of up to 3 percent, but there are insufficient data available to permit final classification of its effectiveness at 1.5 to 3 percent concentrations for long-term OTC use as an antigingivitis/antiplaque agent.

Hydrogen peroxide was isolated by Thenard in 1818 and has been of commercial interest since the mid-nineteenth century. Hydrogen peroxide has been a component of OTC drugs such as topical antiinfectants, canker sore treatments, and earwax softeners. A 3-percent solution of hydrogen peroxide has been widely used as a topical antiseptic agent for suppurative (producing pus) wounds, inflammation of the skin and mucous membranes, by dentists for irrigation during root-canal therapy, and as a mouthrinse for acute necrotizing ulcerative gingivitis. Decomposition of hydrogen peroxide releases large volumes of oxygen, approximately ten times the volume of the solution. A 30-percent solution has been used for bleaching nonvital pulpless teeth.

The Advisory Review Panel on OTC Oral Cavity Drug Products classified hydrogen peroxide as a Category I ingredient for short-term use in oral wound cleansing and debriding in concentrations from 1.5 to 3 percent in aqueous solution (47 FR 22760 at 22906, May 25, 1982). Ten percent carbamide peroxide in anhydrous glycerin, which releases 3 percent hydrogen peroxide, is also classified in Category I. Hydrogen peroxide is listed in the USP (Ref. 130).

i. Safety. The Subcommittee evaluated the toxicity and mutagenicity of hydrogen peroxide. The toxicity data suggested that 1.5 to 3 percent hydrogen peroxide in aqueous solution has a low toxicity. When ingested in large doses, hydrogen peroxide produces esophagitis and gastritis (Ref. 131). Few primary systemic toxic effects are expected at low concentrations because hydrogen peroxide decomposes in the oral cavity (Ref. 132) and bowel before absorption can occur.

The acute toxicity of hydrogen peroxide depends on the concentration tested, with more concentrated solutions being relatively more toxic than dilute solutions. In rats, concentrations of 0.25 percent to 0.5 percent hydrogen peroxide added to drinking water decreased growth and increased mortality within 6 weeks (Ref. 133). Decreased body weight was seen in Osborne-Mendel rats given 0.45 percent hydrogen peroxide in drinking water for 5 months, but this decreased body weight was regained within 2 weeks after replacing the hydrogen peroxide-containing drinking water with tap water (Ref. 134). The decreased body weight was possibly attributed to decreased liquid intake when hydrogen peroxide was provided in the drinking water. In case studies, fatal poisoning (Refs. 135 and 136) has been reported for ingestion of hydrogen peroxide at concentrations exceeding 3 percent or excessive ingestion of 3 percent hydrogen peroxide. Generally, ingestion of household peroxide (3 to 9 percent) causes no significant toxic effects (Refs. 137, 138, and 139).

The LD_{50} of hydrogen peroxide has been established by Ito *et al.* (Ref. 140) as 1,567 mg/kg body weight in rats dosed with a 5-percent solution. The low acute toxicity of hydrogen peroxide is confirmed by unpublished data indicating an LD_{50} of 5,000 mg/kg body weight for 6 percent hydrogen peroxide in rats (Ref. 141).

Teratogenic activity has not been demonstrated for hydrogen peroxide (Ref. 142). Hydrogen peroxide can be absorbed through the oral mucosa (Ref. 143) and epidermis (Ref. 144), but the exposure of the oral cavity to hydrogen peroxide is generally limited since it undergoes rapid decomposition. After 1 minute of brushing, less than 20 percent of the hydrogen peroxide introduced into the oral cavity can be recovered (Ref 145).

In the oral cavity, toxic effects of hydrogen peroxide vary from pulpal alterations (Ref. 146) to gingival lesions (Refs. 147 and 148) and oral irritation in rats (Ref. 149) under certain conditions. Adding a 1- to 1.5-percent solution to drinking water resulted in apparent enamel demineralization in rats over an 8-week period (Ref. 149). This effect on enamel was possibly due to the hydrogen-ion (pH) concentration of the solution used rather than true carious lesions. In addition, no enamel solubility was found from an in-vitro experiment using a 1.5-percent aqueous solution on human enamel (Ref. 141).

The Subcommittee's discussion of mutagenicity is not intended to be a complete review of the literature concerning the mutagenic nature of hydrogen peroxide, but is intended to point out the apparent mutagenic safety concerns associated with hydrogen peroxide. Any mutagenic role of hydrogen peroxide will be further discussed with sodium bicarbonate and hydrogen peroxide in combination.

Numerous reports indicate a mutagenic role for hydrogen peroxide (Refs. 150, 151, and 152). Reviews on the genotoxicity of hydrogen peroxide can be found in reports by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (Refs. 153 and 154) and in an overview of hydrogen peroxide genotoxicity presented at the Subcommittee meeting on December 4, 1995 (Ref. 155).

Hydrogen peroxide can produce hydroxyl radicals which are reactive but short-lived (Refs. 155 and 156). In vitro superoxide and hydroxyl radicals caused chromatic exchanges in mammalian cells and preneoplastic changes (Refs. 153 and 154). Although hydroxyl radicals and singlet oxygen can damage DNA in vitro, the genotoxic potential of hydrogen peroxide depends on the proximity of unprotected DNA. In vitro genotoxicity tests enhance the opportunity for DNA damage and are conducted in cells with defective DNA repair systems. Genotoxic effects are not seen with hydrogen peroxide in the presence of protective enzyme systems that are normally present intracellularly, in the presence of iron chelating agents, and in the presence of hydroxyl radical scavengers.

The mechanism of mutagenesis through superoxide radical production was also suggested by MacRae et al. (Ref. 157). In contrast to most of the references available, Taylor et al. (Ref. 158) suggested that hydrogen peroxide itself and not hydroxyl radicals was responsible for DNA strand breaks in epithelial and fibroblast cultures. Most carefully controlled in vitro studies have shown that the participation of transition metal ions, such as iron or copper, is required for DNA damage to occur (Ref. 159).

In some bacterial mutagenesis studies, hydrogen peroxide was found to be a weak mutagenic agent (Refs. 160 through 167). Many strains are not sensitive to hydrogen peroxide and hydroxyl radicals and mutations are only seen in certain bacterial strains that are sensitive to oxidative damage (Ref. 168). The addition of an external enzymatic metabolic source resulted in abolition of the weak genotoxic effects seen in sensitive bacterial strains. These enzyme sources are normally present throughout the body, and the presence of detoxifying enzymes may explain the lack of genotoxicity seen in whole animals that have been administered hydrogen peroxide. In the oral cavity, salivary peroxidase serves as the initial line of defense against hydrogen peroxide (Ref. 169).

Additional studies were conducted to evaluate systemic effects of long-term administration of hydrogen peroxide, and the endpoint measured was sister chromatic exchange (SCE), a very sensitive assay for genotoxic damage. Hydrogen peroxide was administered to hamsters for 6 months at 70 mg/kg (Ref. 170) and to mice for 3 months (Ref. 171). In both studies, there was no increase in SCE formation following long term ingestion of hydrogen peroxide. A single administration of a carbamide peroxide-containing dentifrice to rats at 1,000 mg/kg daily for 5 days did not increase the incidence of SCE (Ref. 172). Woolverton also examined two commercial carbamide peroxide-containing dental products for micronucleus formation. After two exposures, these products did not increase the incidence of micronucleated erythrocytes (Ref. 173).

Similar results were seen in a micronucleus assay for chromosomal damage in mice that were given hydrogen peroxide intraperitoneally or in drinking water at 0.6 percent for 2 weeks (Refs. 174 and 175). The SCE and micronucleus studies consistently demonstrated a lack of genotoxicity following hydrogen peroxide ingestion or intraperitoneal injection.

Hydrogen peroxide was reported to promote carcinomas in rodents following intraperitoneal injections (Ref. 176) and through its addition to drinking water (Refs. 177, 178, and 179). Duodenal hyperplasia has been found in the rat model following the addition of 1.5 to 3 percent hydrogen peroxide to drinking water (Ref. 176). Ito *et al.* (Ref. 140) observed similar toxicity with higher doses of hydrogen peroxide. In mice with reduced catalase activity, hyperplastic and neoplastic duodenal nodules were found (Ref. 179). Ito's report of the carcinogenicity of hydrogen peroxide has been evaluated by FDA toxicologists who concluded that the results of the study did not provide sufficient evidence to designate hydrogen peroxide as a carcinogen (53 FR 53176, December 30, 1988). Similar conclusions were drawn by a panel of toxicologists who reviewed the potential carcinogenicity of hydrogen peroxide for the International Agency for Research on Cancer (IARC) (Refs. 180 and 181).

A long-term study was conducted in F344 rats in which hydrogen peroxide was administered in drinking water for 18 months at concentrations of up to 0.6 percent, the maximal tolerated dose in F344 rats (Ref. 182). All surviving animals were sacrificed at 24 months of age. Hydrogen peroxide ingestion in the 0.6-percent hydrogen peroxide group was 677 mg/kg/day for females and 433 mg/kg/day for males, with a total ingestion of 72.7 g hydrogen peroxide in females and 81.4 g hydrogen peroxide in males during the course of the study. There was no evidence of carcinogenicity at any organ site in this study following hydrogen peroxide ingestion.

In Syrian hamsters, applications of 3 percent and 30 percent hydrogen peroxide produced pathogenic changes associated with preneoplastic lesions. Preneoplastic lesions are reversible following cessation of exposure (Ref. 178). When combined with DMBA, a known carcinogen, hydrogen peroxide, at a concentration of 30 percent, appeared to augment the carcinogenic effects associated with DMBA (Ref. 183). No carcinogenicity was seen in this study resulting from hydrogen peroxide alone at concentrations of 3 or 30 percent.

Marshall et al. (Ref. 184) conducted two carcinogenesis studies of 16 weeks and 20 weeks in hamsters to compare the effects of similar dentifrices with and without the combination of hydrogen peroxide and sodium bicarbonate in the presence of DMBA. The authors reported that the results demonstrated that an oral product containing hydrogen peroxide and sodium bicarbonate was not carcinogenic and that the combination did not enhance the tumorigenicity of DMBA. In summary, these robust animal studies (Refs. 183 and 184) indicate that hydrogen peroxide does not increase the incidence of oral cavity tumors in combination with a known carcinogen.

Several studies challenge the carcinogenesis of hydrogen peroxide. Cell culture experiments rich in catalase show a marked decrease in the mutagenic effects of hydrogen peroxide (Refs. 185 and 186). Further, variations exist between species in their ability to control the destructive effects by the release of catalase and reduced glutathione (Ref. 187). The mutagenic potential of hydrogen peroxide as measured by production of hydroxyl radicals in the presence of Fe2+ has also been shown to be concentration dependent in a Chinese hamster cell line (Ref. 188). Additional mechanistic studies (Refs. 189 and 190) also suggested that the gel and paste phases of a toothpaste reduce the formation of free radicals. A generous supply of catalase in the oral cavity and studies demonstrating that hydrogen peroxide is rapidly degraded in the oral cavity indicate that hydrogen peroxide is unlikely to have a mutagenic potential at concentrations up to 3 percent (Ref. 191).

The ECETOC 1992 Joint Assessment of the toxic effects of hydrogen peroxide (Refs. 153 and 154) had the following conclusions: (1) Hydrogen peroxide concentrations of less than 1 percent do not appear to have gastrointestinal (GI) tumor-promoting potential; (2) chronic ingestion of 0.1 to 0.15 percent hydrogen peroxide causes an inflammatory response in gastroduodenal tissue of mice; (3) the mutagenicity of hydrogen peroxide in bacteria is a function of the genotype of the strain; (4) hydrogen peroxide has genotoxic potential only through the direct exposure of hydroxyl radicals on target DNA; (5) catalase reduces or abolishes the mutagenic response to hydrogen peroxide; (6) in vivo, many factors may contribute to the reduction of bioavailable hydrogen peroxide for systemic genotoxic action; (7) the possibility of genotoxic effect on cells that directly contact hydrogen peroxide at the site of application cannot be ruled out; and (8) no data are available to fully evaluate chronic toxicity and resulting carcinogenic potential of hydrogen peroxide.

The rate of decomposition of hydrogen peroxide in the oral cavity was determined in adults, children, and xerostomics. Hydrogen peroxide decomposition was so rapid that it was difficult to establish a rate of decomposition. In all cases, less than 27 percent of the hydrogen peroxide introduced into the oral cavity was present after 1 minute of brushing with dentifrices containing up to 3 percent hydrogen peroxide (Ref. 145). Most residual hydrogen peroxide would be expectorated with the dentifrice after brushing, leaving very little for ingestion. Based on clinical studies and adverse event reporting, the lack of irritation to soft tissues of the oral mucosa following use of hydrogen peroxide-containing dentifrices provides further evidence of the safety of long-term use of hydrogen peroxide-containing dental products.

Hydrogen peroxide presents safety concerns at concentrations above 3 percent because of the lack of controlled studies conducted with concentrations between 3 percent and 30 percent hydrogen peroxide. Available evidence indicates that acute toxic effects encountered with high concentrations of hydrogen peroxide (i.e., 30 percent) are rapidly repaired, leaving no deleterious effects. The discussion above mentions only some of the many published articles detailing the mutagenic potential of this ingredient. Despite some safety concerns, the gathering of appropriate clinical data outweighs the currently documented risks, which are inconclusive. While the experimental data suggest a mutagenic effect of hydrogen peroxide, the Subcommittee's review of current data indicates that, at concentrations of up to 3 percent in oral care products, the risk appears to be especially minimal and hydrogen peroxide is safe for its intended use.

ii. *Effectiveness.* Because of the preponderance of anaerobic and microaerophilic microorganisms associated with most forms of periodontal disease, the testing of oxygenating agents to inhibit or kill these microorganisms is understandable. The primary killing mechanism for hydrogen peroxide is through the release of oxygen. Unfortunately, the action is short-lived and inhibited by organic matter.

Hydrogen peroxide added to a mouthrinse has been shown to increase the release of hypothiocyanate into saliva. Hypothiocyanate has been reported to be a bacteriostatic agent against some microbial species (Refs. 192 and 193) through the activation of the lactoperoxidase system (Ref. 194). The addition of hydrogen peroxide to human whole saliva resulted in increased amounts of hypothiocyanate and this effect was concentration dependent (Ref. 195). This study also showed that the concentration of hydrogen peroxide was critical to obtain optimum bacteriocidal effect. Incubation time for inhibitory effects required several minutes, which may be a significant stumbling block in utilizing exogenous hydrogen peroxide through this mechanism of action. Another study of the lactoperoxidase/hypothiocyanate antimicrobial mechanism found that rinsing with a solution containing hydrogen peroxide can readily produce hypothiocyanate, although the amount was dependent on the volume and pH of the rinse and the concentration and pH of the hydrogen peroxide (Ref. 196).

In a 2-week, crossover study, Wennstrom and Lindhe (Ref. 197) found that a hydrogen peroxide-containing mouthrinse effectively prevented the colonization of several morphological groups of microorganisms, e.g., fusiforms, filaments, motile and curved rods, and spirochetes. These groups have been repeatedly associated with several forms of periodontal diseases. Plaque and gingivitis scores were also markedly reduced. The concentration of hydrogen peroxide released was not determined. In another short-term study, a 1.5-percent hydrogen peroxide rinse significantly reduced both plaque and gingivitis scores over the 7-day test period (Ref. 198). In a study using a rat model in which test animals on a high cariogenic diet were inoculated with plaqueforming microbial species, a 10-percent urea (carbamide) peroxide gel and 1 percent hydrogen peroxide solution significantly reduced the accumulation of plaque (Ref. 199). A 3-week study using 10 percent urea (carbamide) peroxide gel compared with a placebo showed a significant decrease in gingivitis but no comparable reduction in plaque scores (Ref. 200). The authors suggested that the oxygenating effects of the test solution produced an environment unsuitable for the microbial species responsible for the development of gingivitis. Similar results were found in another 3-week study using 10 percent urea peroxide gel (Ref. 201).

In contrast, a 3-week study comparing 1 percent hydrogen peroxide, 0.12 percent chlorhexidine, and a placebo rinse found little effect of the hydrogen peroxide on gingivitis scores and no demonstrable effects on plaque scores (Ref. 202). A 2-week study using a 1.5-percent hydrogen peroxide rinse compared to a placebo showed no benefit from the hydrogen peroxide either as a rinse or when delivered by an irrigation system (Ref. 203).

Testing of an 11-percent urea (carbamide) peroxide gel in a 3-month study (Ref. 204) and a 6-month study (Ref. 205) showed that plaque scores were significantly reduced when compared to conventional oral hygiene toothpaste controls. However, no effect on gingivitis could be determined in either study. In an 18-month study comparing a 1.5-percent hydrogen peroxide rinse with a fluoridated rinse

in conjunction with toothbrushing in subjects undergoing orthodontic treatment, a clear benefit was found for the hydrogen peroxide rinse group (Ref. 206). The rinse appeared to prevent the accumulation of plaque and the subsequent development of gingivitis. However, once plaque formed, the experimental rinse did not reduce the established plaque and gingivitis. In contrast, a 24-week study comparing a 1.5-percent hydrogen peroxide rinse with water rinses did not find a significant reduction in either plaque scores or in papillary bleeding scores (Ref. 207). A 2-year study comparing a 1.5-percent hydrogen peroxide rinse with a 0.1-percent chlorhexidine rinse, but without a placebo control, found a reduction in sulcus bleeding but not plaque scores for the hydrogen peroxide group (Ref. 208).

The Subcommittee concludes that there is a lack of well-controlled studies of sufficient length to draw firm conclusions regarding the effectiveness of hydrogen peroxide. The clinical data suggest that hydrogen peroxide may positively effect plaque and gingivitis scores, but the data are contradictory, lacking well-controlled clinical studies of adequate length. Further studies are needed to determine the value of this ingredient as an antiplaque agent. Optimizing the concentration, required exposure time, and best delivery vehicle would be major steps forward. The potential positive effect as an active ingredient is suggested by the current data. However, long-term efficacy is unknown.

d. *Sanguinaria extract*. The Subcommittee concludes that sanguinaria extract at 0.03 to 0.075 percent concentration is safe, but there are insufficient data available to permit final classification of its effectiveness in an oral rinse or dentifrice dosage form as an OTC antigingivitis/antiplaque active ingredient.

Sanguinaria extract is prepared by warm acidulated alcoholic extraction of the rhizome of *Sanguinaria canadensis* (more commonly known as blood root or puccoon), followed by precipitation with a metal salt. Six principal benzophenanthridine alkaloids are present in the extract with sanguinarine (50 percent) and chelerythrine (25 percent) being the major ones. Sanguinaria extract is a bright orange, free-flowing, amorphous powder that is hygroscopic and electrostatic. It is soluble at 25° C in methanol to 1 percent weight per weight (w/w), in chloroform to 0.75 percent w/w, in water or water buffered with one percent citric acid to 2 percent w/w. Sanguinaria extract exhibits a pH dependent lipophilicity and partitions to a significant extent into the lipid phase of a lipid/water mixture above pH 6.5. Sanguinaria extract has been described in several pharmacopeia (Refs. 209 and 210) and textbooks (Ref. 211). Uses include relief of spongy and red gums and in OTC cough syrups as an expectorant. Sanguinaria extract was introduced into homeopathic practice in 1837.

i. *Safety*. Safety studies addressing acute toxicity, irritation potential, sensitization potential, reproductive toxicity, birth defect potential, chronic organ toxicity, and carcinogenic potential were conducted in animals using sanguinaria extract and sanguinarine chloride.

The acute toxicity of sanguinaria extract was determined by oral gavage to Sprague-Dawley rats with doses from 500 to 3,000 mg/kg. In one study (Ref. 212), the oral LD_{50} of sanguinaria extract was 1,440 mg/kg. This suggests that sanguinaria extract is probably poorly absorbed orally. The lethal dose of sanguinaria extract in two Cynomolgus monkeys was above 50 mg/kg. The acute dermal LD_{50} in a limited study using 10 adult New Zealand rabbits was greater than 200 mg/kg body weight. Acute inhalation toxicity of sanguinaria extract (2.2 mg/liter) in 10 rats resulted in mortality in 3 of 5 males and no females. Gross pathology examination revealed no lesions or abnormalities. The LD_{50} from two studies of sanguinarine chloride determined by oral gavage in rats was 1,525 and 1,663 mg/kg. The intravenous LD_{50} in rats was 28.7 mg/kg, and the intraperitoneal LD_{50} in mice was 17.7 mg/kg.

Studies concerning the multidose subchronic toxicity of sanguinaria extract (Refs. 213, 214, and 215) and sanguinarine chloride (Refs. 216, 217, and 218) were conducted in rats and monkeys at doses ranging from 5 to 405 mg/kg for 2 to 13 weeks. In a 4-week oral gavage study in monkeys (Ref. 215), 100 mg/kg of sanguinaria extract was determined to be the appropriate high-dose for a subsequent 13-week toxicity study in monkeys. A 13-week gavage study in monkeys (Ref. 216) with 0 to 60 mg/kg showed no treatment-related toxicity except minor GI irritation of limited duration. The study suggested a NOAEL of 30 mg/kg per day once tolerance is achieved. A 13-week oral gavage study in rats (50 to 400 mg/kg per day) (Ref. 214) showed evidence of dose-related toxicity, principally involving GI irritation and body weight loss at all dosage levels. Mortality was observed at doses of 100 mg/kg per day and above, with a NOAEL of less than 50 mg/kg per day. Administration in the diet appears to protect against GI irritation. A 4-week dietary toxicity study in rats (5 to 405 mg/kg per day) (Ref. 213) showed a group mean body weight loss at 405 mg/kg. Based on these studies, evidence of minor treatment-related toxicity associated with sanguinaria extract and sanguinarine chloride is limited to GI irritation.

Pharmacokinetic studies assessing metabolism, disposition, distribution, and elimination of sanguinaria extract and sanguinarine chloride were conducted in rats and mice (Refs. 219, 220, and 221). The metabolism of sanguinaria extract was tested in vitro in rat and rabbit liver homogenates and in vivo in 10 human subjects for at least 6 months (Ref. 219). Results indicated that no benz[c]acridine (50 parts per billion (ppb) detection limit) was formed in the rat or rabbit liver homogenates. Neither benz[c]acridine (1 ppb detection limit) nor sanguinarine chloride (25 ppb detection limit) was found in the urine of the human subjects.

Studies evaluating the biological disposition of radiolabeled sanguinarine chloride in rats (Ref. 220) and mice (Ref. 221) suggested low absorption, with excretion of over 50 percent (mice) and 88 percent (rats) of the total dose in feces. Less than 1.0 percent (rats) and 0.9 percent (mice) was excreted in the urine.

Analysis of rat tissues collected 96 hours following oral administration of 5 mg/kg indicated a total recovery of approximately 6.1 percent of the administered radioactivity. Excretion via urine, feces, and expired air accounted for 95.1 percent of the administered dose in the 96-hour post-administration period. Blood levels in the rat achieved less than 1.5 percent of the net dose administered orally, peaking around 8 hours and declining to near 1 hour levels by 96 hours.

Expired air accounted for an average of 18.3 percent (mice) and 6.0 percent (rats) of the dose administered. The nature of the blood radioactive residues and excreted ^{14}C-carbon was not determined. An overall mean recovery in mice of 97.89 percent of the ^{14}C-carbon during the 96 hours following oral administration of sanguinarine chloride labeled at one and/or both methylene-dioxy groups suggests that a substantial portion of the radiolabeled test product may be transformed into nonlabeled benzophenanthridine metabolites. These results suggested that sanguinarine chloride is satisfactorily recovered after oral or intravenous administration.

A cardiovascular study in dogs treated intravenously with sanguinarine

chloride (0.075 mg/kg) demonstrated no treatment-related effect on heart function or cardiovascular health (Ref. 222) at a dose 30 times the maximum daily absorbed dose expected from brushing and rinsing.

Sanguinaria extract was tested in a fertility/reproduction study in rats (Ref. 223), in developmental toxicity studies in rats and rabbits (5 to 400 mg/kg per day) (Refs. 224, 225, and 226), and in a perinatal/postnatal study in rats (5 to 60 mg/kg per day) (Ref. 227). The NOAEL level of sanguinaria extract was 25 mg/kg per day for development toxicity in rabbits, and 15 mg/kg per day for maternal toxicity. Sanguinaria extract had no effect on fertility, reproduction, or fetal and neonatal development in rats and rabbits at doses below those resulting in general toxicity in the adult animals.

Mutagenicity studies were conducted with both sanguinaria extract and sanguinarine chloride with in vitro methods using microorganisms and mammalian cells in culture and in vivo in mice. Weak positive responses were elicited only in the bacterial assay using Salmonella typhimurium (Ames assay) in the presence of metabolic activation (Ref. 228). Studies of sanguinaria extract were negative in the bacterial assay with Escherichia coli (Ref. 229), in an unscheduled DNA synthesis assay in rat primary hepatocytes (Ref. 230), and in a micronucleus cytogenetic assay in mice (Ref. 231). An Ames test for metabolites of sanguinaria extract in rat urine using *S. typhimurium* was negative. Studies of sanguinaria chloride were negative in other Ames assays with *S. typhimurium* (Ref. 232), and *Saccharomyces cerevisiae* (Ref. 233) with and without metabolic activation. Two mammalian cell assays (Ref. 234) with sanguinarine chloride, including a Chinese hamster ovary (CHO)-hypoxanthine-guanine phosphoribosyltransferase (HGPRT) forward gene mutation assay and unscheduled DNA synthesis assay in rat primary hepatocytes (Ref. 235) provided results that were equivocal or uninterpretable. Neither study, however, gave a positive mutagenic response. The CHO assay is historically difficult to conduct and interpret.

Long-term (90 to 98 weeks) carcinogenicity studies (Ref. 236) by gavage at dosages of 0 to 60 mg/kg per day sanguinaria extract in rats did not produce treatment-related preneoplastic or neoplastic lesions to suggest a carcinogenic effect. Dosage at 40 mg/kg per day did not produce toxicity and is considered the NOAEL dosage. A lifetime diet carcinogenicity study of sanguinaria extract was evaluated in rats (8 to 200 mg/kg per day) (Ref. 237). No test related hematological, biochemical, or urological changes were observed at any dosage level. No test article related macro- or microscopic pathology changes were observed. A 200 mg/kg per day dosage level can be considered the NOAEL level.

Two controlled 13-week subchronic studies done in monkeys and dogs (Ref. 238) examining ocular toxicity provided no evidence that sanguinaria extract or sanguinarine chloride affected intraocular pressure or produced any other ophthalmologic changes.

Human exposure to sanguinarine with twice daily use of toothpaste and oral rinse has been estimated to be 0.056 mg/kg per day (Ref. 238). Comparison of doses tested in animal studies with human doses expected from use of toothpaste or oral rinse appears to support the use of sanguinaria extract at a significantly higher concentration than contained in currently marketed products.

Ten animal safety studies conducted between 1982 and 1984 were submitted for dentifrice formulas containing 300 to 2,000 µg/mL of sanguinaria extract. None of the studies tested the currently marketed toothpaste formula containing 750 µg/mL of sanguinaria extract. Acute oral toxicity was greater than 20 g/kg in rats for a toothpaste formula containing 300 µg/mL of sanguinaria extract, and 5 g/kg in rats for a formula containing 500 µg/mL of sanguinaria extract (Refs. 239, 240, and 241). Primary skin and eye irritation studies carried out in rabbits (Refs. 242 and 243) demonstrated mild irritation reaction when a toothpaste formula containing less than 750 µg/mL was tested. Mild mucosal irritation was observed when a toothpaste formula containing 300 µg/mL of sanguinaria extract was tested in cheek pouches of hamsters (Refs. 244 through 248).

Two clinical studies (Refs. 249 and 250) demonstrated only mild mucosal irritation in test subjects. No differences were noted in the severity of lesions between the test and control groups.

Eleven clinical studies of animal safety conducted between 1983 and 1987 (Ref. 251) were submitted. Because modification of the oral rinse formulation from pH 3.2 to pH 4.5 began in 1989, none of these studies provided animal safety data on the currently marketed oral rinse (pH 4.5).

Based on data on the oral rinse formula containing 450 to 1,000 µg/mL sanguinaria extract at a pH of 3.2, no mucosal irritation was noted in the hamster cheek pouch (Refs. 252 and 253) or albino guinea pig studies (Ref. 254). No signs of toxicity or pharmacological effects were observed in test animals when a rinse formula of 450 µg/mL sanguinaria extract at pH 3.2 was tested (Ref. 255).

Four human studies conducted between 1982 and 1985 evaluated the irritation and sensitization potential of dentifrice formulas containing sanguinaria extract using a repeated insult patch test design involving a 2-percent aqueous slurry (Refs. 256 through 259). These studies demonstrated no induction of irritation or allergic contact dermatitis. An exaggerated use study (Ref. 260) using an earlier formula (300 µg/g sanguinaria extract) demonstrated no irritation or sensitization in soft oral cavity tissues. Two 6-month studies on a toothpaste containing sanguinaria and sodium monofluorophosphate (Refs. 261 and 262) showed no adverse effects on oral hard or soft tissues. Soft tissue examinations included inspection of the lips, tongue, hard and soft palate, gingiva, mucobuccal fold areas, inner surface of the cheeks, and sublingual areas. Although testing of the microbial flora was inconclusive in one study (Ref. 261), sanguinaria did not promote overgrowth through the development of resistant microbial strains.

A 6-month, double-blind, randomized study using a dentifrice containing 0.075 percent sanguinaria extract (Ref. 263) showed no significant oral irritation or adverse reactions. A 1-week exaggerated use study showed that 18 of the 28 subjects experienced mucosal sloughing (Ref. 264).

Although nine human safety studies were presented, only one study (Ref. 265) tested the currently marketed oral rinse containing 300 µg/mL of sanguinaria extract at pH 4.5. However, this study tested the efficacy of the formula and was not designed to test the safety of the oral rinse. Three of the remaining eight studies showed that repeated application of the earlier oral rinse formula at pH 3.2 under a semiocclusive patch test did not induce clinically significant irritation or evidence of induced contact dermatitis in humans (Refs. 266, 267, and 268). This earlier rinse formula gave no evidence of localized or generalized clinical manifestations in test subjects in two of the 7-day exaggerated use studies (Refs. 269 and 270). The Subcommittee concludes that sanguinaria extract at 0.03 to 0.075 percent concentration in an oral rinse or dentifrice dosage form is safe.

ii. *Effectiveness.* The Subcommittee reviewed controlled clinical studies ranging from 1 week to 6 months in duration. Three short-term studies (two 1 week and one 1 month) had equivocal results between the active and placebo toothpaste preparations. Of the three

studies that tested the currently marketed toothpaste containing 750 µg/g of sanguinaria extract, only one 6-month, double-blind study (Ref. 271) demonstrated a significant decrease in plaque at 3 months. Results from this study also showed that gingival index scores in the active group were significantly lower than the placebo group at 28 weeks. The other two studies were short-term studies of 1 and 4 weeks (Refs. 272 and 273) in which no differences were detected between the active and placebo groups. A 10-week study (Ref. 274) showed that the toothpaste formulation containing 300 µg/g of sanguinaria extract reduced plaque and gingival bleeding, but the zinc chloride in the formulation diminished the plaque-reducing effect. It was not clearly documented whether zinc chloride affects the effectiveness of the currently marketed toothpaste. Based on the short-term clinical studies, the effectiveness of the toothpaste containing 750 µg/g sanguinaria extract in plaque and gingivitis reduction cannot be determined. The effect of zinc chloride on the effectiveness of the toothpaste also needs further study.

Five studies used a toothpaste formula containing 750 µg/g sanguinaria extract and 0.8 percent sodium monofluorophosphate (Refs. 263, 264, 273, 275, and 276). Equivocal results were noted in two 6-month studies (Refs. 263 and 276) and in a 1-week study (Ref. 264). One toothbrushing study (Ref. 273) compared the effect of eight toothpaste formulations on plaque and gingivitis in school children. Because the study design concerning the control product and subject selection was inadequate, this study did not support effectiveness. One-way analysis of variance (ANOVA) showed that the differences between groups were not statistically significant. In addition, no significant differences in plaque or gingivitis reduction were noted between groups using a fluoride toothpaste containing zinc chloride plus sanguinaria extract and a dentifrice containing zinc chloride without sanguinaria extract.

A 1-week, exaggerated use effectiveness study (Ref. 275) tested three regimens of the toothpaste and oral rinse on plaque reduction. The study design and protocol employed did not allow accurate testing of the effectiveness of the toothpaste. Based on all of the data submitted, none of the studies provided evidence of effectiveness.

The Subcommittee evaluated 26 additional controlled clinical studies (Ref. 277). Seven of the 26 studies (Refs. 265 and 278 through 283) provided equivocal results. The remaining 19 studies (ranging from 1 to 8 weeks), conducted for various reasons, evaluated proper dosage, clinical study designs, optimal plaque and gingival indices to be employed, product safety, effectiveness of the regimen (toothpaste and oral rinse combination use), and the role of zinc chloride in plaque reduction.

Among the 19 studies, 9 tested the effectiveness of an oral rinse with a final pH of 4.5. Some short-term clinical trials, employing the 7-day exaggerated use study design, demonstrated statistically significant differences between an earlier rinse product (pH 3.2) and the placebo control in plaque reduction only. However, the only two long-term, 6-month studies testing the effectiveness of this earlier rinse product (pH 3.2) did not demonstrate any effectiveness in plaque or gingivitis reduction when compared to a placebo. The 7-day exaggerated use study design was validated as a screening test for formulation development (Ref. 284). In addition, studies investigating the role of zinc chloride in the effectiveness of the oral rinse provided confusing and controversial results. Two 1-week studies (Refs. 285 and 286) demonstrated that no significant difference in plaque reduction was observed between a sanguinaria extract and zinc chloride rinse and a rinse without sanguinaria extract. The effect of zinc chloride alone was only mildly less than that obtained with the combination of sanguinaria extract and zinc chloride. However, a 2-week, experimental gingivitis, crossover study (Ref. 287) demonstrated that the oral rinse with sanguinaria extract and zinc chloride performed significantly better than the placebo in plaque reduction. The effect on gingivitis was equivocal.

One study trial (Ref. 288) evaluated the effect of the oral rinse on viable microorganisms after a single 60-second rinse. The rinse exhibited a selective effect on anaerobic organisms without adversely affecting aerobes or alpha-hemolytic streptococci. No long-term studies were available.

While some data exist on the short-term effectiveness of the sanguinaria extract oral rinse or dentifrice, the Subcommittee evaluated selected studies that supported the effectiveness of the oral rinse used in combination with one of the sanguinaria toothpaste products. Five short-term (1 to 9 weeks) studies (Refs. 265 and 289 through 292) demonstrated reductions in plaque or gingivitis. Four 6-month studies also produced significant differences for the active regimen compared to placebo (Refs. 293 through 296). However, these nine studies varied substantially in design and formulation of the test dentifrice and oral rinse combinations. In studies prior to 1984, low dose toothpaste (300 µg/mL sanguinaria extract) and pH 3.2 oral rinse were used, whereas studies conducted since 1988 have included the 750 µg/g sanguinaria extract toothpaste and a pH 4.5 oral rinse. Even if effectiveness were demonstrated for the combined regimen, the contribution of sanguinaria extract alone is not clear.

The in vitro efficacy of the individual active components was also investigated. In vitro MICs of sanguinaria chloride and sanguinaria extract were tested against 176 clinical isolates and 43 reference strains of oral bacteria (Ref. 297). MIC's for sanguinaria chloride ranged from 16 to 32 µg/mL for all but 7 reference isolates. MICs for sanguinaria extract ranged from 16 to 24 µg/mL for all strains except *Wolinella succinogenes* and one strain of *Wolinella curva*. For fresh isolates, MIC's for sanguinaria chloride and sanguinaria extract ranged from 16 to 32 µg/mL. Laboratory tests were also conducted on sanguinaria and fluoride-containing toothpaste to evaluate the bioequivalence of the product to positive controls. Tests included bioavailability, rat caries fluoride stability (Ref. 298), remineralization/demineralization, and in vivo bovine enamel fluoride uptake (Ref. 299). These tests are consistent with the required biological testing procedures for fluoride dentifrices (October 6, 1995, 60 FR 52474 at 52510). Results obtained from these studies indicated that the sanguinaria/fluoride toothpaste formula was biologically equivalent to the clinically-tested control in promoting remineralization, promoting fluoride uptake into artificial enamel lesions, reducing the effects of acid challenge on enamel, and reducing caries in the rat caries model. Sanguinaria extract and zinc chloride were also shown not to interfere with fluoride bioavailability uptake profiles with decalcified enamel qualitatively comparable to profiles obtained from sound enamel.

The Subcommittee concludes that, although mild staining and oral irritation may occur, sanguinaria extract at 0.03 to 0.075 percent concentration is safe. However, given the wide variations in study designs, test product concentrations and formulations, placebo controls, and statistical analyses, conclusions cannot be drawn regarding the effectiveness of sanguinaria extract as an OTC antigingivitis/antiplaque agent.

e. *Sodium bicarbonate*. The Subcommittee concludes that sodium

bicarbonate is safe, but appears to have relatively poor efficacy as an OTC antigingivitis/antiplaque agent, requiring high dosages and extended exposure time to have a reasonable chance at affecting the oral flora and clinical parameters.

Sodium bicarbonate has been used as an antacid as well as advocated as an ingredient in both toothpastes and mouthrinses. It has been generally regarded as a bactericidal agent that generates a hypertonic (causing water to flow out of the cell) environment, leading to disruption of the fluid equilibrium of the cell and dehydration, plasmolysis (cell shrinkage due to loss of water by osmosis), and eventual cell death.

i. *Safety*. Sodium bicarbonate is GRAS for use in foods (21 CFR 184.1736). Sodium bicarbonate is listed as an OTC antacid up to a maximum daily dose of 200 milliequivalent (mEq) bicarbonate ion (21 CFR 331.11(k)(1)). The usual dose is 1 to 5 g, providing up to 60 mEq. In OTC mouthrinse applications, sodium bicarbonate has been determined to be safe and effective for use as a debriding ingredient (47 FR 22712 at 22907, May 25, 1982). Ingestion of large amounts of sodium bicarbonate causes several blood chemistry changes, including increased sodium levels, resulting in toxic effects that produce hypernatremia (excessive amount of sodium in the blood) (Refs. 300, 301, and 302). The LD_{50} is 7.57 to 8.9 g/kg body weight for the rat.

Sodium bicarbonate does not appear to be teratogenic or mutagenic using conventional testing, with no discernable effects on fetal survival in several species. It does not produce photosensitization, acute ocular irritation, or skin irritation by standard methods.

ii. *Effectiveness*. Few studies examine the effectiveness of sodium bicarbonate as a single active ingredient. Sodium bicarbonate has been found to be bactericidal to several oral microorganisms (Ref. 303). The authors suggest that the killing effect might be more than an osmotic imbalance created within the cells. This study showed several disturbing aspects about the effectiveness of this ingredient. For killing to be effective, relatively long periods of exposure were required, ranging from several minutes to hours. While a comparison to other antimicrobial agents is not intended as a criteria for effectiveness, sodium bicarbonate had a 10-fold poorer MIC range compared to sodium fluoride and a 1,000-fold poorer MIC range compared to sodium lauryl sulfate. In a study examining the effects of sodium bicarbonate on *S. mutans*, osmotic disruption occurred through salt concentration dependent cell lysis (Ref. 304).

In a 20-day experiment on rats, sodium bicarbonate applications were ineffective at reducing plaque accumulations (Ref. 305). In a 6-week study comparing the effects of a toothpaste containing sodium bicarbonate with a standard fluoride toothpaste, no increase in effectiveness was observed (Ref. 306). In a similar 8-week study, no difference was observed in either plaque or gingivitis scores between the control and sodium bicarbonate test toothpaste (Ref. 307).

The Subcommittee concludes that sodium bicarbonate is safe, but there are insufficient data available to determine its effectiveness as an OTC antigingivitis/antiplaque agent.

f. *Sodium lauryl sulfate*. The Subcommittee concludes that sodium lauryl sulfate is safe at concentrations of 0.1 to 5 percent, but there is insufficient evidence to support its effectiveness as an antigingivitis/antiplaque active ingredient. The Subcommittee notes, however, that sodium lauryl sulfate is a safe and effective foaming ingredient in toothpaste.

Sodium lauryl sulfate is a synthetic detergent that acts as an anionic surfactant to lower surface tension. Sodium lauryl sulfate is available commercially as a viscous liquid, paste, or powder. It may contain small amounts of other sodium alkyl sulfates, although it consists mostly of sodium lauryl sulfate with a molecular weight of 288.4 and the formula $CH_3(CH_2)_{10}CH_2OSO_3Na$. It is soluble in water and alcohols. It binds to positively charged tooth surfaces and positively charged side groups of proteins. Protein binding may lead to denaturation (loss of biological activity) through conformational changes in the molecule. It is stable in alkaline solutions and will hydrolyze (split into fragments by addition of water) at room temperature below a pH of 5 (Ref. 308).

Sodium lauryl sulfate is used in cosmetics such as shampoos, deodorants, facial makeup, shaving preparations, and bath products, and in various oral care products. It is approved as a multipurpose food additive (21 CFR 172.822). Its ubiquity in personal care products can be estimated by a 1981 FDA Cosmetic Product Formulation List that shows it as an ingredient in 703 products (Ref. 308). In oral care products, sodium lauryl sulfate is used as a foaming agent and is frequently combined with other ingredients. It is found in mouthrinses and dentifrices, usually in concentrations of 5 percent or less (Refs. 308 and 309). In most mouthrinses, it is found in concentrations of less than 1 percent. In skin care products, concentrations of sodium lauryl sulfate may range up to 50 percent. In the last two decades, sodium lauryl sulfate has replaced most other surfactants previously used for oral care drug products. It is estimated that 4 to 5 million pounds of sodium lauryl sulfate are used annually in the United States for oral health care products alone (Ref. 309).

The estimated daily intake of sodium lauryl sulfate of about 1 to 10 mg originates, in part, from personal products (including oral hygiene products), foods, and drinking water. Personal products account for about one-half or less of this intake (Ref. 310).

i. *Safety*. Extensive safety data, both in animals and humans, show that sodium lauryl sulfate has a very low level of toxicity at doses used in oral health care products, is rapidly metabolized through the liver, and has no genotoxic or teratogenic effects (Ref. 311).

1. *Absorption and excretion*. Sodium lauryl sulfate is poorly absorbed through the epithelial lining of the skin and mucosal surfaces. Aqueous radio-labeled sodium lauryl sulfate was applied to guinea pig skin in vivo by rubbing for 10 minutes, followed by washing and application of a nonocclusive dressing for 24 hours (Ref. 308). Most of the radioactivity was recovered on the skin at the experimental site, in the washing fluid, and in the dressing. Radioactivity of 0.1 percent was recovered from exhaled air and urine. No radioactivity was found in the internal organs, feces, or carcass. The studies concluded that the presence of a strong anionic terminal group impaired sodium lauryl sulfate penetration through the skin.

Rat skin was exposed for 15 minutes to radio-labeled (25 millimolar (mM)) sodium lauryl sulfate. Expired carbon dioxide, urine, feces, and skin were monitored for 24 hours. Autoradiography showed heavy concentrations of sodium lauryl sulfate on the skin surface and in the hair follicles. Quantifiable levels of sodium lauryl sulfate were also recovered in the urine (Ref. 308).

If linear alkyl sulfates, including sodium lauryl sulfate, are deposited on the skin after a wash and rinse application, only a small amount actually penetrates the skin (Refs. 312 and 313). Sodium lauryl sulfate is rapidly absorbed through the intestine of mammals, rapidly metabolized through the liver, and is excreted in the

urine. Sodium lauryl sulfate is oxidized to carboxylic acid with butyric acid-4-sulfate as the major metabolite (Ref. 314).

2. *Acute toxicity*. Sodium lauryl sulfate has an LD_{50} in rats ranging from 0.9 to 1.6 g/kg with a mean of around 1.3 g/kg (Refs. 315 and 316). Studies (Ref. 308) indicated that sodium lauryl sulfate is slightly toxic. Signs of toxicity included diuresis, diarrhea, lacrimation, salivation, tremors, convulsions, sedation, anaesthesia, and death.

Intraperitoneal administration of sodium lauryl sulfate (25 or 50 mg/kg body weight per day for 3 days) decreased the level of some cytochrome P450 species (Ref. 317), stimulated haem-oxygenase activity (Ref. 318), and affected serum lipids (Ref. 317). The concentrations of sodium lauryl sulfate and the routes of administration in these studies were specifically designed to induce toxic effects, including death, and have little in common with human exposure to this ingredient with normal use of mouthrinses and dentifrices.

3. *Chronic toxicity studies*. Rats fed a diet containing up to 2.25 percent sodium lauryl sulfate for 13 weeks demonstrated enlarged liver cells and increased liver weight, as well as elevated levels of alkaline phosphatase and glutamic pyruvic transaminase. These changes were considered to represent accommodations to the increased work load required for the metabolism of sodium lauryl sulfate. Other changes noted included nonspecific enlargement of the kidneys, increased water consumption, and enlarged intestinal lymphatics. The sodium lauryl sulfate level below which no changes could be detected was 0.14 percent of the dietary intake, or 116 mg/kg body weight (Ref. 319). Another study found the ''no change'' level to be 0.1 percent (Ref. 316).

In a 16-week feeding study in rats, daily doses of different percents of sodium lauryl sulfate in the diet had different results: 8 percent resulted in death, 4 percent in significant growth retardation, and 2 percent in some growth retardation that was not statistically significant (Ref. 320). In a 1-year study in dogs, a 2-percent dietary intake of sodium lauryl sulfate caused some weight loss. The ''no change'' level was 1 percent (Ref. 308).

The toxicology of alkyl sulfates has been extensively reviewed (Refs. 321 and 322). The Subcommittee notes several hypothetical examples (Ref. 313) that place the above findings in the context of human subject users. In the unlikely event of a 20-kg child ingesting 10 mL of a mouthrinse containing 0.3 percent sodium lauryl sulfate daily, over a 13-week period, the daily dose ingested would be 1.5 mg/kg body weight. Based on a ''no change'' level of 116 mg/kg in the rat feeding study, the safety factor is 77-fold (Ref. 319). The safety factor in a 50-kg adult ingesting 1 mL of the mouthwash daily would be over 1,900. Based on the 1-year study in dogs (Ref. 308), the safety factors for the child and adult would be greater than 500 and 13,000, respectively.

4. *Reproduction toxicity*. Teratogenic studies in rats (Refs. 323 through 326) revealed no evidence of teratogenicity. Some embryotoxicity was noted at high doses that were severely toxic to the dams.

5. *Mutagenic potential*. Neither in vivo (Refs. 327 and 328) nor in vitro (Refs. 329 and 330) assays resulted in any increase in chromosome aberrations. There is no evidence that sodium lauryl sulfate incorporated in oral health care products is a teratogenic or mutagenic risk in humans.

6. *Skin irritation*. At concentrations of 2, 10, and 20 percent, sodium lauryl sulfate produces a Draize skin irritancy test score compatible with that of a primary skin irritant (Ref. 308). The 1 to 6 percent concentrations of sodium lauryl sulfate applied to human skin under an occlusive patch for 21 days were irritating to the skin. However, no irritancy potential could be detected in the absence of the occlusive patch (Ref. 331). Therefore, open application of sodium lauryl sulfate produces little, if any, irritation at these concentrations.

7. *Ocular irritation*. The 10 percent sodium lauryl sulfate applied to the rabbit eye caused corneal damage if washing was delayed or withheld. A 1-percent sodium lauryl sulfate application caused little irritation and no corneal damage (Refs. 309, 321, and 322).

8. *Oral irritation potential*. Sodium lauryl sulfate solutions in concentrations of 0.1 to 1 percent in 12 percent ethanol were swabbed for 30 seconds 4 times daily for 4 days on the oral mucosa of rats. Only mild cheilitis (inflammation of the lips) and sloughing were observed (Ref. 332). A single application of 0.2 percent sodium lauryl sulfate to the oral mucosa of rats did not produce any detectable changes, whereas increased cellularity was observed with a 2-percent application in half of the animals. After 3 weekly applications, the cellular reaction decreased (Ref. 333).

The Subcommittee concludes that, based upon the results of the extensive toxicity tests (only some of which are referenced above), sodium lauryl sulfate does not constitute a risk to consumers in the concentrations found in oral health care products. The widespread use of sodium lauryl sulfate in numerous oral health care products, as well as in foods and other personal products, without any reported side effects attributable to normal use, further supports the safety of this ingredient.

ii. *Effectiveness*. The Subcommittee concludes that there are insufficient data available to permit final classification of the effectiveness of sodium lauryl sulfate as an antigingivitis/antiplaque agent.

Sodium lauryl sulfate is used in oral health care products because of certain desirable properties, which include: (1) Decreasing surface tension (Refs. 334 and 335), (2) affinity for enamel surfaces, leading to masking of receptor sites for bacterial proteins (Ref. 336), (3) emulsification of food and bacterial components (Refs. 334 and 337), (4) inhibition of selective enzymes that help form dental plaque (Refs. 337, 338, and 339), (5) affinity for bacterial proteins and ability to denature them (Ref. 337), (6) disruption of cell membranes (Ref. 340), (7) inhibition of plaque formation through decreased surface tension and competition with negatively charged bacterial cells for binding sites on the tooth surface (Ref. 341), and (8) optimization of antibacterial properties of certain zinc salts (Ref. 340).

These properties of sodium lauryl sulfate contribute to its usefulness to loosen and remove food particles (Refs. 342 through 349). Some of these properties also allow sodium lauryl sulfate to inhibit the formation of dental plaque (Ref. 350), exert a mild antibacterial effect (Ref. 351), and provide consumers with the feeling that tooth surfaces are smooth and clean and their breath is fresher (Ref. 352).

In examining the results of clinical trials involving sodium lauryl sulfate, the types of products containing this ingredient and the characteristics that make it desirable for a particular product should be considered. Because of differences in formulations and the presence of other ingredients, it may be difficult to determine to what extent sodium lauryl sulfate contributes to some of the beneficial effects claimed for marketed products. For example, a major objective for mouthrinse users is to reduce oral malodor. However, it is difficult to compare the effect of rinses containing sodium lauryl sulfate to those that do not, since flavoring agents are obvious confounding factors (Refs. 352 through 357). The most common oral health care products that contain sodium lauryl sulfate include

mouthrinses, prebrushing rinses, and dentifrices.

Mouthrinses are designed to provide cosmetic and/or therapeutic benefits. The major desirable characteristics of sodium lauryl sulfate are its affinity for enamel surfaces and its ability to reduce surface tension, which theoretically should interfere with dental plaque formation and provide a clean tooth feeling. Prebrushing rinses rely on these characteristics for additional emulsifying activity, thereby maximizing dental plaque removal that is largely the result of bristle action. Finally, because of its properties as a surfactant, sodium lauryl sulfate is frequently used in toothpastes as a foaming agent. Its superior cleansing properties compared to soap as a toothpaste ingredient were reported as early as 1937 (Ref. 358).

In general, human mouthrinse studies have shown a moderate reduction in plaque formation in the test groups using sodium lauryl sulfate in various formulations, as compared to a control group using no sodium lauryl sulfate. No significant difference was observed between the test and control groups in gingivitis studies.

Typical plaque and gingivitis scores from two representative studies are shown below. The scores at the end of these studies represent plaque and gingivitis score changes from a zero baseline, following an initial prophylaxis:

TABLE 12.—PLAQUE AND GINGIVITIS SCORES FROM THE BARONS STUDY (REF. 359)

Study	Group (n)	Baseline	End
		Plaque scores	
Test Product	Test (13)	0	2.86
(0.3% SLS)	Water (13)	0	5.13
Net plaque reduction: 44%			
		Gingivitis scores	
	Test (13)	0	0.88
	Water (13)	0	0.90
Net gingivitis reduction: 2% (not significant)			

TABLE 13.—PLAQUE AND GINGIVITIS SCORES FROM THE PRETARA-SPANEDDA STUDY (REF. 348)

Study	Group (n)	Baseline	End
		Plaque scores	
Test Product	Test (7)	0	2.20
(0.3% SLS)	0.1% chlorhexidine (9)	0	2.43
	Water (9)	0	4.78
Net plaque reduction: 54%			
		Gingivitis scores	
	Test (7)	0	0.93
	0.1% chlorhexidine (9)	0	1.03
	Water (9)	0	1.17
Net gingivitis reduction: 21% (not significant)			

The statistically significant reductions in plaque scores in these studies, as compared to a water placebo, were not accompanied by a statistically significant reduction in gingivitis scores.

No convincing evidence exists to support the effectiveness of prebrushing rinses, because the net beneficial effect of the rinses as compared to placebo is clinically insignificant. One of the products tested in the Truelove study (Ref. 349) (see Table 14 of this document) contains a number of ingredients other than sodium lauryl sulfate (Ref. 360). However, sodium lauryl sulfate is listed as the only active component. The results of this study indicated that prebrushing rinsing with two rinses that contain sodium lauryl sulfate as the active ingredient is no more effective than rinsing with a suitable sodium lauryl sulfate-free placebo.

TABLE 14.—PLAQUE/GINGIVITIS SCORES FROM THE TRUELOVE STUDY (REF. 349)

Agent	Prebrush score	Postbrush score
Test product (0.25% SLS)	2.56	1.11
Other product (0.3% SLS)	2.94	1.23
Placebo	2.50	1.16

The results of the Emling study (Ref. 361) suggested a somewhat greater plaque score reduction with the test product containing 0.25 percent sodium lauryl sulfate than the placebo (see Table 15 of this document). However, gingivitis scores were not measured in this study or in several other unpublished studies with the same experimental protocol that produced similar results (Refs. 362 and 363).

TABLE 15.—PLAQUE SCORES FROM THE EMLING STUDY (REF. 361)

Agent	Prebrush score	Postbrush score
Test product (0.25% SLS)	3.12	2.05
Placebo	3.09	2.82

In addition, Beiswanger et al. (Ref. 364) were unable to detect a statistically significant difference in the degree of plaque reduction between active and placebo rinses.

Van Dyke *et al.* (Ref. 365) also monitored gingival changes under conditions of prebrushing rinsing. They reported statistically significant reductions of plaque scores for both the placebo and the test rinse as compared to baseline scores. Although there was a statistically significant advantage of the test rinse over the placebo (1.61 versus 1.84 mean score) at interproximal surfaces for plaque scores, these differences were not clinically significant. Further, there were no differences in gingivitis scores before and after treatment, or between test and placebo scores.

Kohut and Mankodi (Ref. 366) found no difference between test and placebo prebrushing rinses, either in the degree of plaque or gingivitis reduction. Similar results were reported by Singh (Ref. 367) and by Pontier et al. (Ref. 368) in children undergoing orthodontic treatment. In a 6-month clinical study, Lobene *et al.* (Ref. 369) failed to show that a test product containing 0.25 percent sodium lauryl sulfate was superior to a placebo in reducing plaque, gingivitis, or calculus.

The Subcommittee concludes that sodium lauryl sulfate is effective to facilitate the removal of food and other particulate material and provide a clean tooth feeling, primarily through its surfactant properties and its affinity for binding to tooth surfaces. Sodium lauryl sulfate appears to have a minor inhibitory effect on plaque formation, following an initial dental prophylaxis. Although sodium lauryl sulfate has antibacterial properties in vitro, it is not clear to what extent this antibacterial effect is exerted in vivo. The antiplaque effect of sodium lauryl sulfate is at best moderate. Sodium lauryl sulfate does not have a significant effect on gingivitis. The role of sodium lauryl sulfate as a facilitator of plaque removal when used in a prebrushing rinse is marginal and does not result in any beneficial clinical improvement, such as gingivitis reduction or inhibition of calculus formation. Sodium lauryl sulfate is a safe and effective foaming ingredient when used in toothpaste.

The Subcommittee concludes that sodium lauryl sulfate at 0.1 to 5 percent concentration in an oral rinse or dentifrice dosage is safe, but that there are insufficient data available to permit final classification of its effectiveness as an antiplaque and antigingivitis agent.

g. *Zinc citrate*. The Subcommittee concludes that zinc citrate is safe, but there is insufficient evidence to support its effectiveness as an OTC antigingivitis/antiplaque agent.

Zinc citrate has a chemical formula of $Zn_3(C_6H_5O_7)_2$ and is prepared from zinc carbonate and citric acid. It is described as a dihydrate, odorless powder, that is slightly soluble in water (Ref. 370). Based on the known abilities of zinc to inhibit crystal formation and of citrate to inhibit crystal aggregation, zinc citrate replaced zinc chloride (highly effective but with a disagreeable taste) as a toothpaste ingredient to inhibit dental calculus formation (Ref. 371). Zinc citrate trihydrate ($Zn_3(C_6H_5O_7)_23H_2O$) has been used to inhibit supragingival calculus formation.

i. *Safety*. Zinc is ubiquitous in our environment and is an essential trace element in humans. Its role in humans continues to be the subject of investigation. The overall safety of zinc citrate has been well and extensively documented (Ref. 372). Acute toxicity studies in animals have shown zinc citrate to be only slightly toxic. Zinc citrate fed to rats for up to 13 weeks produced toxic effects only at high levels. No toxic effects were observed when toothpaste containing up to 10 percent zinc citrate was fed to rats and dogs for up to 18 months. In humans, zinc salts are considered relatively nontoxic (Ref. 372).

Zinc citrate had no adverse effects on fertility, the fetus, or neonate in rats and rabbits (Ref. 372). This finding correlates with published findings on other zinc salts. No mutagenic effects have been reported from in vivo studies. Zinc does not have genotoxic effects or pose a carcinogenic hazard at levels normally found in the body (Ref. 372). The oral irritation potential of toothpastes containing zinc citrate is no greater than that of other marketed toothpastes.

ii. *Effectiveness*. The Subcommittee reviewed five short-term clinical studies, two 6-month studies, and a 3-year trial assessing the effect of zinc citrate on gingivitis (Ref. 373). The five studies had in common a 21-day experimental period in which subjects, following a 4-week period of tooth cleaning and oral hygiene instruction, refrained from brushing one lower quadrant of teeth. An impression of each lower tooth arch was made and a plaster mold prepared. A plastic ''tooth shield'' was heated and vacuum fitted to

the plaster models. Subjects were instructed to place a measured quantity of dentifrice into the indentations in the tooth shield twice daily prior to its insertion in the mouth, and brush the remaining teeth. Plaque and gingivitis were assessed after 21 days. Various concentrations of zinc citrate in toothpaste or other ingredients alone or in combination with zinc citrate were used as well as placebos, which were not as effective as active ingredients. Because these studies were not randomized clinical trials, they cannot be considered as evidence of the effectiveness of zinc citrate.

The first 6-month study by Hefti and Marks (Ref. 374) was conducted to evaluate the relative effectiveness of a hydrogen peroxide/baking soda/fluoride/zinc citrate dentifrice with a commercially available fluoride dentifrice and a commercially available fluoride antitartar dentifrice. This was essentially a supragingival calculus study where subjects were selected based on having a score of at least 6.0 on the Volpe-Manhold Calculus Index at the time of screening. Clinical exams during the trial period were done at 45, 90, and 180 days. The Modified Gingival Index by Lobene et al. (Ref. 112) was used for gingival assessment. Only simple means for the 6 months assessment were given for the 3 groups of 60 to 63 subjects. A simple p value was given, indicating the multiingredient product and the other antitartar toothpaste group had statistically lower scores than the fluoride-only commercially available toothpaste. Three means were given for 45 and 90 days, plus one p value, showing similar results. No information was provided about subject characteristics, inclusion or exclusion criteria other than Volpe-Manhold Calculus Index scores, examiners, compliance, indicators of measurement error or uncertainty, or blinding. The conclusions concerning zinc citrate effectiveness were based on a multiagent product compared to other agents/ingredients.

The second 6-month clinical study (Ref. 375) included 295 subjects selected from a population of 330 adults of which 311 fulfilled strict dental and medical health requirements. No further details on health requirements were given. No information was provided about the study population, e.g., age, sex, education, and socioeconomic status. Inclusion criteria included a gingival index score greater than 0.5 but less than 2.5 on a scale of 0 to 3. One-third of the qualifying subjects were selected for plaque collection, which was performed prior to disclosing for the plaque assessment. There was no information on how these subjects were selected.

The products used were described as supplied by the sponsor in identical two-chamber, 5.2 oz pump dispensers, each with one of three three-letter codes. The report (Ref. 375) describes the three as ''negative control dentifrice,'' ''experimental dentifrice,'' and ''experimental dentifrice.'' An accompanying summary identified the products only as ''dual-phase dentifrices containing stannous salts and/or zinc citrate.'' One of the three-letter codes was identified only as ''the zinc citrate-containing dentifrice.'' Thus, there was no information about the composition and concentration of ingredients or details about differences in color, odor, and taste in the products tested. The Subcommittee does not believe this study adhered to strict criteria for a double-blind study because the following appeared in the report: ''Except for some complaints about the taste and staining associated with experimental dentifrice 'ABC,' the products were favorably received.'' These complaints were associated with only one of the three tested products. This suggests that one product differed from the others in taste and staining and, therefore, the study was not a double-blind study.

Examiners were described only as ''experimental examiners, who participated in a calibration exercise prior to initiating the investigation, performed the same assessments at each examination.'' The report did not discuss the number of examiners and their background, whether calibration was successful, or testing for intra-examiner and inter-examiner reliability.

Mean gingival index scores plus standard error were given for each of the three groups at baseline, 3 months, and 6 months (279 of 295 subjects completed 6 months). All scores were reduced from baseline at 3 and 6 months. The dentifrice containing zinc citrate was statistically significantly different ($p<0.03$) from the ''control'' group. Mean scores at 3 months were 0.87±.02 for the control dentifrice, 0.83±0.2 for the test dentifrice without zinc citrate, and 0.81±.02 for the test dentifrice containing zinc citrate. At 6 months the scores were 0.92±0.2 for the control dentifrice, 0.86±0.2 ($p<0.04$) for the test dentifrice without zinc citrate, and 0.85±.02 ($p<0.04$) for the test dentifrice containing zinc citrate. The study does not provide evidence that a clinically significant improvement in gingival index scoring was due to zinc citrate.

The 3-year trial (Ref. 376), of which results from the first 2 years were submitted, was a caries study. The main objectives of the trial were to establish the reduction of caries increments caused by increasing the level of sodium monofluorophosphate and to investigate whether the inclusion of 0.5 percent zinc citrate affected caries increments. Three thousand children with a mean age of 12.5 years and all within a 1-year age range were recruited. Two clinicians assessed all subjects, who were then randomly assigned to one of six toothpaste groups. One-half of the subjects used a toothpaste containing zinc citrate. Plaque (using Greene and Vermillion's Simplified Oral Health Index (OHI-S)) and gingivitis (Loe and Silness Gingival Index) were assessed each year. Six teeth were assessed: One molar, premolar, and incisor in each arch, at four surfaces on each tooth.

Differences between cumulative mean scores for groups using toothpastes with and without zinc citrate were calculated. One examiner showed nonstatistically significant differences for years 1 and 2 and a second examiner showed statistically significant differences. When pooled together, the small differences were statistically significant. There was no other information about examiner calibration or testing for intra and interexaminer reliability. Clinically significant effects due to zinc citrate could not be determined from this study.

The Subcommittee's criteria for data submitted from randomized clinical trials include presenting information on all of the major study components, e.g., the protocol (study population, agents, outcomes, rationale for statistical analysis), methods of randomization, concealment of allocation to study group, and method of blinding. Results should be presented with appropriate indicators of measurement error or uncertainty, avoiding dependence solely on statistical hypothesis testing, such as the use of p values, which fail to convey important quantitative information. Based on these criteria, the Subcommittee concludes that the data submitted were insufficient to permit final classification of the effectiveness of zinc citrate as an OTC antigingivitis/antiplaque agent.

2. Category III Combinations of Active Ingredients

Data to demonstrate safety and effectiveness as an antigingivitis/antiplaque agent will be required in accordance with the general guidelines on safety and effectiveness in section II.H of this document.

Alkyl dimethyl amine oxide and alkyl dimethyl glycine

Hydrogen peroxide and povidone iodine

Hydrogen peroxide and sodium bicarbonate

Hydrogen peroxide, sodium citrate, sodium lauryl sulfate, and zinc chloride

Peppermint oil and sage oil

Polydimethylsiloxane and poloxamer

Stannous pyrophosphate and zinc citrate

a. *Alkyl dimethyl amine oxide and alkyl dimethyl glycine*. The Subcommittee concludes that there is insufficient evidence to support the safety and effectiveness of the combination of alkyl dimethyl amine oxide and alkyl dimethyl glycine as an OTC antigingivitis/antiplaque agent. This combination consists of two amphoteric (having both acidic and basic properties) quaternary ammonium inner salt surfactants said to have broad spectrum antimicrobial activity.

i. *Safety*. An acute oral toxicity study (Ref. 377) of a 3-percent solution of alkyl dimethyl amine oxide and alkyl dimethyl glycine calculated that the LD_{50} in Sprague-Dawley rats was greater than 6,000 mg/kg. Necropsy observations included slight intestinal hemorrhage, slight liver discoloration, and slight to severe lung congestion.

An additional acute toxicity study in beagle dogs (Ref. 378) was difficult to evaluate because the dosages were stated in mL/kg but the concentration of the solution was not stated. Although there did not appear to be a constant pattern at necropsy, all of the dogs displayed abnormal findings, such as cortical congestion of the mesenteric lymph nodes, white nodules on the gall bladder mucosa, and consolidation of the lungs with a yellow-colored mucoid material in the bronchi.

A series of dermal toxicity studies was carried out. Again, because the concentration of the liquid used was not stated, these studies were difficult to evaluate. In one study (Ref. 379), the dermal toxicity of a 3-percent solution of the combination of alkyl dimethyl glycine and alkyl dimethyl amine oxide was evaluated on abraded skin of rabbits. Two of 20 animals displayed minimal reaction. An additional study (Ref. 380) reported that 3.6 percent of an applied dose was absorbed through rabbit skin.

Two dermal sensitization studies were carried out in guinea pigs (Refs. 381 and 382) and appeared to have diverse results. In one study (Ref. 381), the investigator concluded that there was no evidence suggesting the combination of these ingredients can act as a sensitizer in the guinea pig. However, it was unclear what concentration of the test material was used. In the second study (Ref. 382), it was concluded that repeated topical exposures of guinea pigs to a 3–percent solution of these ingredients has the potential to induce mild dermal sensitization.

Based on the results of a *Salmonella*/microsome mutagenesis assay (Ref. 383), the authors concluded that the combination of alkyl dimethyl amine oxide and alkyl dimethyl glycine inhibits the growth of microorganisms at some concentrations. Although small increases were observed in several strains of *S. typhimurium*, the authors stated that these increases were not reproducible and were attributed to random fluctuations that do not represent a mutagenic response to the test product. The test, therefore, has some limitations.

Eye and vagina1 irritant tests have also been conducted. A 3-percent solution of alkyl dimethyl amine oxide and alkyl dimethyl glycine was judged to be a mild irritant in the eyes of dogs and a severe irritant in rabbits (Ref. 384). In an additional study conducted by a different institution, it was concluded that a 12.5-percent solution was not an irritant to rabbits. Results from vaginal irritation studies (Ref. 385) concluded that these ingredients produced an ''acceptable'' vaginal irritation score. However, it was unclear which concentrations were tested and what is an ''acceptable'' score. Six preparations appear to have been examined, but no information was presented on how they differed in composition.

The data also included a series of studies (Refs. 386, 387, and 388) evaluating a 10-percent solution of alkyl dimethyl amine oxide and alkyl dimethyl glycine as a body wash in nursing home patients. The evaluations appear to be largely subjective or gathered from interviews. Adverse effects were not observed.

Dentists gave the combination of these ingredients to subjects to use as a mouthrinse (Refs. 389 and 390). Overall adverse effects, including tingling, mucosal irritation, stain, and a peppery sensation on the tongue, were reported by 0.5 to 0.8 percent of users. Other dentists (Ref. 391) reported adverse effects in 1.3 percent of subjects.

The effects of the combination of these ingredients on mammalian cells were examined using a chromium release assay from human leukemic cells (H6–60). The release of chromium occurred at concentrations of 0.025 to 0.005 percent. As the report notes, ''these findings are of some concern since the effective window approximates the MIC for several bacterial species'' (Ref. 392).

ii. *Effectiveness*. A number of studies have been carried out to assess the effects of this combination on the growth of oral bacteria and on the ability of oral microorganisms to produce acid from glucose.

The combination of alkyl dimethyl amine oxide and alkyl dimethyl glycine exhibits an antimicrobial effect against a wide range of microorganisms (Ref. 393). *Lactobacillus casei* is highly susceptible and is inhibited by as little as a 0.0004-percent solution. Several isolates of *Pseudomonas* are highly resistant to the combination. In general, the effect against gram-positive organisms was independent of pH. In contrast, the effect against gram-negative organisms was influenced by pH values. A 0.5-percent concentration of these ingredients completely inhibited bacterial glycolysis for 7 hours and inhibited the adherence of *S. sobrinus* to michrome wires. A lower concentration (0.05 percent) had less effect.

Twelve subjects (Ref. 394) rinsed with various concentrations of alkyl dimethyl amine oxide and alkyl dimethyl glycine and other preparations with only 2 days allowed between testing each material. Concentrations of 0.1 percent or higher reduced the population of total cultivable flora and total *Streptococcus* populations for at least 1 hour post rinse. Concentrations of 0.2 and 0.5 percent inhibited glycolysis in salivary sediment for several hours.

A clinical study involving 84 females and 42 males (aged 20 to 49) used a 0.25-percent solution (pH 6.8) of this combination (Ref. 395). Subjects were divided into one of three groups using a placebo, the test ingredients, or a positive control. Gender distribution was not disclosed. Following a complete prophylaxis, subjects rinsed twice daily for 6 weeks with 20 mL of solution. Subjects were instructed to continue their normal oral hygiene throughout the study. Plaque was assessed using Turesky modification of the Quigley-Hein Index. Mean plaque scores at the end of the study were as follows: Placebo, 2.53 ± 0.56 (2.44 ± 0.38), test ingredients, 2.05 ± 0.58 (2.45 ± 0.36), and positive control, 1.96 ± 0.33 (2.46 ± 0.31). An F test (test for equality of variances) comparison of the final three numbers showed statistica1 differences. An F test between the test solution and the positive control showed no statistically significant difference. No other statistical tests were reported. Gingivitis was not assessed.

A brief report (Ref. 396) claimed that a toothpaste containing these

ingredients reduced plaque formation by 43 percent in 15 subjects who used these ingredients for 7 days. Gingivitis apparently was not assessed. The report lacked essential information.

In a combined animal and human study (Ref. 397) and a separate human study (Ref. 398), a toothpaste containing 1 percent of the combination of alkyl dimethyl amine oxide and alkyl dimethyl glycine applied topically three times weekly had no effect in preventing caries.

In a more recent single-blind, randomized, crossover study in 20 subjects (Ref. 399), the effects of four ingredients, including the combination of alkyl dimethyl amine oxide and alkyl dimethyl glycine, were compared with saline in preventing plaque regrowth. Subjects rinsed twice daily for 1 minute and suspended normal oral hygiene measures. Plaque was scored using a plaque index and plaque area assessment. The combination of alkyl dimethyl amine oxide and alkyl dimethyl glycine was significantly less effective than the other three agents tested, but was more effective than saline. Gingivitis was not assessed.

Based on the data submitted, the Subcommittee concludes that there is insufficient evidence to support the safety and effectiveness of the combination of alkyl dimethyl amine oxide and alkyl dimethyl glycine as an OTC antigingivitis/antiplaque agent.

b. *Hydrogen peroxide and povidone iodine.* The Subcommittee has determined that there is insufficient evidence to support the safety and effectiveness of the combination of hydrogen peroxide and povidine iodine as an OTC antigingivitis/antiplaque agent.

i. *Safety.*

The Subcommittee concludes that hydrogen peroxide is safe at concentrations of up to 3 percent. Because the final concentration of hydrogen peroxide in this combination is 1.5 percent when the separately packaged solutions are mixed, the Subcommittee considers this portion of the combination to be safe. The povidone iodine component of the combination (5 percent final concentration), however, raises several safety concerns, including acute and chronic toxicity.

1. *Acute toxicity study.* An acute toxicity study (Ref. 400) was performed on rats to determine the LD_{50} iodine concentration. Ten animals were dosed with 5 g/kg with no fatalities occurring. The data established that povidone iodine is not considered toxic when the LD_{50} is greater than 5 g/kg. The only noted toxic effect at this level was hydronephrosis (distention with urine) of the kidneys of two male rats.

2. *Oral mucosal toxicity study.* Oral mucosal toxicity was also examined in rats (Ref. 401). A solution containing 1.5 percent hydrogen peroxide and 5 percent povidone iodine was applied three times daily for 7 days to the oral mucosa of 12 albino rats. Two other groups of 12 rats were exposed to the components individually. While there were animals in each group that did not gain weight normally, the differences between the groups were not significant. In the group that received the combination of ingredients, 5 of the 12 animals showed signs of acute iodine toxicity, including lethargy, diarrhea, and abnormalities in the GI tract. These signs suggest possible acute toxicity in humans due to iodine overdose. These abnormalities were not noted in the two groups exposed to hydrogen peroxide or povidone iodine solutions individually. No negative control group was included.

3. *Acute dermal toxicity study.* In an acute dermal toxicity study, a 10-percent povidine iodine solution mixed with 3 percent hydrogen peroxide at 2 g/kg of body weight was applied to 10 albino rats (Ref. 402). Skin reactions were recorded as slight, but 8 of 10 animals showed lethargy, nasal discharges, diarrhea, and other signs of GI disturbances. All 10 animals survived, showing only mild dermal irritation. The investigators defined the test mixture as nontoxic because the LD_{50} was greater than 2 g/kg of body weight.

4. *Eye irritation study.* An eye irritation study was conducted on six albino rats by placing a standard mixture of 10 percent povidine iodine and 3 percent hydrogen peroxide (Ref. 403) into the conjunctival sac and scoring by the Draize technique at 1, 2, and 3 days after dosing. The test mixture was determined to be an irritant, causing iritis and moderate conjunctival irritation in five of six animals.

5. *Chronic toxicity study.* Chronic toxicity is also of concern because of the activity of iodine on the thyroid. A 6-month prospective study in 50 subjects to assess thyroid function and iodine levels following prolonged exposure to the mouthrinse showed that iodine levels were significantly elevated in test subjects with increased protein bound iodine in blood and in urine samples (Ref. 404). In general, thyroid function tests remained within normal limits. These tests included serum thyroxine (T4), free T4, triiodiothyronine (T3), and free T4 index measurements. A small but significant rise in the serum thyroid stimulating hormone (TSH) was consistently noted. The investigators suggested that this small increase in serum TSH should be considered a normal physiological adaptive response to increased iodine intake and had no adverse effects on the subjects. While the study was a good first step in establishing the safety of chronic use of the test solution, there were several concerns. The total number of healthy subjects was relatively small and may not reveal possible side effects in a larger population. While the investigators considered increased TSH without concomitant serious side effects as a sign that subjects were able to tolerate increased iodine, an alternative interpretation is that the increased TSH was an early indication of a thyroid system that is not functioning properly. A larger and perhaps longer study is needed.

6. *Chronic use test in compromised thyroids.* Although a second much smaller study examined the effects of chronic use of a mouthrinse containing hydrogen peroxide and povidone iodine in subjects with compromised thyroids, the number of subjects was completely inadequate to establish possible side effects.

7. *Mutagenicity tests.* Tests to determine the mutagenicity of povidone iodine were carried out using the Salmonella/microsome mutagenesis assay, a micronucleus test in rats, and a rat hepatocyte DNA repair assay (Refs. 405, 406, and 407). While the tests indicated cell toxicity, they did not indicate a mutagenic effect. A cytotoxicity study examining the cytotoxic effects on Chinese hamster ovary cells was also reported (Ref. 408). The study concluded that the combination rinse is cytotoxic at a concentrations of 2,500 µg/mL. The report indicated that when a metabolic activation mixture with the appropriate buffer and cofactors was added to the assay, the test rinse was no longer considered cytotoxic. The report did not elaborate on the possible ramifications of these results.

In order to evaluate the acute toxicity studies submitted, the Subcommittee examined iodine toxicity in general. Acute toxicity of iodine tincture (2 percent iodine and 2.4 percent sodium iodine in a 50 percent ethanol solution) has been recorded at levels relevant to the concentration of povidone iodine (5 percent) in this combination. Fatal events have occurred when as little as 30 mL of tincture of iodine have been ingested (Ref. 409). Acute toxic effects produce local actions in the GI tract. Iodine is corrosive, but is also readily inactivated by foodstuffs. When large concentrations of iodine are ingested,

resulting shock and tissue hypoxia have been noted (Ref. 409). Ingestion of lesser amounts can cause gastroenteritis, abdominal pain, and diarrhea that may be bloody. Nausea and vomiting are common with ingested iodine.

The current product labeling recommends that children under 12 be supervised while using the product and warns against use by pregnant or nursing mothers, those with iodine sensitivity, and those with a history of thyroid disorder. Because of the potential toxic side effects, the labeling should include a warning that the product should not be used by children, women of child-bearing years, or anyone suffering from a thyroid disease, disorder, or ailment. Subjects considering long-term use of these ingredients should consult their physician to determine if any conditions exist that might contraindicate use.

ii. *Effectiveness*.

1. *Six-month studies*. Two 6-month studies (Refs. 410 and 411), a 3-week study (Ref. 412), a 6-week study (Ref. 413), and a brief review of the antimicrobial effects of mouthrinses on dental plaque (Ref. 414) were submitted. The 6-month studies (Refs. 410 and 411) were designed similarly, using subjects admitted according to common exclusion criteria. Subjects received a thorough prophylaxis and were then assigned to one of four groups using a test rinse containing hydrogen peroxide and povidone iodine, a rinse containing only one of these ingredients in distilled water, or a distilled water placebo. Because the subject pool was divided into four groups, each group had a relatively limited number of subjects. Ninety total subjects completed one study (Ref. 410) with 23 in the test rinse group, and 96 subjects completed the other study (Ref. 411) with 23 in the test rinse group. Clinical assignments included measurements of plaque using the Turesky modification of the Quigley-Hein Plaque Index and the Papillary Bleeding Scoring, which attempts to quantitatively assess inflammation and bleeding at the interproxima1 sites.

Several troubling aspects of the protocol jeopardized the value of the studies from the start. The overall sample size was immediately halved by including groups that used only hydrogen peroxide or only povidone iodine. The control rinse was substantially different from the test rinse and did not contain a placebo vehicle. The protocol for both studies included professional subgingival irrigation at 3-week intervals throughout the study. Further, subjects were instructed not to rinse, drink, or eat anything for 30 minutes following the rinsing procedure.

Results from the two 6-month studies failed to provide convincing clinical data in support of the tested ingredients. For example, while one study showed borderline significant plaque index score differences, the other study did not. Neither study reported the overall gingival index (bleeding index) scores. It appears that there were no significant differences overall for the gingival index in either study. Instead, only scores for sites greater than or equal to three were chosen for analysis. While both studies suggested that significant differences could be determined in this limited and skewed selection of sites, p values for these comparisons were unclear or not reported. Because use of the test solution did not significantly affect plaque buildup in at least one of the studies, it is possible that the positive effect on the gingival condition was due to the subgingival irrigation professionally administered every 3 weeks during the test period. If the test solution altered the subgingival flora but did not significantly change the supragingival flora, the most likely contributing factor would be the professional irrigation.

Further, the two studies were tabulated differently and the results were somewhat difficult to compare. One study compared sites while the other study examined differences between subjects. The number of sites used in these analyses was unclear or unstated. The investigators in one study chose sites over subjects for analysis because of the variation in the number of sites between subjects with a bleeding index greater than 3. Therefore, it is possible that one or only a few subjects had many sites and the remaining subjects had few sites that qualified. Such a distribution could produce results that realistically represent only a few subjects within the group rather than the group itself. As with several other important aspects of these studies, p values and standard errors for specific comparisons were often unclear or unstated.

The studies included a limited number of samples for microbiological examination. The investigators in both studies utilized selective media along with other microbiological assays. Both study reports indicated that opportunistic pathogens (*Candida* and enteric bacteria) did not establish themselves in any of the test groups sampled. The test solution samples tended to show fewer presumed periodontal pathogens compared to control samples. However, the number of periodontal pathogens was generally quite low or absent depending on the species studied. While the microbiological data hold some interest, the use of professional subgingival irrigations throughout the studies made interpretation of the microbiology data difficult.

The design of these studies made definitive conclusions very difficult, with no consistent or convincingly significant clinical effect on plaque or gingivitis. The toxicology data suggested that the combination is safe, but doubts linger. An appropriately sized study of healthy and thyroid-compromised subjects should be considered using a placebo that more closely resembles the test product. Subjects should not be instructed to refrain from eating, drinking, or rinsing and professional irrigation should not be included, as such procedures might significantly alter the results.

2. *Three and 6-week studies*. Two short-term studies of 3 and 6 weeks (Refs. 412 and 413) showed significant improvement in the clinical parameters reported. However, several ingredients reviewed by the Subcommittee, including some formulations of hydrogen peroxide, have shown positive short-term results only to fall short in long-term studies.

Based on these studies, the Subcommittee finds that there is insufficient evidence to support the safety and effectiveness of the combination of hydrogen peroxide and povidone iodine as an OTC antigingivitis/antiplaque agent.

c. *Hydrogen peroxide and sodium bicarbonate*. The Subcommittee concludes that the combination of sodium bicarbonate and hydrogen peroxide at concentrations up to and including 3 percent hydrogen peroxide is safe, but there are insufficient data available to permit final classification of the effectiveness of the combination as an antigingivitis/antiplaque agent.

i. *Safety*. Hydrogen peroxide can produce hydroxyl radicals in the presence of iron (Fe+2) or copper (Cu+1) (Refs. 188 and 189) and in vitro studies have shown that sister chromatic exchanges can be produced by hydroxyl radicals. Experimental and clinical data are sparse demonstrating a significant mutagenic effect with the combination of hydrogen peroxide and sodium bicarbonate in oral health care products. Experimental and clinical data, however, do not demonstrate a significant mutagenic potential with the combination of hydrogen peroxide and sodium bicarbonate in oral health care products (Refs. 145, 188, and 189). The rapid decomposition of hydrogen peroxide in the presence of sodium

bicarbonate (Ref. 145) further reduces the likelihood of a mutagenic effect occurring with combination products.

A 1989 mutagenicity study by Kuhn et al. (Ref. 415) tested varying concentrations of a gel containing levels of hydrogen peroxide up to 100 µg/plate in a bacteriological assay for toxicity and mutagenicity on several strains of *S. typhimurium*. The results showed no toxic or mutagenic effects on the strains tested, which was approximately 100 times greater than the optimal mutagenic response seen with aqueous hydrogen peroxide. This result is in contrast to other studies using strains of *S. typhimurium* that showed mutagenic action associated with hydrogen peroxide (Refs. 163, 168, and 416). This result is also in agreement with studies conducted with peroxide formulated in dental products that are uniformly not mutagenic in oxidant-sensitive bacterial strains (Refs. 172 and 417).

After 1 minute of brushing, recovery of hydrogen peroxide in the presence of baking soda was less than 5 percent of the amount introduced into the oral cavity (Ref. 145). Identical results on hydrogen peroxide decomposition were seen in control subjects and subjects with impaired salivary flow.

Using a rat animal model, a combination of sodium bicarbonate and hydrogen peroxide incorporated into a toothpaste vehicle was tested for oral mucosa irritancy by Meyers et al. (Ref. 418). The particular formulation was found to be a mild-to-moderate irritant. However, the test toothpaste was found to be less irritating compared to a common fluoridated toothpaste used as a control. Unfortunately, the concentrations of ingredients did not appear to be listed, including the concentration of sodium bicarbonate and hydrogen peroxide. These results do not agree with those reported by Marshall et al. (Ref. 184), in which no irritation was found to the oral mucosa of hamsters administered a dual phase hydrogen peroxide and sodium bicarbonate dentifrice containing 0.75 percent or 1.5 percent hydrogen peroxide and 5 percent or 7.5 percent sodium bicarbonate once-daily, five times per week for up to 20 weeks.

Two animal studies examined the potential for oral mucosal irritation by hydrogen peroxide in combination with sodium bicarbonate (Ref. 184). No mucosal irritation was observed after administration of a hydrogen peroxide and baking soda dentifrice once daily, five times a week for 20 weeks. These results support clinical and consumer studies that show no evidence of oral irritation following use of dentifrices containing a combination of these ingredients. A study by Kuhn et al. (Ref. 419) used a combination of 10 percent sodium bicarbonate and 1.5 percent hydrogen peroxide. The study included exposure of the test animals to DMBA, a known carcinogen, and evaluated if any of the test compounds (including this combination) resulted in additional carcinomas. The test and control compounds were administered in a 20-week cheek pouch mucosal irritation study and no additional carcinogenic effects from the test combination were found. These results and those seen in a second hamster bioassay (Ref. 184) are contrary to those of Weitzman et al. (Ref. 183) who found that, when combined with DMBA, hydrogen peroxide, only at a concentration of 30 percent, appeared to augment the carcinogenic effects associated with DMBA. No augmentation of the carcinogenic effects of DMBA was seen with 3 percent hydrogen peroxide in the Weitzman study (Ref. 183), whose results support the previous observations that concentrations of hydrogen peroxide of 3 percent or less are safe for use in the oral cavity.

In a 9-month human trial with concentrations of 10 percent sodium bicarbonate and 1.5 percent hydrogen peroxide used as a dentifrice, Truelove (Ref. 420) found no increase in yeast concentrations in test subjects compared to subjects using a standard fluoridated dentifrice.

There are reports in the literature of excessive use of these compounds producing marked gingival detrimental changes, although these lesions appear to be easily correctable (Refs. 421 and 422).

ii. *Effectiveness*. The value of the combination of hydrogen peroxide and sodium bicarbonate has led to a continuing debate within the dental research and clinical communities. An in vitro MIC and minimal bactericidal concentration (MBC) study found that both ingredients were weak bacteriocidal agents, with sodium bicarbonate requiring extremely high dosages to cause bacterial cell death (Ref. 423). Varying outcomes resulted from the concentration of ingredients, with some mixtures inhibiting/killing while other concentrations produced a synergistic effect. In one study, a combination of 3 percent hydrogen peroxide, 0.5 g of sodium bicarbonate, and 10 g sodium chloride was tested on 10 experimental and 10 control subjects who had moderate periodontitis and were carefully scaled and root planed at the beginning of the study (Ref. 424). The experimental subjects had the test ingredients administered at home with a toothbrush and at biweekly professional irrigations. Sites in the test group also received iodine applications. The results indicated that following scaling and root planing, and with a carefully monitored oral hygiene regimen including sodium chloride and iodine in addition to the hydrogen peroxide and sodium bicarbonate, a reduction of several clinical periodontal parameters occurred after 3 months of treatment. This study suggested a significant effect on the oral flora could be achieved by subgingival irrigation with these chemicals.

In a 3-week study (Ref. 425), a 1.5-percent hydrogen peroxide and a 2-percent sodium bicarbonate mouthrinse was tested in a positive and negative parallel-control study. The results indicated significant control of gingivitis and gingival bleeding compared to the negative control. The rinse compared favorably to the positive control 1.2 percent chlorhexidine rinse. The Subcommittee found that the study only evaluated efficacy up to 3 weeks, and long-term results are unknown.

Using a split-mouth design, Greenwell et al. (Ref. 426) tested the effect of this combination (hydrogen peroxide, sodium bicarbonate, and salt water) against standard oral hygiene methods. The effects on commonly monitored indices suggested no significant effect over the standard oral hygiene control except where initial therapy was not instituted. However, these subjects were diagnosed with treated or untreated periodontitis, and the study was limited to 8 weeks.

In a similar study, four subjects with early periodontitis used either a fluoridated paste or an experimental paste containing 3 percent hydrogen peroxide and sodium bicarbonate in a splitmouth study design. Over the 3-week test period, no discernible differences between the groups could be identified (Ref. 427). Similar results were found in a 3-month study in which the test ingredients (hydrogen peroxide and sodium bicarbonate) were applied with a toothpick (Ref. 428).

In a 2-year study in which salts and hydrogen peroxide mixture was compared to conventional oral hygiene methods, no discernible differences could be found using phase contrast microbiological parameters (Ref. 429). In another 2-year study, no positive clinical effects were discernible from the use of the combination of test ingredients (hydrogen peroxide, sodium bicarbonate, and sodium chloride) compared to conventional oral hygiene methods (Ref. 430). The 4-year data from the same subject group showed the same results as seen at 2 years (Ref. 431). As in the study noted above (Ref.

426), the subjects in this large-scale, long-term study had diagnosed early periodontitis. Keyes et al. (Refs. 432 and 433), in uncontrolled and poorly documented reports, indicated reductions in signs and symptoms associated with periodontal diseases when using a regimen consisting of a thick mix of sodium bicarbonate slightly moistened with a few drops of water and 3 percent hydrogen peroxide.

Because of a lack of properly designed studies showing conclusively that the combination of hydrogen peroxide and sodium bicarbonate is effective, this combination of ingredients does not appear to present any added benefit to oral hygiene products. Further, most reports indicated that the two ingredients were no better at controlling plaque and gingivitis than products currently on the market which do not contain these ingredients. Moreover, many of the published references exploring the effects of these ingredients tested small numbers of subjects, did not employ controls, and/or used subjects with inappropriate disease entities, such as mild to moderate periodontitis. Many of the published references instituted a variety of professional cleanings, irrigations, instructional oral hygiene sessions, and additional possibly active ingredients during the test periods, thus further clouding the already contradictory results. Several studies did not disclose the concentrations of either ingredient, making it difficult to make conclusions.

d. *Hydrogen peroxide*, sodium citrate, sodium lauryl sulfate, and zinc chloride. The Subcommittee concludes that the combination of these ingredients is safe, but there is insufficient evidence to permit final classification of its effectiveness as an OTC antigingivitis/antiplaque agent. The Subcommittee is aware of three formulations of a combination of hydrogen peroxide, sodium citrate, sodium lauryl sulfate, and zinc chloride. All of the active ingredients have potentially useful properties when included in a mouth rinse.

Hydrogen peroxide (0.595 to 1.5 percent). Hydrogen peroxide is used for its antibacterial and foaming properties (see section III.C of this document).

Sodium citrate (0.024 to 0.12 percent). Sodium citrate is used as an astringent and to enhance the antibacterial activity of zinc chloride.

Sodium lauryl sulfate (0.06 to 0.15 percent). Sodium lauryl sulfate is used for its emulsifying and antiplaque formation properties (see section III.C of this document).

Zinc chloride (0.016 to 0.08 percent). Zinc chloride is used for its antibacterial properties and its ability to reduce plaque accumulation and acid production by plaque bacteria. Zinc has also been shown to be effective in inhibiting calculus formation by interfering with the conversion of amorphous calcium phosphate to more crystalline calcium phosphate compounds and their growth (Ref. 434). The antibacterial effect of zinc salts may be enhanced in the presence of sodium lauryl sulfate.

i. *Safety*. Because the above ingredients are used in combination, the safety and efficacy of these ingredients must be examined under conditions of combined use.

Toxicity in animals. Acute oral toxicity tests in rats (Ref. 435) indicated that one of the three formulations (it is not clear from the protocol which one), is relatively nontoxic. The purpose of the study was to assess the toxicity of the combination of ingredients administered orally as a single dose to Sprague-Dawley rats, followed by a 14-day observation period. The combination was administered by oral gavage to five male and five female rats at a dose of 40 g/kg of body weight. Over the following 14 days all animals survived in apparent good health, although they exhibited hunched postures and loose stools for the first 2 days. No abnormal findings were observed at necropsy. This dose is considerably higher than the likely intake by subjects using these ingredients in a rinse.

In another study on the effect of topical application of this formulation to hamster cheek pouches, 76 hamsters were divided into 3 groups of 22 animals each, with equal numbers of males and females, and a fourth group of 10 animals. The test group received daily topical applications of the test formulation to their cheek pouches for a 30-day period. The negative control group received comparable applications of water. The positive control group received 5 percent sodium lauryl sulfate. An additional group of 10 animals received a fixed combination of essential oils and water. At the end of the 30-day period, the cheek pouches were examined clinically and histologically. The results indicated no evidence of mucosal irritation in the form of epithelial damage, inflammation, hyperplasia, atrophy, or hyperkeratosis when compared to the water control (Ref. 436).

Another hamster study of 30-days duration compared topical applications of the test formulation to abraded and non-abraded hamster cheek pouches with application of 0.12 percent chlorhexidine gluconate, 1, 2, and 3 percent hydrogen peroxide, 5 percent sodium lauryl sulfate, and tap water. The animals on the test formulation gained weight normally and did not demonstrate any evidence of mucosal irritation in the form of inflammation, epithelial ulceration, hyperplasia (abnormal multiplication of cells in a tissue), atrophy, or hyperkeratosis (enlargement of the keratin layer due to increase in cell size), as compared to the water control. The test formulation did not interfere with the healing of abraded pouches (Ref. 436).

ii. *Effectiveness*.

1. *Mechanism of action*. It is not clear how this complex mixture behaves under conditions of normal use. One formulation contains 0.6 percent hydrogen peroxide and is dispensed in a single bottle. In the other two formulations, the rinses are dispensed in two bottles, one of which contains hydrogen peroxide. The directions state that the contents of the two bottles should be mixed just prior to rinsing. According to the data, these latter two formulations have 2.5 to 3 times the concentration of the active ingredients found in the first formulation and are combined with 1.5 percent hydrogen peroxide versus 0.6 percent hydrogen peroxide used in the first formulation. One of the latter two rinses also has 5 times as much zinc chloride as the first rinse. The proportions of the ingredients vary among the three formulations, but are generally found in relatively low concentrations. The concentration ranges for the active ingredients are as follows: Hydrogen peroxide, 0.595 to 1.5 percent; sodium citrate, 0.024 to 0.12 percent; sodium lauryl sulfate, 0.06 to 0.15 percent; and zinc chloride, 0.016 to 0.08 percent (Ref. 437).

2. *In vitro studies*. Study 1 evaluated the effect of the combination formulation on acid production by *S. mutans* and included three experimental groups: (1) *S. mutans* in an enriched growth medium (control), (2) *S. mutans* in an enriched growth medium exposed for various durations to the combination formulation with a 1:4 dilution, (3) *S. mutans* in an enriched growth medium exposed for various durations to the combination formulation with a 1:8 dilution. After a 5-minute exposure, the cells were centrifuged, washed, resuspended in combination formulation-free medium, and incubated. The viability of the bacterial cells was not affected by the exposure to the formulation, and the formulation did not kill the bacteria during a 5-minute exposure. However, acid production by *S. mutans* was inhibited for 8 hours as a result of the

5-minute exposure, as compared to the control (Ref. 438).

Study 2, carried out by Drake et al. (Ref. 439), was designed to determine the antimicrobial activity of the combination formulation. A spectrum of oral microorganisms was exposed to various dilutions of the combination formulation (1:2 and 1:128) for times varying from 5 minutes to 2 hours. MIC's varied among the species tested. Periodontal pathogens, including *P. gingivalis*, *F. mucleatum*, *E. corrodens*, and *A. actinomycetemcomitans*, were among the more susceptible of the species tested, with MICs between dilutions of 1:64 and 1:28. Streptococci tended to be less susceptible. Under this protocol, *S. mutans* was inhibited by dilutions as low as 1:32, whereas in the previous study the combination formulation appeared to be ineffective even at dilutions as low as 1:4 (Ref. 438). This apparent discrepancy with study 1 is likely due to the longer exposure time of the bacteria in study 2 (up to 2 hours). Exposures of 15 minutes at a dilution of 1:4, or 5-minutes at a dilution of 1:2, were needed to kill all *S. mutans* cells in this study. Because mouthrinses are seldom used clinically for more than 30 to 60 seconds, it is doubtful that these results reflect the antibacterial effect of the mouthrinse in actual use.

3. *Human clinical trials.* One 6-week, blinded, parallel clinical trial compared the relative efficacy of two of the three combination formulations on plaque and gingivitis in a human adult population (Ref. 438). Subjects were divided into three groups, using either a commercial toothpaste and toothbrush (control), the ''regular strength'' (single-bottle) formulation and a commercial toothpaste and toothbrush, or the orthodontic strength'' (twin-bottle formulation not containing five times the concentration of zinc chloride) and a commercial toothpaste and toothbrush. Following the baseline examination, each subject was instructed to brush twice a day and, if assigned to a mouthrinse, to use the rinse after brushing. Baseline and 6-week data included the Loe and Silness Gingival Index recorded on six surfaces per tooth, and Turesky's modification of the Quigley-Hein Plaque Index. A mean score per subject was calculated for each index. The results are in Table 16.

TABLE 16.—GINGIVAL INDEX AND PLAQUE INDEX SCORES FROM THE GROSSMAN STUDY (REF. 438)

Experimental Groups	Baseline Gingival Index	6-week Gingival Index	Baseline Plaque Index	6-week Plaque Index
Group 1 (control)	1.52	1.40	20.76	18.56
Group 2 (1-bottle)	1.48	1.32	19.91	11.73
Group 3 (2-bottle)	1.47	1.33	19.15	12.84

Although the reduction in gingival index score was statistically significant for all three groups, the clinical significance of this reduction was marginal at best. There were no statistically significant differences among the three groups. The plaque index reduction was statistically significantly better for the mouthrinse groups than for the control group. However, the control group lacked a placebo rinse to determine whether the difference in plaque reduction was due to the rinsing effect or to some of the active ingredients in the test rinses. The degree of plaque reduction for any of the groups is of questionable clinical significance, because it did not result in any meaningful reduction of the gingivitis score.

In another double-blind clinical study (Ref. 440), 119 adults were fitted with a toothshield (for either the right or left mandibular quadrant) that was designed to prevent toothbrushing from disturbing plaque accumulation. All subjects received an initial prophylaxis and were assigned to one of three experimental groups, each of which brushed their teeth (except for the shielded quadrant) once a day and used a different mouthrinse formulation twice a day for 1 minute. The final examination took place after 3 weeks, and 102 subjects completed the trial. Two rinses were variations of the two-phase system formula used in the 1-bottle and 2-bottle formulations. The third formulation was a control rinse dispensed as a two-phase system. The results show no statistically significant differences in gingival index scores or bleeding sites among the three experimental regimens, either on the shielded or nonshielded teeth.

Plaque scores (Modified Turesky Plaque Index) were higher on shielded versus nonshielded teeth. The plaque scores after 3 weeks were lower for the two test rinses compared to the control rinse for both shielded and nonshielded teeth. However, the differences in plaque scores, while statistically significant, were not clinically significant.

TABLE 17.—DATA FOR SHIELDED TEETH FROM THE BESSELAAR LABS STUDY (REF. 440)

Experimental Groups	Modified Plaque Index Baseline	Mean ± Std. Error 3-Week
Data for Shielded Teeth		
Group 1 (Test 1)	2.21 ± 0.08	2.73 ± 0.08
Group 2 (Test 2)	2.14 ± 0.09	2.61 ± 0.09
Group 3 (Control)	2.15 ± 0.09	3.03 ± 0.09
Data for Nonshielded Teeth		
Group 1 (Test 1)	1.95 ± 0.07	1.76 ± 0.07
Group 2 (Test 2)	1.88 ± 0.08	1.63 ± 0.09

TABLE 17.—DATA FOR SHIELDED TEETH FROM THE BESSELAAR LABS STUDY (REF. 440)—Continued

Experimental Groups	Modified Plaque Index Baseline	Mean ± Std. Error 3-Week
Group 3 (Control)	1.91 ± 0.07	2.24 ± 0.06

The study results indicated that the test rinses had a marginal effect, at best, on plaque reduction, because plaque scores actually increased for all groups on shielded teeth, although less so, for the experimental rinses. None of the tested rinses had any effect to prevent development of gingivitis.

Data collected in individual dental offices by dental practitioners (Ref. 437) had no protocols and lacked the basic requirements for controlled, randomized clinical trials. Therefore, these data were of questionable value.

The Subcommittee concludes that this combination of ingredients is safe, but there are insufficient data to support its effectiveness as an OTC antigingivitis/antiplaque agent.

e. *Peppermint oil and sage oil*. The Subcommittee concludes that peppermint oil and sage oil are safe, but there are insufficient data to classify the effectiveness of the combination as an OTC antigingivitis/antiplaque agent.

Peppermint oil is described as the volatile oil distilled with steam from the fresh overground parts of the flowering plant *Mentha piperita linne*, rectified by distillation and neither partially nor wholly dementholized (Refs. 441 and 442).

Sage oil is derived from the dried leaves of the plant *Salvia officinalis*, which contains the essential oil (Ref. 443). It is described as having carminative and astringent properties and is used as a flavoring agent. It is used with other volatile agents in preparations for respiratory-tract disorders, and in mouthwashes and gargles for disorders of the mouth and throat. It is also used in homeopathic medicine.

Both peppermint oil and sage oil were reviewed by the Advisory Review Panel on OTC Oral Cavity Drug Products, which classified them as inactive ingredients (47 FR 22760 at 22764).

i. *Safety*. Peppermint oil has been used as a food flavoring for many years (21 CFR 182.20). Safety studies on peppermint oil continue to the present. For example, Spindler and Madsen (Ref. 444) conducted a toxicity study in rats giving peppermint oil orally to groups of rats at dosage levels of 0, 10, 40, and 100 mg/kg body weight. Some encephalopathy and nephropathy were seen at the highest dose. The authors determined a NOAEL of 40 mg/kg body weight per day.

Immunotoxicity testing of commonly used food flavoring ingredients including peppermint oil was reported (Ref. 445). Humoral and cell-mediated immune responses in mice were evaluated. Only at very high dose levels did peppermint oil increase mortality rate and reduce survival time in the host resistance assay, but it did not significantly alter humoral immunity.

Toothpaste and mouth rinse products containing both peppermint oil and sage oil were tested on the skin of rabbits with either no or slight-to-moderate irritant effects reported. Oral toxicity in rats showed no gross post mortem change. No untoward irritation or sensation relative to the oral mucosa was reported (Ref. 446).

ii. *Effectiveness*. The Subcommittee concludes that there are insufficient data from controlled studies to permit final classification of the effectiveness of peppermint oil and sage oil as OTC active ingredients for the reduction of plaque and gingivitis.

A single-blind study (Ref. 447) showed significantly less bleeding and less plaque in 25 dental students following 1 month use of the test toothpaste and oral rinse compared to 25 students using the placebo. However, all the relatively young dental students (age 25.5 ± 2.1 years) began with relatively low initial scores.

Although several efficacy studies of a toothpaste and an oral rinse containing peppermint oil and sage oil have been conducted (Ref. 448), these studies lack various aspects of double-blind, well-controlled research.

f. *Polydimethylsiloxane and poloxamer*. The Subcommittee concludes that these ingredients are safe, but there are insufficient data available to permit final classification of the effectiveness of the combination of polydimethylsiloxane and poloxamer as an OTC antigingivitis/antiplaque agent. The active ingredient is polydimethylsiloxane (dimethicone, simethicone), a fully methylated linear siloxane polymer used for its antifoaming properties in a number of marketed ingestible products such as antacids and certain foods (21 CFR 176.200). In order to insure the emulsification of the active ingredient, poloxamer, a polymer of polyoxyethylene, is used as a nonionic surfactant.

Polydimethylsiloxane combines readily with a number of other ingredients and has been packaged into different formulations (including sprays, mouthrinses, and dentifrices) and incorporated into oral hygiene devices (such as floss and interdental stimulators) and chewing gum. The ratio of the poloxamer to the polydimethylsiloxane varies from 100:1 in rinses to 1:1 in chewing gums. Concentrations range from 0.4 to 4 percent for liquid and gel emulsions, including toothpastes, and .01 to 0.2 g per use for interdental cleansing devices coated with solid emulsion, as well as chewing gum and mints.

i. *Safety*.

1. *Toxicity in animals*. Toxicity data in animals (Ref. 449) and humans (Ref. 450) indicate that polydimethylsiloxane has minimal toxicity. The biological safety of polydimethysiloxane has been tested by subdermal, intramuscular, and subcutaneous administration at greatly exaggerated dose levels in rats for periods of up to 26 weeks and further followups of up to 2 years. Monitoring included hematological and urinary chemistry, clinical parameters, and gross and microscopic anatomy. No effect was noted on the survival, body weights, clinical chemistry, hematology, urine chemistry, organ weights, or gross and microscopic anatomical features of the test animals that could be related to the tested product (Ref. 449). Acute toxicity testing of the poloxamer indicated minimal or no side effects from exaggerated doses via ingestion and intraocular administration of the tested products (Ref. 449).

The combination of poloxamer and dimethicone, packaged as a gel, was tested for acute oral toxicity in rats and in a 20-day hamster cheek pouch application study. At a dose level of 10 g/kg of body weight no deaths were observed in the rat study. If this combination were toxic, at this dose level it would have been expected to kill one half or more of the animals. Additionally, no abnormal changes were observed in the cheek pouches after topical applications of 0.1 mL of the combination three times daily for 4 weeks.

2. *Toxicity in humans*. No human toxicity data were submitted because poloxamer and dimethicone are categorized as safe (Ref. 450). The long-term use of the ingredients in antacids,

antiflatulents, and as an additive to certain foods without any report of harmful effects indicates that this combination is safe in the dosages and formulations in current use. The estimated daily intake varies from 0.2 g or less for sprays, gels, dentifrices, rinses, or dental floss to a high of 0.4 g per breath mint or candy (Ref. 451).

ii. *Effectiveness.*

1. *Mechanisms of action.* This combination acts by reducing the surface energy of the tooth (Ref. 452). Glantz (Ref. 453) showed a rapid increase in plaque formation with increasing surface energy in an in vitro assay. By reducing the surface energy with various surfactants, the rate of dental plaque build up can be theoretically reduced, particularly in the initial stages of dental plaque formation.

2. *Results from human clinical trials.* In general, most of the human studies have shown a marginal reduction in plaque formation in the test groups, using assorted formulations, as compared to the placebo or control group. In those studies that monitored gingivitis, no detectable difference in gingivitis was observed between the test and control groups.

TABLE 18.—TYPICAL PLAQUE SCORES FROM REPRESENTATIVE STUDIES MEASURING CHANGES FROM A BASELINE WITH OR WITHOUT AN INITIAL PROPHYLAXIS (REF. 454)

Study	Groups(n)	Baseline	End	Mean Difference
Study 1986–01	Test(10)	1.83	2.04	0.21
(OTC vol. 210259)	Control(10)	1.78	2.10	0.31
Study 1986–02	Test(13)	0	1.62	T vs C[1]
(OTC vol. 210259)	Control(13)	0	1.78	0.16
Study WHOIT–1990	Test(32)	0	2.06-2.16	T vs C
(OTC vol. 210259)	Control(32)	0	2.30	0.14–0.24
Study WHD–001	Test(30)	2.75	2.73	0.02
(OTC vol. 210259)	Control(30)	2.62	2.69	0.06
Study 47–01	Test(30)	0	1.87	T vs C
(OTC vol. 210260)	Control(30)	0	2.11	0.24
(Gingival Index score)	Test(30)	0	1.47	T vs C
	Control(30)	0	1.56	0.09

[1]T vs C means Test versus Control.

The protocols differed significantly from one another, as did the formulations of the test products. Nevertheless, it was clear that the differential effect on plaque scores between test and controls, while statistically significant, was not clinically relevant. Nor was it likely that the reduction in plaque scores is responsible for any potential cosmetic benefits that might be claimed. Therefore, it is misleading to claim that this combination has a plaque inhibitory effect. Such a claim might suggest a beneficial therapeutic or at least a cosmetic effect. While the plaque claim may be technically correct, the marginal nature of the effect is unlikely to have any clinically significant benefit, either therapeutic or cosmetic.

g. *Stannous pyrophosphate and zinc citrate.* The Subcommittee concludes that this combination of ingredients is safe, but there is insufficient evidence of its effectiveness as an OTC antigingivitis/antiplaque agent. Stannous pyrophosphate has the chemical formula $Sn_2P_2O_7$ and is a free flowing, odorless white to offwhite powder (Ref. 455). The commercial form of stannous pyrophosphate is anhydrous stannous pyrophosphate. This ingredient has been used in a dentifrice based on prior demonstrated antibacterial effects, which have been ascribed to the soluble stannous ion.

Because of reported antiplaque and anticalculus effectiveness, zinc citrate was combined in a dentifrice with stannous pyrophosphate (see discussion of zinc citrate chemistry in section III.C of this document).

i. *Safety.* Based on animal studies and human use, the two ingredients used in the combination do not appear to present a risk in terms of acute toxicity, chronic toxicity, reproduction toxicity, genotoxicity, carcinogenicity, phototoxic sensitization, or oral irritation. Oral ecology studies were done to ensure that long-term use of antimicrobial agents does not result in a significant change in the balance of the normal flora. In a 21-day experimental gingivitis study by Watson, Jones, and Richie (Ref. 456) and a 6-month clinical trial by Jones et al. (Ref. 457), following use of a dentifrice containing stannous pyrophosphate (1 percent) and zinc citrate (0.5 percent), there were no significant changes in plaque flora, no increase in opportunistic organisms in saliva, and no development of resistance.

ii. *Effectiveness.* Data on the clinical effectiveness of a fluoride toothpaste containing stannous pyrophosphate (1 percent) and zinc citrate (0.5 percent) included four studies: (1) An 18-hour plaque growth inhibition test, (2) a 21-day experimental gingivitis trial, (3) a 12-week motivational brushing trial, and (4) a 6-month normal use clinical trial.

The plaque growth inhibition studies used an 18-hour protocol described by Harrap (Ref. 458) to test the effect of the combination dentifrice on plaque growth in vivo. Lloyd (Ref. 459) reported that the formulation reduced plaque significantly compared to a placebo toothpaste, showing the antimicrobial activity of the two

ingredients when formulated into a dentifrice.

A 21-day experimental gingivitis study by Saxton and Cummins (Ref. 460) enrolled 37 subjects who were brought to a state of no gingival inflammation following 4 weeks of repeated professional cleaning and oral hygiene instruction. One posterior lower segment of tooth was covered with a vacuum-formed tooth shield as described by Bosman and Powell (Ref. 461). Subjects were instructed not to brush that segment of the tooth, which was covered when the subjects cleaned the remainder of their dentition. The tooth shields also served as carriers for the daily application of the control and test toothpastes. Assessment of inflammation and bleeding was done at baseline and at 3 weeks. Mean scores were significantly lower for the test group at 3 weeks, which was interpreted as the test dentifrice being better in delaying development of gingivitis.

A 12-week motivational brushing trial by Gaare et al. (Ref. 462) included 81 adult subjects described as receiving a prophylaxis and motivation at baseline and then using the combination dentifrice at least twice daily. Plaque index and GI scores improved at 6 weeks; plaque scores continued to improve at 12 weeks; and bleeding scores were maintained at 12 weeks.

A 6-month normal use clinical study by Saxton et al. (Ref. 463) enrolled 268 subjects, with 251 completing the trial. Clinical assessments were made at baseline and at 1, 4, and 6 months. Tooth scaling and polishing were done after baseline assessments, which included plaque index by Loe (Ref. 464), modified gingival index by Lobene (Ref. 112), extrinsic stain indices by Lobene (Ref. 465), supragingival calculus by Volpe (Ref. 466), and gingival bleeding by Ainamo and Bay (Ref. 467). The results at 6 months showed no difference in mean plaque scores and no difference in mean modified gingival index scores. Gingival bleeding was statistically significantly lower for the test group ($p<0.01$) as was the mean calculus scores ($p<0.01$). Tooth staining area mean scores were statistically significantly higher ($p<0.05$) and the stain intensity mean score was also higher ($p<0.00$) for the test group. It was reported that 17 percent of the test group observed tooth staining for themselves. Tongue staining was clinically detectable in approximately 40 percent of test dentifrice subjects compared to approximately 10 percent of control dentifrice subjects (53 versus 15 subjects at 6 months).

The Subcommittee concludes that the combination of stannous pyrophosphate (1 percent) and zinc citrate (0.5 percent) in a dentifrice is safe. However, there are insufficient data to permit final classification of its effectiveness as an OTC antigingivitis/antiplaque agent.

IV. Analysis of Impacts

FDA seeks specific comment regarding any substantial or significant economic benefit or impact that this proposed rule would have on manufacturers or consumers of antigingivitis/antiplaque drug products. Comments regarding the benefit or impact of this proposed rule on such manufacturers or consumers should be accompanied by appropriate documentation. The agency will evaluate any comments and supporting data that are received and will assess the economic impact of this proposed rule in the preamble to the proposed rule.

V. Paperwork Reduction Act of 1995

FDA tentatively concludes that the labeling requirements in this document are not subject to review by the Office of Management and Budget because they do not constitute a ''collection of information'' under the Paperwork Reduction Act of 1995 (44 U.S.C. 3501 *et seq.*). Rather, the labeling statements are a ''public disclosure of information originally supplied by the Federal government to the recipient for the purpose of disclosure to the public'' (5 CFR 1320.3(c)(2)).

VI. Environmental Impact

The agency has determined under 21 CFR 25.31(a) 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.

VII. Request for Comments

The agency is providing interested persons a period of 90 days to submit written or electronic comments to the Dockets Management Branch (see **ADDRESSES**) regarding this advance notice of proposed rulemaking. Three copies of all written comments are to be submitted. Individuals submitting written comments or anyone submitting electronic comments may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document and may be accompanied by a supporting memorandum or brief. The agency is also providing interested persons a period of 150 days to submit comments replying to comments regarding this advance notice of proposed rulemaking. Received comments may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

VIII. References

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List of Subjects in 21 CFR Part 356

Over-the-counter drugs, Antigingivitis/antiplaque drug products.

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 356 (as proposed in the **Federal Register** of May 25, 1982 (47 FR 22760), the **Federal Register** of January 27, 1988 (53 FR 2436), the **Federal Register** of September 24, 1991 (56 FR 48302), and the **Federal Register** of February 9, 1994 (59 FR 6084)) be amended as follows:

PART 356—ORAL HEALTH CARE DRUG PRODUCTS FOR OVER–THE–COUNTER HUMAN USE

1. The authority citation for 21 CFR part 356 is revised to read as follows:

Authority: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

2. Section 356.3 is amended by adding paragraphs (o) and (p) to read as follows:

§ 356.3 Definitions.

* * * * *

(o) *Antigingivitis drug*. A drug applied to the oral cavity to help reduce or prevent gingivitis.

(p) *Antigingivitis/antiplaque drug*. A drug applied to the oral cavity to help reduce or prevent gingivitis and dental plaque.

3. Section 356.13 is added to subpart B to read as follows:

§ 356.13 Antigingivitis active ingredients.

The active ingredient of the product consists of stannous fluoride 0.454 percent in a compatible dentifrice base.

4. Section 356.15 is added to subpart B to read as follows:

§ 356.15 Antigingivitis/antiplaque active ingredients.

The active ingredient of the product consists of any of the following when used within the dosage limits and in the dosage form established for each ingredient:

(a) Cetylpyridinium chloride 0.045 to 0.1 percent in a mouthrinse with at least 72 to 77 percent available cetylpyridinium chloride.

(b) Eucalyptol 0.092 percent in a mouthrinse when combined in accordance with § 356.26(p).

(c) Menthol 0.042 percent in a mouthrinse when combined in accordance with § 356.26(p).

(d) Methyl salicylate 0.060 percent in a mouthrinse when combined in accordance with § 356.26(p).

(e) Thymol 0.064 percent in a mouthrinse when combined in accordance with § 356.26(p).

5. Section 356.24 is amended by redesignating the text as paragraph (a) and by adding paragraph (b) to read as follows:

§ 356.24 Package-size limitations.

* * * * *

(b) Due to the toxicity associated with fluoride active ingredients in § 355.10 of this chapter, the following package-size limitations are required for antigingivitis drug products containing stannous fluoride:

(1) *Dentifrices*. Dentifrice (toothpaste) packages shall not contain more than 276 milligrams (mg) total fluorine per package.

(2) *Exception*. Package size limitations do not apply to antigingivitis/antiplaque drug products marketed for professional office use only and labeled in accordance with § 355.60 of this chapter.

6. Section 356.26 is amended by adding paragraphs (p), (q), (r), and (s) to read as follows:

§ 356.26 Permitted combinations of active ingredients.

* * * * *

(p) The ingredients identified in § 356.15(b), (c), (d), and (e) may be combined in a hydroalcoholic vehicle containing 21.6 to 26.9 percent alcohol in a mouthrinse provided the product is labeled according to § 356.65.

(q) The antigingivitis/antiplaque active ingredient identified in § 356.15(a) or the combination of ingredients identified in § 356.26(p) may be combined with any single anticaries active ingredient identified in § 355.10 of this chapter.

(r) The antigingivitis active ingredient identified in § 356.13(a) or the antigingivitis/antiplaque active ingredient identified in § 356.15(a) or the combination of ingredients identified in § 356.26(p) may be combined with any single tooth desensitizer active ingredient identified in § 356.22.

(s) The antigingivitis/antiplaque active ingredient identified in § 356.15(a) or the combination of ingredients identified in § 356.26(p) may be combined with any single anticaries active ingredient identified in § 355.10 of this chapter and any single tooth desensitizer active ingredient identified in § 356.22.

7. Section 356.65 is added to subpart C to read as follows:

§ 356.65 Labeling of antigingivitis/antiplaque drug products.

(a) *Statement of identity*. The labeling of the product contains the established name of the drug, if any, and identifies the product as ''antigingivitis'' or ''antigingivitis/antiplaque'' (optional: may include dosage form, e.g., dentifrice, toothpaste, mouthrinse).

(b) *Indications*. The labeling of the product states, under the heading ''Uses,'' one or more of the phrases

listed in this paragraph (b), as appropriate. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this part, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) *For all antigingivitis products.* The labeling states ''[bullet][1] helps [select one of the following: 'control,' 'reduce,' or 'prevent'] [select one or more of the following: '[bullet] gingivitis,' '[bullet] gingivitis, an early form of gum disease,' or '[bullet] bleeding gums'].''

(2) *For antigingivitis products containing stannous fluoride.* The labeling states the indication in paragraph (b)(1) of this section and/or the following: ''[bullet] helps interfere with harmful effects of plaque associated with gingivitis''.

(3) *For all antigingivitis/antiplaque products.* The labeling states ''[bullet] helps [select one of the following: 'control,' 'reduce,' 'prevent,' or 'remove'] plaque that leads to [select one or more of the following: '[bullet] gingivitis,' '[bullet] gingivitis, an early form of gum disease,' or '[bullet] bleeding gums'].''

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) *For all antigingivitis and antigingivitis/antiplaque products.* (i) ''Stop use and ask a dentist[2] if [in bold type] [bullet] gingivitis, bleeding, or redness persists for more than 2 weeks [bullet] you have painful or swollen gums, pus from the gum line, loose teeth, or increasing spacing between the teeth. These may be signs or symptoms of periodontitis, a serious form of gum disease.''

(ii) The following warnings shall be used in place of the general warning statements required by § 330.1(g) of this chapter.

(A) ''Keep out of reach of children under 6 years of age.'' [highlighted in bold type]

(B) ''If more than used for [select appropriate word: 'brushing' or 'rinsing'] is accidentally swallowed, get medical help or contact a Poison Control Center right away.''

(2) [Reserved]

(d) *Directions.* The labeling of the product states, under the heading ''Directions,'' the following directions for use:

(1) *For antigingivitis dentifrice products containing 0.454 percent stannous fluoride in a paste dosage form with a theoretical total fluorine concentration of 850 to 1,150 parts per million identified in § 355.10(c)(1) of this chapter.* ''[bullet] adults and children 2 years of age and older: brush teeth thoroughly, preferably after each meal or at least twice a day, or as directed by a dentist or doctor [bullet] instruct children under 6 years of age in good brushing and rinsing habits (to minimize swallowing) [bullet] supervise children as necessary until capable of using without supervision [bullet] children under 2 years of age: ask a dentist or doctor''.

(2) *For antigingivitis/antiplaque oral rinse products containing 0.045 to 0.1 percent cetylpyridinium chloride.* ''[bullet] adults and children 12 years of age and older: vigorously swish 20 milliliters of rinse between your teeth twice a day for 30 seconds and then spit out. Do not swallow the rinse. [bullet] children 6 years to under 12 years of age: supervise use [bullet] children under 6 years of age: do not use''.

(3) *For antigingivitis/antiplaque oral rinse products containing the combination of ingredients in § 356.26(p).* ''[bullet] adults and children 12 years of age and older: vigorously swish 20 milliliters of rinse between your teeth twice a day for 30 seconds and then spit out. Do not swallow the rinse. [bullet] children 6 years to under 12 years of age: supervise use. [bullet] children under 6 years of age: do not use''.

(e) *Other information.* The labeling of the product contains the following information under the heading ''Other information'':

(1) *For antigingivitis dentifrice products containing stannous fluoride.* The labeling states ''[bullet] this product may produce surface staining of the teeth. Adequate tooth brushing may prevent these stains which are not harmful or permanent and may be removed by a dentist.''

(2) *For antigingivitis/antiplaque oral rinse products.* The labeling states ''[bullet] this rinse is not intended to replace brushing or flossing''.

8. Section 356.66 is amended by adding paragraphs (b)(10), (c)(5), and (d)(3) to read as follows:

§ 356.66 Labeling of combination drug products.

* * * * *

(b) * * *

(10) *For permitted combinations identified in § 356.26(p).* The labeling of the product states, under the heading ''Uses,'' one or more of the indications for antigingivitis/antiplaque active ingredients in § 356.65(b)(3), or the following: ''[bullet] helps [select one of the following: 'control,' 'inhibit,' or 'kill'] plaque bacteria that contribute to the development of [select one or more of the following: '[bullet] gingivitis,' '[bullet] gingivitis, an early form of gum disease,' or '[bullet] bleeding gums'].''

(c) * * *

(5) *For permitted combinations identified in § 356.26.* The warnings in § 356.65(c) should be used.

(d) * * *

(3) For permitted combinations identified in § 356.26. The directions in § 356.65(d) should be used.

9. Section 356.92 is added to subpart D to read as follows:

§ 356.92 Testing of antigingivitis/antiplaque drug products.

The following testing should be conducted on the product formulation, a standard formulation with effectiveness documented by clinical trials, and a negative control.

(a) *Cetylpyridinium chloride rinse.* One of the following tests should be conducted:

(1) Determine the in vitro antimicrobial activity of the product against representative plaque organisms commonly associated with gingivitis. Representative organisms include, but are not limited to, typed stains of: *Actinomyces viscosus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Candida* species, *Streptococcus mutans*, and gram negative enteric rods. Testing to determine a product's in vitro antimicrobial activity should include minimal inhibitory concentration (MIC) assays, or 30-second kill-time studies, as appropriate.

(2) Demonstrate the availability of the active ingredient using a Disk Retention Assay (DRA).

(3) Demonstrate the biological activity of the product using an ex vivo Plaque Glycolysis and Regrowth Model (PGRM).

(b) *Combination of ingredients identified in § 356.26(p).* One of the following tests should be conducted:

(1) Determine the in vitro antimicrobial activity of the product using 30-second kill-time studies with both standard laboratory strains and

[1] See § 201.66(b)(4) of this chapter for definition of bullet symbol.

[2] For these products, the word ''dentist'' should be substituted for ''doctor'' in the heading ''Stop use and ask a doctor if'' required by § 201.66(c)(5)(vii) of this chapter.

wild-type organisms obtained from saliva sampling. Representative organisms include, but are not limited to, typed stains of: *Actinomyces viscosus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Candida* species, *Streptococcus mutans*, and gram negative enteric rods. Kill-time testing should be conducted using an exposure time of 30 seconds in the presence of exogenous protein. An initial inoculum of 1 percent transmission should be used.

(2) Demonstrate the in vivo activity of the product in a short-term experimental gingivitis study of at least 2 weeks duration. Formulation comparability in this test is established if the new mouthrinse formulation satisfies the ''at least as good as'' statistical criteria for both plaque and gingivitis with respect to the clinically tested standard, or another generally accepted statistical test of clinical comparability. The criterion for study validation is statistically significant differences in plaque and gingivitis between the clinically tested standard and the negative control.

(c) *Stannous fluoride dentifrice*.

(1) In addition to tests required by § 355.70 of this chapter, testing should include an in vitro determination of the antimicrobial activity against representative plaque organisms commonly associated with gingivitis. Representative organisms include, but are not limited to, typed stains of: *Actinomyces viscosus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Candida* species, *Streptococcus mutans*, and gram negative enteric rods. Testing to determine a product's in vitro antimicrobial activity should include MIC assays, 30-second kill-time studies, or plaque biofilm assays, as appropriate.

(2) Demonstrate the biological activity of the product ex vivo using PGRM.

(d) *Test modifications*. The formulation or mode of administration of certain products may require modification of the testing procedures in this section. In addition, alternative assay methods (including automated procedures) employing the same basic chemistry or microbiology as the methods described in this section may be used. Any proposed modification or alternative assay method shall be submitted as a petition in accordance with § 10.30 of this chapter. The petition should contain data to support the modification or data demonstrating that an alternative assay method provides results of equivalent accuracy. All information submitted will be subject to the disclosure rules in part 20 of this chapter.

Dated: May 12, 2003.

Jeffrey Shuren,

Assistant Commissioner for Policy.

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