DENTAL PRODUCTS PANEL ADVISORY COMMITTEE

OTC PLAQUE PRODUCTS SUBCOMMITTEE (NONPRESCRIPTION DRUGS)

12/16-17/96

Food and Drug Administration
Dental Products Panel Advisory Committee
OTC Plaque Products Subcommittee (Nonprescription Drugs)

Holiday Inn Bethesda
8120 Wisconsin Avenue
Bethesda, MD 20814

December 16, 1996

Agenda

Topic: The committee will discuss xylitol, C31G-Therasol, and the effectiveness of menthol, thymol, methyl salicylate, and eucalyptol.

8:30 a.m.	Call to Order and Announcements	Robert Genco, D.D.S., Ph.D. Chair
8:35 a.m.	Conflict of Interest Statement	Kennerly K. Chapman Executive Secretary
8:45 a.m.	Open Public Hearing	
	The Effectiveness of Essential Oil Containing Mouthrinses	Michael L. Barnett, D.D.S. Senior Director, Dental Affairs Worldwide Consumer Healthcare Research & Development Warner-Lambert Company
9:45 a.m.	Committee Discussion and Questions	
10:00 a.m.	Break	
10:15 a.m.	Subcommittee Review of Ingredients	
	Efficacy of menthol, thymol, eucalyptol, and methyl salicylate	Stanley Saxe, D.M.D., M.S.D.
	Xylitol	Sheila McGuire, D.D.S.
	C31G-Therasol	William Bowen, Ph.D., D.Sc.

11:00 a.m.	Open Committee Discussion
12:00 noon	Lunch
1:00 p.m.	Open Committee Discussion
5:00 p.m.	Adjourn

Food and Drug Administration
Dental Products Panel Advisory Committee
OTC Plaque Products Subcommittee (Nonprescription Drugs)

Holiday Inn Bethesda
8120 Wisconsin Avenue
Bethesda, MD 20814

December 17, 1996

Agenda

Topic: The committee will discuss microdent, and continue its discussion on sodium lauryl sulfate. In addition, the subcommittee will continue its discussion and vote on cetylpyridinium chloride, stannous fluoride, hydrogen peroxide, and sodium bicarbonate. If necessary, the subcommittee will continue its discussion of the effectiveness of menthol, thymol, methyl salicylate, and eucalyptol.

8:30 a.m.	Call to Order and Announcements	Robert Genco, D.D.S., Ph.D. Chair
8:35 a.m.	Conflict of Interest Statement	Kennerly K. Chapman Executive Secretary
	Open Public Hearing	
8:45 a.m.	Safety Concerns-Hydrogen Peroxide	R. William Soller, Ph.D. Senior Vice President and Director of Science & Technology Nonprescription Drug Manufacturers Association (NDMA)
9:15 a.m.	Stannous Fluoride Safety And Effectiveness	Stephen McClanahan, Ph.D. Section Head, Clinical Proctor & Gamble Co.
9:30 a.m.	Cetylpyridinium Chloride Safety and Effectiveness	Matthew J. Doyle, Ph.D. Associate Director and Senior Researcher, Proctor & Gamble Co.

9:40 a.m.	Committee Discussion and Questions	
10:00 a.m.	Break	
10:15 a.m.	Subcommittee Review of Ingredients	
	Microdent 200 Sodium Lauryl Sulfate-safety	Max Listgarten, D.D.S.
	Hydrogen peroxide (continuation)	Eugene Savitt, D.M.D
	Open Committee Discussion and Summary Review	
	• Hydrogen Peroxide • Cetylpyridinium chloride	Eugene Savitt, D.M.D.
	• Sodium bicarbonate	Robert Genco, D.D.S., Ph.D.
	• Stannous fluoride	William Bowen, Ph.D., D.Sc.
12:00 noon	Lunch	
1:00 p.m.	Open Committee Discussion	
5:00 p.m.	Adjourn	

DENTAL PRODUCTS PANEL (NONPRESCRIPTION DRUGS)
OTC PLAQUE PRODUCTS SUBCOMMITTEE ROSTER

November 25, 1996

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LISTERINE
(MENTHOL, THYMOL, METHYL SALICYLATE AND EUCALYPTOL) EFFICACY

Warner-Lambert Company
170 Tabor Road
Morris Plains, New Jersey 07950

201 540-2000
Cable Address WARNLAMBERT MORRISPLAINS
Telex 136424 WARNERLAMB MRPS

June 11, 1991

Division of OTC Drug Evaluation (HFD-210)
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857

Attention: Dr. William Gilbertson

Re: Listerine Antiseptic
Docket No. 81N-0033
Over-the-Counter Dental and Oral Health Care
Drug Products for Antiplaque Use; Safety and
Efficacy Review

Dear Dr. Gilbertson:

Pursuant to the request for data on ingredients contained in products making claims for plaque and gingivitis published in the Federal Register of September 19, 1990 (55 FR 38560-38562), the Warner-Lambert Company herewith submits data on Listerine antiseptic mouthwash. The data submitted support the safety and efficacy of the Listerine antiseptic formulation, a fixed combination of four essential oils; menthol, thymol, methyl salicylate and eucalyptol, for the prevention and reduction of supragingival plaque and gingivitis.

Clinical Studies

The submission consists of twenty-one human clinical trials in which the safety and efficacy of Listerine antiseptic for the prevention and reduction of supragingival plaque and gingivitis has been evaluated. In addition to confirming the safety of the product, these studies conclusively establish the efficacy of Listerine antiseptic for the above conditions.

Virtually no adverse effects were experienced by any of the subjects and no shift in the balance of the oral flora or increase in presumptive or opportunistic pathogens was observed. Differences in plaque and gingivitis scores between the test and control groups were statistically significant in all but one of the trials. The longer term six and nine-month clinical trials were conducted according to the testing guidelines established by the American Dental Association's (ADA) Council on Dental Therapeutics (CDT). These guidelines, developed by the ADA after rigorous review by the scientific community, establish comprehensive "state of the art" testing criteria for the evaluation of products making plaque and gingivitis reduction and prevention claims. A copy of the ADA guidelines is enclosed in this submission. We recommend that the Panel adopt these ADA guidelines as the standard by which all antiplaque/antigingivitis products will be judged. The enclosed studies demonstrate conclusively that Listerine antiseptic, when used in conjunction with a program of regular oral hygiene and routine professional care is highly effective for the prevention and reduction of supragingival plaque and gingivitis.

American Dental Association Acceptance

The American Dental Association Council on Dental Therapeutics is recognized throughout the world as an authoritative, professional group and it is relied upon by numerous regulatory bodies to develop criteria and standards for oral hygiene and dental products. The responsibility of the Council is to establish criteria for safe and effective dental products, evaluate their safety and efficacy, and review and approve labeling and advertising for accepted products. The program for the evaluation of products with claims for the prevention and treatment of plaque and gingivitis was formally established in 1984. The testing guidelines, which were established after several years of peer review by the scientific community, require that clinical safety and effectiveness be demonstrated in two independent studies of at least six months' duration which meet multiple strict study requirements.

Data on the safety and effectiveness of Listerine antiseptic meeting the Council's stringent criteria were submitted to the American Dental Association for review. After rigorous scientific evaluation by the Council and its expert scientific consultants, Listerine antiseptic was awarded the Council's Seal of Acceptance on June 22, 1987. The Seal represents the ADA Council's determination that Listerine antiseptic is safe and effective in helping prevent and reduce supragingival plaque accumulation and gingivitis when used in a conscientiously applied program of oral hygiene and regular professional care. The Listerine antiseptic formula is the first and only major nonprescription mouthwash formula to receive this distinction.

Self Diagnosis and Self Treatment

The request for data solicits comments on the appropriateness of OTC products for the prevention and reduction of plaque and gingivitis. It is well documented and accepted by qualified experts that the microflora of the mouth are responsible for diseases of the gingiva and periodontium. It is also axiomatic that all persons have plaque and that gingivitis is nearly endemic in the United States affecting between 75 and 90 percent of all adults. Thus, plaque accumulation may be viewed as a sign of poor oral health and a precursor of more severe conditions. Its presence is almost inevitable and everyone can assume that they have some plaque accumulation. Moreover, the signs of gingivitis - red, swollen, readily bleeding gums - can easily be recognized visually.

With respect to self treatment, it should be noted that Listerine antiseptic labeling and advertising explicitly recommend that the product be used as an adjunct to routine oral hygiene practices, including tooth brushing and flossing, and regular professional care. Listerine antiseptic is clearly represented as an adjunct to and not as a replacement for any of these practices. This is specifically stated in the ADA Seal of Acceptance statement found on every package of Listerine antiseptic and in Listerine antiseptic promotional and advertising material. The ADA statement reads:

> *"Listerine Antiseptic has been shown to help prevent and reduce supragingival plaque accumulation and gingivitis when used in a conscientiously applied program of oral hygiene and regular professional care. Its effect on periodontitis has not been determined." Council on Dental Therapeutics, American Dental Association.*

The ADA statement clearly communicates that the use of Listerine is part of a comprehensive oral hygiene program. In truth, advertising of consumer products like Listerine antiseptic in a conscientious manner has significantly increased consumer awareness of plaque and gingivitis.

Safety Data

The four essential oils in Listerine antiseptic; menthol, thymol, eucalyptol and methyl salicylate have previously been reviewed by numerous FDA OTC Drug Review panels and all have found them to be safe for their intended uses. The OTC Oral Cavity Drug Products Advisory Review Panel, which specifically reviewed oral health care drugs, recognized the safety of these four essential oils for topical use on the mucous membrane of the mouth and throat.
The applicable Federal Register notices reporting on findings of safety by the

several earlier Panels that reviewed Listerine antiseptic are included in our attached submission.

Additionally, it should be noted that the four essential oils in Listerine antiseptic are all widely recognized to be safe as food ingredients and are commonly used in many food products as flavorings.

The clinical studies included in this submission further document the safety of Listerine antiseptic. These studies demonstrate that daily long term use of Listerine antiseptic for up to nine months produced no adverse effects on soft oral tissue, no formation of calculus, essentially no extrinsic tooth staining and no adverse alteration of the normal oral flora. Additionally, three separate mutagenicity tests, which are also included, employing well recognized and established test methods, showed no potential for Listerine antiseptic to cause mutation. The safety of Listerine antiseptic is also well established in the marketplace. Listerine antiseptic has been marketed for over 110 years and has been used safely by millions of consumers.

Marketing Experience

The Federal Register notice calling for the submission of data provides that products to be reviewed may remain on the market if they have been marketed with the relevant indications to a material extent and for a material time. Listerine antiseptic fully meets these criteria. Listerine has been marketed as an antiseptic mouthrinse since well before the turn of the century. Since its introduction, labeling has represented, at different times, that the product will prevent and reduce plaque and gingivitis. Listerine promotional materials made gingivitis and plaque-related claims before enactment of the 1906 Pure Food and Drugs Act and the 1938 Federal Food, Drug and Cosmetic Act. Promotion for Listerine prior to the enactment of the 1962 Amendments to the Federal Food, Drug and Cosmetic Act recommended the use of the product for gum disease and the plaque which causes it. More recently, since June 1987, when we obtained the ADA Seal, Listerine has been heavily promoted with labels like those submitted for review here.

Since the inception of the OTC drug review program in 1972, additional extensive and comprehensive clinical research, including long term clinical studies following strict ADA guidelines, has been conducted and has clearly confirmed the efficacy of Listerine antiseptic in preventing and reducing plaque and gingivitis. Based on its extensive history of safe use, the recognition of its effectiveness in preventing and reducing plaque and gingivitis and its endorsement by respected scientific bodies, Listerine antiseptic meets the standards of general recognition of safety and effectiveness and the marketing requirements of "material extent" and "material time."

Other Conditions

In addition to the plaque and gingivitis studies mentioned above, we are providing data on the effectiveness of Listerine antiseptic used for other related conditions. These data include studies on the antibacterial effectiveness of Listerine antiseptic when used as a preprocedural rinse in the dental operatory; a study on the antiplaque and antigingivitis effectiveness of Listerine antiseptic when used in an oral irrigation device; a study demonstrating the effectiveness of Listerine antiseptic in enhancing healing after gingival flap surgery; and a study which demonstrates the effectiveness of Listerine antiseptic in reducing Candida albicans, the organism responsible for the development of denture stomatitis. We do not propose to make claims to consumers for these other conditions at this time. We request that the Panel evaluate and allow these claims for communication directly to dentists in professional labeling.

Based on all the above discussed reasons and the extensive clinical data included in this submission, we respectfully request that the Panel take the following actions:

1. *Place the combination of four essential oils (in concentration indicated) in Listerine antiseptic in Category 1 for safety and efficacy.*

2. *Approve the current recognized claims for Listerine, including:*
   - *"kills germs that can cause plaque and gingivitis"*
   - *"helps prevent and reduce plaque and gingivitis"*

3. *Approve the following proposed claims for professional labeling:*
   - *"reduces the level of aerosolized bacteria from dental procedures"*
   - *"reduces the level of salivary bacterial during dental procedures"*
   - *"reduces Candida albicans, the organism responsible for development of denture stomatitis"*
   - *"enhances wound healing after gingival flap surgery"*

4. *Recognize and accept the American Dental Association (ADA) guidelines as the criteria for the conduct of definitive studies required as support for indications for prevention and treatment of supragingival plaque and gingivitis.*

This submission is organized according to the requirements of 21 CFR 330.10. The basic document consists of five (5) volumes which are numbered

1 through 5. There are also four (4) appendices which are numbered as Appendix 1 through Appendix IV for a total submission of 9 (nine) volumes. The appendices are copies of the Flavor Extract Manufacturers Association scientific literature reviews of the GRAS (generally recognized as safe) status of the active ingredients in Listerine Antiseptic. These reviews were previously prepared for the Food and Drug Administration.

Our submission is paginated in consecutive order across all five (5) volumes. The submission page number is located on the bottom right hand corner of each page. It is an 8 digit number configured as 00-000000. The first two digits are always 00, and remaining digits start at 000001 and are consecutive to the end of the document.

The document is organized as follows:

Volume 1 - Sections I, II, III and IV
Volume 2 - Section IV (continued)
Volume 3 - Section V
Volume 4 - Section V (continued)
Volume 5 - Section VI

Each volume will contain a Table of Contents to facilitate the location of specific information. Each Table of Contents line item has a corresponding tab within the document.

Should you have any questions or desire any additional information, please contact the undersigned directly.

Very truly yours,

Robert Kirpitch
Director, Regulatory Affairs
Consumer Products
Research & Development

701GM11

Kills germs that cause Plaque, Gingivitis and Bad Breath

To help prevent and reduce plaque and gingivitis / For bad breath– Rinse full strength for 30 seconds with ⅔ ounce (4 teaspoonfuls) morning and night. If bad breath persists, see your dentist.

Warning: Do not administer to children under three years of age. Keep this and all drugs out of reach of children. Not for ingestion.

Active Ingredients: Thymol .06%, Eucalyptol .09%, Methyl Salicylate .06% and Menthol .04%. Also contains Water, Alcohol 26.9%, Benzoic Acid, Poloxamer 407 and Caramel.

COLD WEATHER MAY CLOUD LISTERINE. ITS ANTISEPTIC PROPERTIES ARE NOT AFFECTED. STORE AT ROOM TEMPERATURE (59°–86°F).

Questions about Listerine Antiseptic? Call toll-free 1-800-223-0182.
In New Jersey call 1-800-338-0326
Consumer Health Products Group, Warner-Lambert Co., Morris Plains, NJ 07950 USA

701GM12

(II) <u>A STATEMENT SETTING FORTH THE QUANTITIES OF ACTIVE INGREDIENTS OF THE DRUG</u>.

The active ingredient in Listerine Antiseptic is a fixed combination of oils consisting of:

Thymol	.06%
Eucalyptol	.09%
Methyl Salicylate	.06%
Menthol	.04%

III. ANIMAL SAFETY DATA

Safety Studies of Listerine and Its Active Components

The following section contains summaries of the published scientific literature on the four active ingredients (eucalyptol, menthol, methyl salicylate and thymol) in Listerine antiseptic. Each section includes a document that was submitted in the original Listerine antiseptic submission to the Oral Cavity Panel in 1972 plus an addendum that updates the published safety literature to the present.

Also enclosed with this submission are four Scientific Literature Reviews, prepared by the Flavor and Extract Manufacturers' Association in 1984 by contract for the Public Health Service, Food and Drug Administration, Department of Health, Education and Welfare. These four reviews summarize the preclinical data used in establishing the active ingredients in Listerine (menthol, thymol, eucalyptol and methyl salicylate) as GRAS (generally recognized as safe) substances in food. These data can be found in appendices I - IV to this submission.

In order to assess the risk potential of Listerine and/or its active components (eucalyptol, menthol, methyl salicylate and thymol), it is necessary to consider the exposure levels associated with the recommended twice daily oral rinsing of 20 milliliters of Listerine.

The concentrations of these active ingredients in Listerine range from 0.04% to 0.09%. At these concentrations, the daily exposure ranges from 16 mg to 36 mg for a 60 Kg person. Considering that Listerine is expectorated after the recommended 30-second rinse, the quantity that is ingested and/or absorbed is substantially less.

Eucalyptol is the chief constituent of oil of eucalyptus and is found as a component of other essential oils. It is also known as cineol or cajeputol. The formula is $C_{10}H_{18}O$ with a molecular weight of 154.24. The boiling point is 176-177°C and the melting point 1.5°C. It is insoluble in water but miscible with alcohol and oils.

The food additive status as a flavoring agent is described in the Code of Federal Regulations.

Animal Toxicology

Acute and subacute studies

Jenner (1) determined the acute oral toxicities of 107 synthetic and natural flavoring materials and related compounds. Using a 2-week observation period in Adult Osborne-Mendel rats, the LD_{50} of eucalyptol was determined to be 2580 mg/kg.

Taylor, et al (2) (in 1917) studied the effects of intraperitoneal injection of eucalyptol in white mice. He found that at .05 mg/100 gms. body weight just one animal survived. At 0.15 mg/100 gms. the animal died in 4 hours; at 0.5 mg/100 gms. and above, the one animal per group died within 10 minutes. He also found that with a subcutaneous injection in the guinea pig at 1200 mg/100 gms the animal died in 12 hours.

The acute oral toxicity in rats for eucalyptol was determined by Brownlee (3) to be 1.68 ml/kg. This author also described the effect of eucalyptol on blood pressure, respiration, and the essential nervous system of the decerberate cat. Dzhumagalieva (4) obtained the following results: (1) no deaths observed when less than 2.5 g/kg. injected subcutaneously to mice, (2) intramuscular LD_{50} of

approximately 2.0 g/kg. in the guinea pig, and (3) dogs tolerated a subcutaneous dose of 1.2 g/kg. but abscesses were noted at injection sites when injected at concentration higher than 40%.

Rob and Field (5) reported the chronic toxicity of various essential oils. These authors noted that eucalyptus oil had weak promoting activity for mouse skin tumors.

Special Studies

A summary of the current knowledge on the carcinogenic activity of various essential oils was given by Homburger (6). Eucalyptus oil was applied to mouse skin; about 10% of the mice treated developed tumors. The tumors were not described in detail.

Jori and Briatico (7) administered eucalyptol subcutaneously to pregnant rats. They found that liver microsomal activity was greatly enhanced in adult rats treated both during and after pregnancy and was also increased in the fetal and newborn offspring.

Human Safety

Reviews

The toxicology of eucalyptus oil was reviewed by MacPherson (8) in 1925. His review indicated an extreme variability in reported toxicity with idiosyncrasies stated to be an important factor. Collapse has followed the taking of 12 drops in or

case while recovery has been noted after the administration of a "cupful" in another case. The author reports 8 fatal cases in Australia with a teaspoonful the minimum fatal adult dose and death commonly occurring following the administration of an ounce or more. Symptoms of overdosage include gastric irritation, lowered blood pressure, skin lesions, breathing difficulties, cyanosis and kidney irritation often followed by coma. Death was stated to occur due to respiratory paralysis. Recommended treatment includes stimulants, emesis and oxygen inhalation.

Gutmann (9) stated in 1932 that essential oils including eucalyptol can act as allergens. He does not, however, provide any specific data.

In 1953, Craig (10) reviewed in depth 50 out of a total of 74 cases of poisoning from volatile oils seen in a 20 year period in Scotland. Four of these 74 cases were due to eucalyptus oil. He also notes that in Great Britain, out of 454 deaths from accidental poisoning, 54 were due to volatile oils. In this same 20 year period one of these latter was due to eucalyptus. In regard to eucalyptus, he points out that it is an uncommon poison in childhood. Three cases are reported here, one in detail; all recovered. Doses varied from 1 teaspoon to 1 ounce. Three major manifestations noted were depressive effect, stertorious breathing due to moisture in the respiratory tract, and myosis. Toxic dose in the human appeared difficult to determine.

Arena (11) states that ingestion of 15 ml. of a volatile oil has caused fatal poisoning.

In Samitz, et al (12) it was reported that eucalyptol as well as menthol, thymol and methyl salicylate were among the less frequent sensitizers. In contrast, Adam (13) cites menthol and eucalyptus as skin irritants often found in o.t.c. medications.

Studies

Perrault (14) tested several agents including eucalyptol for effects on odontoblasts. This study was conducted because these agents have potential value in killing bacteria when dental cavities are filled. Eucalyptol had no effect on the odontoblast whereas alcohol and phenol had deleterious effects, and silver nitrate significantly damaged the pulp as well as affecting the odontoblasts

Case Reports

Several individual case reports due to misuse of eucalyptus were noted in this review. In 1898 Esmonde-White (15) reported that an individual ingested 1-1/2 ounces of commercial eucalyptus oil with no untoward effect. Benham (16) in 1905 reported two cases of inadvertent administration of a teaspoonful of eucalyptus oil to adult males. Each case resulted in partially comatose condition but by the use of emetic, each of the cases recovered uneventfully. Also in this same year, Taylor (17) reported a single case of a 25-year old male swallowing a teaspoonful of eucalyptus oil. This individual had extreme breathing difficulties and mental confusion and became semi-comatose. He was administered stimulants and emetics and recovered.

The following year Davies (18) reported that his wife and son were subject to fainting spells when in the presence of eucalyptus oil vapor. While the author concluded that this was a familial problem, no further data is given. Also in 1906 Smith (19) reported death following the ingestion of approximately 22.5 ml. of eucalyptus oil. Kirkness (20) also reported on 2 cases of poisoning by misuse of oil of eucalyptus. In this case an adult male ingested 2 to 3 teaspoonfuls and an 18 year old female approximately 1 gram; both recovered.

Accidental ingestion of 1.5 grams of eucalyptus oil by a two year old child was reported by Bremer (21) to be followed by diminished breathing rate, weak pulse, and extreme drowsiness. Complete recovery occurred in this case following the administration of ipecac as an emetic. In 1918, Barker et al (22) reported a case where an individual was taking not only eucalyptus oil but myrtol and sandalwood oil and recovered from an overdose of eucalyptus oil. In 1927, Gibbin (23) reported a case where recovery occurred after ingestion of 4 grams. More recently, Gurr (24) reviewed eucalyptus oil poisoning. He reports a case of severe intoxication following ingestion of 4 to 7 ounces by an 18 year old male. This resulted in prolonged coma. Successful management was achieved by use of intravenous mannitol, antibiotics, hemodialysis and peritoneal dialysis.

Recently Bickers, et al. (25) reported the case of a 48 year old woman who intentionally drank over a period of months substantial quantities of a mouthwash containing eucalyptol as one of its ingredients. The patient, known to have acute intermittent porphyria, responded to therapy but had a series of subsequent re-hospitalizations. Each exacerbation occurred after several weeks at home during which time the patient admitted to drinking the mouthwash. The mouthwash was found to be a potent inducer of the rate limiting enzyme in the porphyrin-heme pathway. While Eucalyptol was the major component having this biologic activity, the inducing potency of the mouthwash was not fully accounted for by eucalyptol.

In regard to topical reaction to eucalyptus, the following have been reported: Oppenheim (26) reported the appearance of a rash and erythema in a 35 year old subject following the ingestion of 20 pieces of a cough candy containing eucalyptus.

In 1935 Sezary (27) reported desensitization for 24 year old female who was allergic to tincture of eucalyptus administered by inhalation for sinusitis. Topical application with an unspecified medication relieved the erythema, pain and pruritis. After relief, the patient was desensitized by means of 5 intramuscular injections of an extract of de-albuminized spleen and intravenous sodium hyposulfate.

Pharmaceutical and Chemical Studies

A brief review of the biology of eucalyptus trees and the chemistry of the oil is given by Fauvel (28). Armstrong and his co-workers (29) studied the relationship between vapor pressure of the drug and its concentration merging in the airstre from a nasal inhaler. His results showed that mixtures of eucalyptol and methylamphetamine yielded reduced vapor pressure and thus a lower effective concentration was required for methylamphetamine.

Heffelmann (30) reviewed (as of 1912) the effectiveness of mouthwashes and antibacterial activity as they apply to caries. This author states that mouthwashes should not be acid in nature. This was based on some work he had done on mouthwash and components in regard to mineralization and demineralization of excised teeth. Eucalyptol had no positive or negative effect in this system.

Absorption, Metabolism, and Related Information

Eucalyptol was stated by Meyer (31) to be a substance showing fairly rapid percutaneous absorption.

In respect to the effect of eucalyptol on drug metabolism, Jori (32) studied a number of components of essential oils for their effects on drug metabolism in rats. Eucalyptol administered either subcutaneously or by aerosol increased the in vitro liver metabolism of aminopyrine, p-N- anisol and analine and the in vivo metabolism of pentobarbital. Both modes of eucalyptol administration gave reduced pentobarbital brain levels and reduced sleeping times.

Jori (33) subjected rats to an aerosol inhalation of eucalyptol and found decreased plasma and/or brain levels of amphetamine, pentobarbital and aminopyrine when these drugs were also administered. In addition, the rate of disappearance of aminopyrine from plasma was increased in four of five volunteers treated by eucalyptol aerosol inhalation. This is of interest as eucalyptol aerosol has been used in combination with aminopyrine for treatment of bronchial pulmonary disease. The author suggested that eucalyptol may modify the efficacy of other drugs given concomitantly with respiratory disease therapy. Inducing activity on liver microsomal enzymes was postulated with the mechanism involved. A further study by Jori et al. (34 showed that eucalyptol increases the microsomal activity of rat liver after a single subcutaneous dose, but further doses did not enhance the activity. Eucalyptol did not effect the concentration of cytochrome P_{450} in liver microsomes and thus differs from phenobarbitone. Hohenwallner, et al. (35) showed that eucalyptol given either by aerosol or subcutaneously produced a marked increase in the activity of rat liver glucuronyl transferase. Sodium phenobarbital showed the same effect.

Von Skramlik (36) determined the sensory effects of essential oils following either inhalation, oral administration or application to the skin. All essential oils had an effect on the sense of smell to a differing degree. As far as taste was concerned these effects were limited to a sensation of bitterness of sweetness. Eucalyptus oil also produced a sensation of cold in the mouth and on the skin.

Bournot (37) reviewed certain aspects of taste and smell sensations. This author noted that in order to be smelled, a substance must reach the olfactory nerv ending in the nose. He also discussed the smell and cooling sensations of various essential oils. It was noted that inhalation not only effects sense organs but these oils are absorbed through the respiratory system and can exert their pharmacological effects in various parts of the body. It was further noted that eucalypt is bactericidal and acts as an irritant to the mucosa.

Eucalyptol and other essential oils facilitate narcosis of larvae by aiding the penetration of narcotizing substance according to Dastugue and Brun (38).

Antimicrobial Activity

In Vitro Studies

The phenol coefficient of eucalyptol was reported as equal to 1.0 by Wood (39). Penfold (40) also reports a phenol coefficient for eucalyptol. Rideal and Walker (41) reported that the phenol coefficient of eucalyptus was 1.6. In this partic publication, antibacterial activity as a function of surface tension was determined. Subrahmanyan (42) pointed out that the phenol coefficients will vary for eucalypt depending on the source of the oil. Blum and Blum and Fabian (43) tested eucalyptol against organisms which infect fermented food products. They found that

eucalyptol, as well as thymol, were not the best preservatives and not the worst of those tested.

Jerome and Lechat (44) also determined the phenol coefficient and conducted othe antibacterial tests with eucalyptol. They reported that 10% eucalyptol did not kill in 10 minutes.

In the use dilution test, 2.5% eucalyptol inhibited Brucella but had no apparent effect on Staphyloccus. The effect on Staphyloccus was apparently confirmed using a disc method which showed no inhibition from eucalyptol at 10%. These authors also determined the effect of vapors of essential oils on antibacterial activity. In respect to eucalyptol, it was reported that vapors from 12% dilution and greater were bacteriostatic toward Streptococcus and at 25% and greater bacteriostatic toward Klebsiella. Vapors from pure eucalyptol inhibited Staphyloccus as well as E. coli. In respect to bactericidal activity the 100% vapors killed Streptococcus, Klebsiella, Staphyloccus and E. coli.

Maruzzella and Bloch (45) tested a series of antibiotics along with essential oil combinations for antibacterial effects. The essential oils tested included eucalyptus but individual data on the essential oils alone were not given. The same senior author and Sicurella (46) screened 133 essential oil vapors for in vitro antibacterial activity against 6 test organisms. It was noted that in general gram positive bacteria were more susceptible to vapors than gram negative bacteria. The organisms tested included E. coli, S. aureas, B. subtilis, Strep. faecalis, S. typhosa, and M. avium. In respect to eucalyptus oil rectified NF, he found it was active against E. coli, Strep. faecalis and M. avium. The method used in this investigation was to inoculate test organisms on solidified agar in petri dishes. Filter paper discs saturated with each volatile oil were placed in the center of the dish covers. The dishes were inverted and incubated

at 37° for 24 hours, (72 hours in the case of M. avium). The clear zones on the surface of the agar above the filter paper discs were measured.

A phenol coefficient of 3.4 as tested against E. coli was obtained in a study reported in 1919 (47). In this study, oils from various species of trees and several individual components were evaluated. The phenol coefficient was found to vary with the species and the composition of the oil but cineol, (now known as eucalyptol) was the most active constituent. Penfold and Grant (48) determined the phenol coefficient against S. typhosa of various commercial oils and isolated constituents. All tests were run as 1% solutions. The coefficient of oils from various eycalyptus species varied from 1 to 12, pure eucalyptol showed a coefficien of 3.5, other components of eucalyptus oil varied in their phenol coefficient from under 1 to 22.5.

Rideal (41) studied antibacterial activity as a function of surface tension. Using the Rideal-Walker phenol coefficient technique, the antibacterial activity against S. typhosa was determined. Eucalyptol, in this study, showed a phenol coefficient of 1.6.

Use by the Egyptians of essential oils for the preservation of mummies was noted in a study by Collier and Nitta (49). Eucalyptus, along with other essential oils, was tested by these workers for bactericidal activity against various species using a use-dilution method. Eucalyptus was bactericidal against Streptococcus at a 1:200 dilution, against E. coli at a 1:200 dilution; and against Vibrio at a 1:1000 dilution. Miller (50) also used a phenol coefficient test against S. typhosa. She found that eucalyptol killed this organism within 2.5 minutes at a 1:50 dilution and within 15 minutes at a 1:250 dilution. The phenol coefficient as obtained (using the Tanner method) in this study was 1.44.

The antibacterial action of aromatic chemicals including eucalyptol was studied by Mashimo (51) et al. The dilution of eucalyptus oil required to kill Staphyloccus and the other organisms tested, was around 1:1000.

Maruzzella and his co-workers reported the antimicrobial activity of essential oils including eucalyptol in several studies. In the first study (52) eucalyptol was tested against 11 bacterial species using a filter paper disc method. The essential oil was found to be active against 6 species, namely, S. lutea, B. mesentericus, S. marcescens, E. coli, B. subtilis and Ps. vulgaris. Eucalyptol was also tested against 18 species or fungi using the same technique (53). Eucalyptol was active against 4 species. This group (54) also tested combinations. It appears from this data that the combinations of eucalyptus and methyl salicylate was slightly less active than the individual components.

In Germany, Kellner and Kober have studied several essential oils for their potential use as room disinfectants. In a study of the anti acterial activity of vapors of these essential oils (55), eucalyptol was shown to be effective against all of the organisms studied. These organisms were E. coli, S. typhosa, Neisseria, S. faecalis, Strep. pyogenes, S. aureus, B. megatherium, E. diphtherie and c. albicans. These workers (56) also showed that eucalyptol was effective against these same organisms when tested in the liquid form. Further data has also been reported by this group which confirms the previous data (57).

Schurmann, et al (58) studied the correlation between antibacterial efficacy, peroxide number, and solubility. Eucalyptol demonstrated antibacterial activity in this study. The antibacterial efficacy of eucalyptol has also been reviewed by Goryaev (59).

Several essential oils were studied for antiviral activity by Dunham and MacNeal (60). Eucalyptol was shown to have some inactivating ability versus vaccinea virus. In this study, the antiviral activity was studied by inoculating onto chorio-allantoic membranes of developing chick embryos. Virus suspensions were mixed with the test substances, then inoculated. More recently, Kozhina and Korotkova (61) studied the effect of various preparations made from eucalyptus leaves on type A influenza virus. It was shown that certain extract suppressed virus propogation in chick embryos. The degree of activity was found to be directly proportional to the tannin content and primarily the alcohol soluble tannin fraction. The fractions were not chemically analyzed, however, so the presence or absence of eucalyptol, per se, was not given.

In Vivo Studies

As long ago as 1891, Miller (62) used a mouth rinse technique to measure the antibacterial activity of several essential oils. He found in regard to eucalyptol as well as other essential oils, that they were bactericidal but generally "slow." This author recommends a mouthwash of benzoic acid and saccharin. Henderson (63) recommends eucalyptol, as well as other essential oils such as menthol and thymol, as components of nasal sprays and drops for their antiseptic activity. Eucalyptol was also recommended as a component of mouthwashes for antiseptic activity by Prinz (64). This is also the view of Eichorn (65) who stated that the essential oils including menthol, thymol and eucalyptol are highly suitable for disinfection of the mouth and throat because of volatility and lipid solubility. This author points out that the vapors of these essential oils, including eucalyptol, are endowed with bactericidal action, therefore, good therapeutic effects are achieved with aerosols in laryngitis, pharyngitis, tracheaitis, bronchitis, etc.

Graff reported on a product (66) (Saliva-thymol) which contains eucalyptol as well as menthol and thymol. It also contains other essential oils. He reported antibacterial activity on the combonation but did not do tests on the components. Hunkirchen studied the effects of this product on oral inflammatory disease and found it to be effective (67).

Wyler, et al. (68) studies various preperative oral hygiene procedures for their effect in reducing the number of microorganisms aerosolized into the dental operatory. Listerine Antiseptic, which contains eucalyptol as one of its active ingredients, provided a significant reduction in bacteria when used as a preoperative rinse.

Efficacy

Reviews

In an interesting review, Thompson (69) discussed the history of the eucalyptus tree in California. He pointed out that these trees were planted in California in 1870's and 1880's to combat malaria, at that time thought to be due to miasma or a toxic gas. Thompson gives the background of the botany and the distribution of the trees in California. He cites the possible value of the vapors from the trees, but generally downgrades effectiveness as far as malaria is concerned.

As long ago as 1879, Fluckiger, et al (70) reviewed the history of essential oils. He pointed out that eucalyptol was first described before 1700, and states that it is occasionally administered internally as a stimulant, antispasmodic and diaphoretic and externally as a rubifacient. It is in frequent use. Wood (71) in 1882, gives an early report of toxicological effects primarily observational. He points out that eucalyptol is used in a similar way

as quinine as an antipyretic in fevers. He also cites its use in bronchitis. Somewhat later in 1891, Potter (72) states that eucalyptol is as powerful antiseptic, a stimulating expectorant and an efficient diaphoretic. Therapuetically according to Potter, it is an efficient stomachic, a useful stimulant and disinfectant to the mucus membranes.

Currier (73) cites eucalyptol as a valuable antiseptic. This author stresses prevention of sickness by mouthwash and nasal irrigation. Grosicki (74) cites eucalyptol as an antiseptic component of hemorrhoidal products. In a review of pharyngitis, Smith (75) mentions eucalyptol inhalation as a possible treatment for chronic sore throat. In his review of external analgesics for the APhA Handbook of Non-Prescription Drugs, Dickison (76) lists products with eucalyptol as active ingredients. For essential oils generally, this author states that they are valued as nasal decongestants. He also believes that their placebo effect (odor and feel) may be equal or greater than their physiological value

In a relatively recent review, Greenberg (77) stated that inhalation of eucalyptol as well as menthol and benzoine is still used in tracheitis, sinusitis, and bronchitis to clear air passages and lessen bronchospasm. It is the opinion of this author, that these administrations have no place in asthma therapy.

Barker, et al (78) reviewed the uses of eucalyptus in 1918. He pointed out that it was used for catarrhal conditions of the respiratory tract, as capsules, on sugar or with steam in a vaporizer. This ingredient was also used for chronic inflammation of the genital urinary tract, pulpalgal ulcers and as a counter-irritant in rheumatic disease. It also appeared to be valued as an antipyretic in malaria. Gelenthein (79) in reviewing the common cold in 1934, divided this disease into three stages. For the beginning stage, he advocated antiseptic instilled into the nose and also in the third stage (thick tenacious mucous) an

antiseptic douche of the nose. During the second stage (profuse, watery secretions) he recommends eucalyptol along with menthol and thymol in mineral oil inhaled into the nose as a soothing agent. In a review of mouthwashes, gargles, and lozenges in the British Dental Journal (80) it is stated that volatile oil such as eucalyptol have antiseptic activity but because of their irritant, rubefacient, and locally anesthetic activity, are used in relatively low concentration to avoid local reactions thus resulting in a poor antibacterial effect.

Studies

The effect of eucalyptol as well as other essential oils on the nasal mucosa was studied by Fox. Using an objective technique to measure decongestant activity (81) his study indicated that 5% eucalyptol in oil sprayed in the nose had no effect. It should be noted that this technique involved compression of the soft palate against the pharyngeal wall by the subject, this in order to close off the nose from the throat. An airstream was induced into one nostril and out the other. This technique is likely to give inconsistent and incorrect results. In another study, he (82) found that a 5% eucalyptol solution caused some deleterious changes in the mucus membrane of rabbits when applied directly in oil over periods of up to 9 months. It should be noted that liquid petrolatum used as carrier in this study also produced these changes but to a lesser degree.

Boyd and his co-workers in Canada (83,84,) have studied drugs and other conditions which effect the output and composition of respiratory tract fluid over a number of years. In these studies eucalyptus

oil, as well as other volatile oils were administered via steam inhalation to rabbits and respiratory tract fluid collected. Some volatile oils were found to increase volume and decrease specific gravity when added to a steam vaporizer in normal doses. Larger doses were required for eucalyptus oil but these larger doses led to local inflammation and several animal deaths. This was apparently due to the large volume of ethanol inhaled. Inhaled eucalyptus oil had no mucotropic action at any season in rabbits, and there was no augmentation of the volume of respiratory tract fluid. Later, this group administered eucalyptol by stomach tube to urethanized animals (Boyd and Pierson (85). Eucalyptol was shown to be expectorant in rats, guinea pigs, rabbits, cats and dogs. The effect was not influenced by section of the afferent gastric nerves from which the author concluded that eucalyptol does not act reflexly on the stomach but directly upon the secretory cells of the respiratory tract.

Kramerenko (86) used a eucalyptus suspension for therapy of acute and chronic purulent disease.

EUCALYPTOL

ANIMAL TOXICOLOGY STUDIES

CARCINOGENICITY STUDIES

Eucalyptol was one of 41 food additives from the GRAS list tested for carcinogenicity potential using a mouse pulmomary tumor system developed by Andervont and Shimkin. Fifteen/sex/group, A/He, 6 to 8 weeks old mice were administered eucalyptol intraperitoneally, 3 times weekly, at doses of 100 or 500 mg/kg/day for a total of 24 doses. Twenty-four weeks after the first injection, the mice were necropsied and the lungs were examined grossly and microscopically. In addition the liver, kidneys spleen, thymus, intestine, and salivary and endocrine glands were examined for abnormalities. Tumor incidences were compared between treated and control (untreated and water vehicle) groups.

The incidence of pulmonary tumors in mice given eucalyptol was comparable to the controls, therefore, eucalyptol is not regarded as a pulmonary carcinogen in this test. (1)

REFERENCES

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Menthol is also known as 3-p-menthenol, l-menthol, hexahydrothymol, and "peppermint candy." Its formula is $C_{10}H_{20}O$ and has a molecular weight of 156.26. It is obtained from peppermint or other mint oils or synthetically. The melting point is 41 to 43°C. and boiling point is 212°C, Menthol is slightly soluble in water; very soluble in alcohol and liquid petrolatum, (1, 2, 3, 4, 5).

The food additive status of Menthol as a flavoring agent is noted in the Code of Federal Regulations.

ANIMAL TOXICOLOGY

Acute and Subacute Studies

Macht (6)studied menthol and its isomers in several animal and plant models. In general, he found that the l-isomer was more active than 3-menthol. In the mouse he found that 2000 mg./kg. intra-peritoneally is the lethal dose. In the rat, 1500 mg./kg (i.p.) was lethal. In the cat he found that 34 mg./kg. given intravenously was lethal.

No systemic or local toxicity was shown for peppermint oil (which contains 60% menthol) by Rob and Field (7).

Hazard, et al (8) reported that the rate LD_{50} (by i.p. injection) for natural menthol was 785 mg/kg and for synthetic, 670 mg/kg. In the g.pig the figures were 860 mg/kg for natural and 865 mg/kg for synthetic. In an earlier paper, Wokes (9) reported that in the mouse the LD_{50} was between 3 and 4 gm/kg for each of the isomers, l-menthol, dl-menthol, dl-neomenthol and dl-isomenthol.

In a comprehensive report, Jenner, et al (10) reported on the acute oral toxicity of several food flavorings. The LD_{50} of menthol in the rat was determined to be 3180 mg/kg. The strain of rats used was Osborne-Mendel. An LD_{50} of 3400 mg/kg has also been reported (1).

Flury and Seel (11) compared the toxicity, pharmacological efficacy, and odor and taste of several isomeric synthetic menthols with natural menthol. Seel (12) also reported on the comparative pharmacology of natural and synthetic menthol In this paper, he reported that natural menthol was approximately twice as toxic as any

of the synthetic menthols except neoisomenthol when injected as an oil solution in frogs. The same order of toxicity was also found in white mice and cats. For all menthol preparations tested there was first a transient rise in excitability as well as an elevation in the frequency of respiration and pulse rate, then progressive paralysis. Death was due to paralysis of the respiratory center.

Rakieten and his co-workers (13) studied the subacute effects of menthol vapor, particularly on the upper respiratory tract. Rats were exposed to a variety of menthol vapor concentrations over a period of several months. Inhalation of menthol vapors in a range of concentrations below 0.275 ppm resulted in no toxic effects as determined by growth curves, food consumption and hematology. Examination of tissues showed no significant changes in the mesopharynx, heart, spleen, liver, kidney, eye, skin, ovaries, testes, skeletal muscles and brain as compared to control animals. Animals exposed to the highest menthol concentrations showed changes indicative of lung irritation. Transient erythema of the conjunctiva was exhibited by some animals exposed to menthol at all concentrations.

Special Studies

Jerome and Lechat (14) reported that the cutaneous tolerance of menthol in young animals was satisfactory. They also tested the toxicity of menthol vapors in the rabbit and instilled the oil in the nasal passages. Toxicity was not noted with either of these applications. In a review of the literature on toxicity, mostly in the area of misuse, the authors concluded that it was safe to apply menthol directly in the nose in children above 30 months of age.

Shapiron, et al, studied the effect of menthol containing ointments on wound healing in guinea pigs (15). Camphor was also a component of the test ointment used. It was found that menthol and camphor slightly retarded wound healing in young animals and slightly accelerated it in mature animals. The differences were very small, however.

HUMAN SAFETY

Reviews

Arena (16) reviewed the toxicity of menthol and other essential oils. For essential oils in general he notes their use as skin irritants and that ingestion of 15 ml. of a volatile oil has been known to cause fatal poisoning. Menthol's toxic effects are listed as severe abdominal pain, nausea and vomiting, dizziness, staggering gait, slow respiration, flushed face, sluggishness, sleepiness, and in large amount in children, coma, produced. The treatment suggested is to discontinue use.

There have been several reviews published concerning the safety of menthol. In 1950, Griggs (17) reviewed menthol from a homeopathic standpoint. He described headache, pain around the eyeballs, nasal catharrh, fever blisters in the mouth, and a number of other pains and irritations which could result from the ingestion of menthol. Hewlett reviewed 12 cases of lipoid pneumonia, which were alledgedly due to misuse of oil-based medications containing menthol (18). In Deichmann's text on toxicology (19) it is stated that excess ingestion or inhalation of mentholated products has caused abdominal distress and CNS depression. He cites the treatment as symptomatic.

Samitz (20) in reviewing occupational dermatosis in dentists and allied personnel, reports that menthol, as well as other individual essential oils, are among the less frequent sensitizers. In contrast, menthol is cited by Adams (21) as a skin irritant often found in o.t.c. medications.

Studies

Lesoine (22) described adverse reaction in infants to pharmaceutical preparations containing menthol. He stated that on occasion these reactions involve cyanosis which could lead to death. Other symptoms of menthol poisoning cited by this author included conjunctivitis, tinnitis, erythema, eczema, dermatitis and symptoms of renal damage. Lesoine recommended that a warning be placed on the label of menthol preparations to avoid use on infants and small children.

In contrast, Brueninger, et al (23) surveyed approximately 124,000 infants who received nasal drops containing essential oils, including menthol. No untoward effects were noted. Nasal application of small concentrations, particularly menthol, caused reproducible reactions in breathing and circulation in infants. This reaction did not occur in older children and adults. It was the author's conclusion that although there is no indication for nasal application for these essential oils in infants there is also no safety problem.

Rudzki and Kleiniewska (24) reported on a survey of 1440 patients. In the case of menthol, sensitivity was found more frequently in long term than in short term patients. In 1070 tests using menthol in vasoline, 0.9% showed a positive reaction of some type although none of them were "fully positive." In 229 patients who used menthol less than one year, 0.8% showed positive reactions. Of 313 patients using menthol from 1 to 10 years, the percent of positive reactions was 0.6. For those patients having used the product more than 10 years, in a sample of 144 3.4% were positive.

Case Reports

Several cases of menthol toxicity have been reported. These include two cases of ottitis media resulting from mentholated petrolatum applied to the nose accumulating in the middle ear (Helmus 25 and Dysart 26). Randolph (27) reported that the case of a 26 year old woman who suffered severe headache and nausea as a result of smoking mentholated cigarettes. The author believed this to be a case of individual hypersensitivity rather than a general menthol toxicity. Naus (28) reported that workers in a shop preparing menthol sweets were found to have undergone an abnormal diminution of the smell acuity.

Miscellaneous case reports include stomatitis aphosa following the ingestion of menthol candies (Ochsenius, 29) lipoid pneumonia due to overuse of a product containing both menthol and eucalyptol (Cohen and Schoene, 30), keratitis from a clear gel containing menthol, thymol, and eucalyptol (Dahl, 31), glottal edema (Salatino 32), and methemoglobinemia due to a rectal suppository containing menthol (Hughes, 33). In this latter case, the author stated that benzocaine was the causative agent. Champeau (34) reported a case of a 4 year old female who ingested 6 mg. of menthol and showed intoxication but recovered without consequence. Bruening reported on a fatal intoxication from a menthol-camphor preparation in the nose of an infant (35). In a review of drugs used in pediatric otorhinolaryngology, Reitlinger (36) cited cases of menthol toxicity, including fatalities.

Gronemeyer (37) reported a case of an allergic reaction which appears to be confirmed by the data. Walbott (38) in reviewing therapeutic bronchoscopy and asthma suggested that menthol not be used for endoscopic application due to the possibility of sensitization. Gutmann (39) states that essential oils including peppermint (which contains menthol) can act as allergens.

In a comprehensive case history, Fischer-Wasels (40) reports a fatal shrinking of the lungs from use of menthol oil. In this case, a patient had regularly put 1% menthol in her nose for relief of nasal irritations over a period of several decades. This patient developed difficulty in breathing, thickening of the lungs, slow progressive lung disease, and finally, death. Autopsy showed hard knots in the hilus of lung and oil deposits in alveoli. As much as 10% of the weight of lungs consisted of paraffin oil deposits. No evidence was found that this large amount of menthol had caused any malignancy nor spread to parts of the body other than the lungs. It may be that the paraffin oil was more involved than menthol in this case. Bettecker (40a) reported on two non-fatal cases in which babies aged 12 days and 4-1/2 months presented symptoms of menthol poisoning, specifically laryngospasms, cyanosis and dyspnea after treatment with menthol ointments containing 2.7% and 1% menthol, respectively. The physicians concluded that these preparations should not be recommended for babies and should include a warning against nasal application.

An interesting commentary on the toxicity of menthol appeared in the German scientific literature in 1966. (40b) This concerned the Drug Commission of Germany Physicians' retraction in 1966 of a warning on the use of menthol-containing preparations in babies and young children. In this report the author claims that the original warning in 1964 was due in part to reports from a single physician who had a "menthol phobia" and erroneously attributed many reactions to this compound. The author also noted that any volatile oil or even non-volatile oil or aqueous liquid incorrectly applied directly to the nasal mucosa of infants may cause a respiratory reaction.

Sezary and Tanret reports on the desensitization (41) of a 24 year old female found having a reaction to tincture of benzoin and tincture of eucalyptus but not menthol. Menthol is mentioned as the precipitating agent in cold urticaria by Lobitz (42). Menthol was reported by Laugier, et al (43) to be implicated in some cases of keratosis.

Papa, et al (44) reported a single case of urticaria in a 31 year old female due to menthol with apparent confirmation by oral and skin challenge. This patient used peppermint toothpaste, peppermint candy, and an ointment containing menthol. Urticaria due to menthol has also been reported by McGowan (45). In this case, an 18 year old girl who smoked menthol cigarettes, ate menthol cough drops, used menthol ointments and an aerosol room spray with menthol, responded to the oral challenge of menthol.

Bickers (45a) reported on the unusual case of a 48-year old female suffering from hereditary hepatic porphyria which he claimed was exacerbated by the woman's ingestion over an extended period of time of a mouthwash containing aromatic oils (menthol, thymol, eucalyptol, methyl salicylate) and alcohol. The author conducted avian experiments which showed that the mouthwash and particularly the ingredient eucalyptol were capable of inducing production of the rate limiting enzyme delta-aminolevulinic acid (ALA) synthetase and the symptoms of the above disease. The individual aromatic oils other than eucalyptol did not have any ALA synthetase activity.

Finally, Heistein reported a case of hypersensitivity to menthol (46) resulting in purpuric eruptions on the hands, arms, forearms, etc. This apparently was due to smoking mentholated cigarettes.

PHARMACEUTICAL AND CHEMICAL STUDIES

Wood (47) determined the solubility in alcohol of menthol as peppermint oil and other essential oils. He found that menthol was not particularly soluble. A rapid gas chromatographic technique for quantitative analysis of menthol in pharmaceutical products is reported by Bahjat (48). The chemical and physical properties and tests for impurities in menthol are described in U.S.P. XVII (49). Rhode (50) studied the relationship between the solubility and capillarity and hemolytic effects of menthol and several other essential oils. No particular relationship was shown. For menthol, he reported that 1.0 mM of menthol (0.15 gms/liter) had a water solubility of 2.7. Kabasakalian reported on a study of benzocaine incompatability in throat lozenges (51) in which menthol was shown not to be involved in the instability.

ABSORPTION, METABOLISM, PHYSIOLOGICAL AND PHARMACOLOGICAL EFFECTS

Absorption

Masaki and co-workers studied the factors which influence menthol resorption in the digestive tract (52). Menthol was administered rectally to rats by Grisk and Fisher (53), with menthol subsequently being detected in the lungs. The authors discuss whether the levels found are pharmacologically active but draw no conclusions. Boyner, et al (54) studied an ointment containing menthol and other volatile oils in a petrolatum base. This ointment was placed on a dog's chest and the menthol was detected in the bronchial air 12 hours after application.

Metabolic Studies

Hohenwallener (55) reported that menthol did not produce the marked increase in the activity of rat liver glucuronyl transferase that was observed with eucalyptol. Jori (56) studies the effect of a number of components of essential oils on drug metabolism in rats. Menthol did not increase the in vitro liver metabolism of the several compounds tested. Popper and Delahuga (57) used menthol or a low protein diet to reduce hepatic glycogen which in turn, aggravated fatty lesions due to amino acid deficiency.

It was shown by Elder, et al (58) that menthol exhibits progesterone-like activity in its inhibitory action on liver and kidney aldehyde dehydrogenase activity, in vitro. Under certain circumstances this activity is reflected in an increased rate of oxidation of d-dalactose. Elder also reported that two prepubertal congenitally galactose deficient patients showed increased levels of d-galactose oxidation after treatment with menthol.

The effect of several constituents of essential oils on the rate and activity of drug metabolism in rats was studied by Jori, et al (59). He showed that menthol is inactive in affecting pentobarbital metabolism. Woods, et al (60) using a test chamber, showed that menthol accelerates conditional hypoglycemic response. Menthol was also used in a test procedure to assess glucuronide formation (Arias (61).

Pharmacology

The pharmacology of menthol and its isomers was compared by Macht (6). He found that in the cat the IV lethal dose depressed the respiratory center. In the rabbit, an oral dose of 3 cc/kg. impaired kidney function; at 1.5 cc/kg. dose (oral) this species showed some impaired kidney function but function was recovered after the drug was withdrawn. In the rabbit, a 2.5 cc/kg. I.V. dose also impaired liver function. Macht found, using isolated smooth muscle that menthol had a depressant effect at an application rate of 0.1 cc/2kg. cat. CNS effects were also studied. Excitation was noted which was followed by depression. No convulsive activity was observed although the chemically related camphor shows strong convulsive effects. Macht also showed that menthol was readily absorbed through the skin. Local anesthetic activity was confirmed by application to rabbit eye, frog skin and by intradermal injection to guinea pigs using electrical stimulation as a model. It was found by Heathcote (62) that menthol depressed the isolated heart of the frog and rabbit directly by its action on cardiac muscle. Menthol was also shown to dilate coronary vessels.

Using a frog esophagus model, Das, et al (63) determined that menthol stimulates ciliary motility. A saturated solution was used in this study.

Rakieten (64) studied the effect of menthol on excised ciliated respiratory epithelium from humans, rats and rabbits. He found that menthol solution (0.04%) had no toxic effects compared to Ringer's solution. Some of the conflicting reports in the literature on the effect of menthol on the vascularity of the nasal mucosa were reviewed by Rinaldi (65). This author used rhinomanometry to determine the resistance offered by the nasal fossae to the current of air. His results indicated that spraying with 1% mentholated oil under the nose caused an increase in the thickness of the nasal mucosa as a result of a vasodilitation phenomenon. This result differs from the widespread opinion that menthol has a vasoconstricting action. The effect of menthol on the nasal mucosa was also studied by Szabon, et al (66). In this in vitro study, paraffin plus menthol was compared with paraffin alone. It was found that paraffin alone inhibited ciliary activity but less so than menthol. The data given in this report, however, is not particularly complete and the differences between menthol and paraffin are rather inconclusive.

Le Bourhis and Soenen (66a) studied the psycholetptic effect of several aromatic compounds in the mouse and found that the psychotropic activity of menthol is weak especially when administered orally. The authors further concluded that any such activity is always transient and would most likely not have any effect in man at the concentrations normally found in foods or drink.

Sensory Effects

The effects of menthol on skin, nasal and oral mucosa was studied by Bliss et al (67). When applied to the skin, menthol caused an intense and lasting cooling sensation followed by numbness, with a slight smarting sensation and hyperemia. Local irritation did not proceed beyond the rubefacient stage. In this study, a 20% oil solution was rubbed vigorously in the skin. The application of a 0.5% solution directly to the nasal and oral mucosa was irritating. However, a 0.2% solution was non-irritating and most subjects administered a solution found it non-irritating.

The effect of menthol and other essential oils on taste and odor detection has been studied by several investigators. Bornout (68) reviewed taste and smell sensation and noted that to be smelled the substance must reach the olfactory nerve. He also points out that the smell and cooling sensations of menthol are separate phenomena. He further notes that inhalation not only affects the sense organs, but menthol is also absorbed through the respiratory system and can exert its pharmacological effect in various parts of the body.

Skouby, et al (69) applied menthol at a dose of 4 mcg/ml in 0.9% saline directly into the nose. These workers found that this application decreased the smell threshold by 21 to 50%. These same workers (70) noted that menthol also decreased taste perception at low concentration levels but increased taste perception at high levels. In this study, sensitivity was measured by measuring the galvanic current required to produce an acid sensation.

The sensory effects of essential oils were also studied by Von Skramlik (71) following either inhalation, oral administration, or application to the skin. It was found that all essential oils including menthol have an effect on the sense of

smell. He noted that peppermint oil (which contains menthol) produced a sensation of cold in the mouth and on the skin.

Electrophysical measurements on the lingual nerve of cats and dogs in response to thermal or electrical stimulation of the tongue was studied by Hensel and Zotterman (72). Menthol was shown to produce cold response even when the tongue is maintained at constant warm temperature. When temperature of the tongue is lowered, menthol greatly increases the frequency of cold response. The author surmises that the cooling effect of menthol involves an enzymatic response of cold receptors to menthol.

Hellekant (73) used an electrophysiological technique to study the effect of menthol on taste receptors in cats. He found that menthol in moderate concentrations stimulates response to sodium chloride, acetic acid, and sucrose, among other substances. However, at greater concentrations or during prolonged exposure menthol paralyzes these receptors. In a rat study (using peppermint oil) Phillips (74) showed a positive effect in investigating a hypothesis related to intracranial self-stimulation (facilitation by peroral sensory stimulation).

ANTIMICROBIAL ACTIVITY

In Vitro Studies

The antibacterial activity of menthol alone and in combination with other essential oils has been reported over a period of many years.

In 1930, Collier and Nitta (75) noted that essential oils were used by the Egyptians for the preservation of mummies. These workers tested several essential oils for bactericidal activity against various strains utilizing a use-dilution technique. These workers found that at a dilution of 1:400 menthol was bactericidal versus *Streptococcus*; at 1:200 versus *Staphylococcus* at 1:400 versus *E. coli*; at 1:1800 versus *Vibrio*.

Miller (76) conducted the phenol coefficient test using *S. typhosa* and found that a 1:250 dilution of menthol killed in 2.5 minutes and a 1:750 dilution in 15 minutes. The phenol coefficient obtained in this study (using the Tanner method) was 5.1.

Using the Rideal-Walker (RW) method of determining germicidal activity (modified phenol coefficient), Rideal found that synthetic menthol had a RW coefficient of 0.9 and natural menthol a RW coefficient of 0.4; i.e. synthetic menthol had 90% of the germicidal activity of phenol while natural menthol had 40% of the phenol activity (76a).

Kellner, et al (77) studied the antibacterial activity of many essential oil vapors versus various species. Menthol was found to possess activity against E. coli, S. typhosa, Neisseria, S. fecalis, Strep. pyogenes, S. aureus, B. megatherium, C. diphtheriae, and O. albicans. Against E. coli, natural menthol was not active but synthetic menthol was. These same workers (78) studied the effects of menthol as the oil against the same group of bacteria. Menthol was active against S. fecalis, S. pyogenes, S. aureus and C. diptheriae.

The antibacterial activity of various aromatic chemicals has also been reported by Mashimo, et al (79). Menthol was effective in dilutions of approximately 1:1000 versus Staphylococcus and other organisms. The antibacterial activity of menthol againts 75 strains of Brucella was studied by Zeetti (80). Menthol exerted a bacteriostatic action of varying intensity depending on the Brucella species tested.

Menthol was shown to lyse several species of bacteria but not others by Pacheco and Kosta (81). Among the species lysed were Brucella melitensis, Shigella dysenteriae, Strep, pneumoniae and B. subtilis A. Aerogenes, S. typhosa and several others were not lysed by menthol.

The antibacterial activity of menthol and other essential oils has also been studied by Jerome and Lechat (14). In respect to phenol coefficient they found that 3% menthol did not kill in 5 minutes but that 10% menthol killed in 10 minutes. In the use dilution test, 2.5% menthol prevented Staphylococcus growth; 1.2% menthol inhibited Klebsiella. Using that same test method, menthol was shown to inhibit Staphylococcus at concentrations of 10 and 25%.

In a later study, Collier (82) studied the efficacy of essential oils in killing several bacteria. Oil of peppermint (with menthol) was found to be effective against Streptococci, Gonococci, and Vibrio at dilute concentrations (approximately 1:1000) but ineffective against Staphylocci and E. coli at concentrations of 1:100.

Based on these reports of antibacterial activity, Henderson (83) recommended menthol, as well as other essential oils, as components of nasal sprays and drops for their antiseptic activity. Of interest is the study by Wokes (9) who studied the antibacterial activity of several menthol isomers. In this study, phenol was used as a control to compare the antiseptic activity against B-coli columnus and S. aureus. Against B-coli, natural menthol was 10 to 12 times more effective than phenol. The other menthols showed slightly greater activity than l-menthol. Against Staphylococcus, natural l-menthol was about 8 times more active than phenol; the other isomers again being slightly more active than the natural menthol.

Gershenfeld (84) reviewed the literature on the antibacterial action of menthol and menthol containing oils. He also carried out a study showing that 1% menthol, 1% camphor, and a mixture of 1% menthol with 1% camphor (all in petrolatum) showed no bacteriocidal effect when tested against S. aureus and B typhosus. However, he showed that saturated aqueous solutions of menthol were bactericidal against these two organisms while bacteriastatic action was displayed against B-coli.

Dunham, Wolcott & MacNeal (85) tested several essential oils by inoculation onto the chorio-allantoic membranes of developing chick embryos, using vaccinia virus. Virus suspensions were mixed with the test solutions, then inoculated. The virus retained activity after mixing with ethanol but was somewhat inactivated by menthol as well as the other essential oils tested.

Of interest is Jerome & Lechat's study (14) of the antibacterial activity of menthol vapors. In these studies, bacteria were exposed to the vapors for 24 hours. In measuring bacteriastatic activity, they found that menthol concentrations of 6% and higher produced vapors which prevented growth of Streptococcus whereas it required vapors from 12% solutions or higher to prevent growth of Klebsiella. In the case of Staphylococcus it required solutions of 25% and with E. coli, 100%. Bacteriacidal activity of the vapors were as follows: Streptococcus was killed at 12% solutions and above. Klebsiella at 12% or above. Staphylococcus at 25% and above, and E. coli 100% and above.

Vapors of 133 essential oils were screened by Maruzzella, et al (86) for antibacterial activity using test organisms. The organisms tested were E. coli, S. aureus, B. subtilis, Strep. faecalis, S. typhosa, and Microbacterium avium. The method used was to inoculate agar in petri dishes with test organisms. Filter paper discs were saturated with each volatile oil and placed in the center of the dish covers. Dishes were inverted and incubated at 37° for 24 hours (or for 72 hours in the case of M. avium.) The clear zones on the surface of agar above the filter paper discs were measured. Natural peppermint oil vapors were active against S. aureus, B. subtilis, and M. avium.

The antibacterial activity of menthol vapor has also been studied by Grubb (87). In this case, the effects were tested by inoculating solidified agar with the test organism. Those were inverted and incubated with the material to be tested held in the cover by a cup. Menthol showed 5 to 25% growth inhibition versus S. aureus.

Experiments in white mice were carried out by Kato and his associates to find out whether certain topical drugs were capable of inducing phagocytic activity in endothelial cells of the skin capillaries (87a). Menthol did not produce any such activity.

Myers (87b) studied a series of common volatile oils for in vitro fungicidal activity against various yeasts and yeastlike organisms. Thymol was found to be the most active compound possessing marked antifungal activity. Menthol was not similarly active.

In Vivo Studies

In 1891 Miller (88), using a mouth rinse technique with menthol (in peppermint oil) showed antibacterial but generally "slow" activity. Wood, in his study of volatile oils in alcohol (47) stated that oil of peppermint was feeble as an antiseptic.

In a review of the common cold, Barnett (89) advocated antibacterial treatment including the painting of the throat with an antiseptic menthol solution. Menthol's utility as an antiseptic is mentioned by Brockhaus (90). In his review of antibacterial agents for the mouth and throat, Eichorn (91) states that ethereal oils

such as menthol are highly suitable for disinfection of the mouth and throat because of the volatility and lipid solubility. He also points out that their vapors are endowed by bactericidal action therefore good therapeutic effects are achieved with aerosols in laryngitis, pharyngitis, tracheaitis, bronchitis, etc. In a recent publication, Hall (92) studied a topical acne product in which menthol was included in both the active product and the placebo. The active product was more effective.

Prinz and Greenbaus (93) recommend menthol, as well as other essential oils as components of antiseptic mouthwashes.

Jerome & Lechat (14) also reported a crossover study in infants comparing anti-bacterial activity of menthol with tyrothricin. Menthol was found as active as tyrothricin towards the infections studied. They also observed that menthol liquified the secretions and showed expectorant activity.

Graf (94) studies a product containing various essential oils including menthol for antibacterial activity. No data was given on individual components. This same product was shown by Hunkirchen (95) to be useful in showing relief of inflammatory changes in the mouth.

Currier (96) in 1924 cited menthol as a valuable antiseptic. This author stressed prevention of sickness by mouthwash and nasal irrigation with solutions containing menthol as well as other essential oils. Grosicki (97) cites menthol as an antiseptic ingredient in hemorrhoidal products.

Of several evaluations of menthol-containing products in antibacterial studies, Jones. et al (98) studies a mouthwash which contained menthol and thymol, phenol, sodium phenolate and sodium borate. This mouthwash was compared with saline and no treatment for the control of bacteremia associated with tooth extraction. The mouthwash was significantly superior to saline and was recommended for routine use. In a continuation of this study, Cutcher, et al (99) observed a 30.7% reduction in the number of bacteremias in those using the mouthwash as compared to those

using a control rinse. The irrigation plus rinse procedure was superior to rinse alone. Novick and Sodhi (100) compared this same mouthwash with placebo and penicillin. Throat cultures were taken and counts of S. hemolytycis were made. The mouthwash reduced counts of this bacteria 40 to 70% in 24 hours. Placebo had no effect, while penicillin reduced the count by only 17%.

General Efficacy Reviews

Several general references cite the utility of menthol. As long ago as 1879, Fluckiger (101) described menthol as a component of peppermint oil. This text states that a water or alcohol solution of peppermint oil is a "grateful stimulant" and a frequent adjunct to other medicines. In 1891, Potter (102) mentioned menthol's antiseptic activity and described it as locally anesthetic and acting as a vascular stimulant. This author cites menthol as useful in external application for neuralgia and as an antiseptic. In more recent times, The Medical Letter (103) stated that menthol in anesthetic sprays and wipes, because of its cooling effect along with its anesthetic activity is useful in products for temporary relief of itching. In the chapter on external analgesics by Dickison (104) for the APhA Handbook of Non-Prescription Drugs the author states that menthol has mild anesthetic and counter irritant actions, that it stimulates nerves for perception of cold but depresses pain reception. In describing the utility of essential oils generally, Dickison states that the placebo effect, odor and feel, may be equal or greater than the physiological value. He also notes that some essential oils including menthol, are valued as nasal decongestants.

Lamy and Rotkovitz (105) reviewed the common cold and its management. Menthol was mentioned in the context of its use in room sprays and its anesthetic action was acknowledged. However, the authors question whether it is possible for patient to get a large enough dose from a room spray. Finally, the utility of menthol is cited in Merck Index (3), U.S. Dispensatory (4) and Remington's Pharmaceutical Sciences (5).

Gellentheien (106) in his early review of treatment of the common cold divided the cold into three stages. For the second stage, consisting of diffuse watery

secretions, he recommends menthol, as well as thymol and eucalyptol in mineral oil inhaled into the nose as a soothing agent. In discussing a controversy over gargling, Lieberman (107) mentions the use of mentholated solution for the prompt relief of sore throat pain. Also, in a relatively early editorial review of the common cold, Perlman (108) mentions menthol as a component of medicated vaporizers but condemns these as not being particularly useful.

Analgesic, Anesthetic, Antipruritic Efficacy

Many materials were surveyed for topical anesthetic activity in human subjects by Tainter, et al (109). Subjective techniques with proper controls were used. 5% menthol in ethanol was shown to be very effective but irritating with some sloughing of tissue noted. Dastugue and Brun (110) found that menthol and other essential oils facilitate narcosis of larvae by aiding penetration of the narcotizing substance.

White, et al (111) recently studied the topical analgesic effect on induced muscular pain of an ointment containing menthol and methylsalicylate. The subjective response was also determined. In this study it was found that neither the active drug nor the placebo altered skin resistance. However, the active product decreased the muscle action potential whereas the placebo did not. The test ointment also produced a feeling of warmth and reduced pain whereas the placebo showed neither of these effects.

The cooling sensation of menthol when applied to the skin was confirmed by Von Czetch-Lindenwald (112). Menthol had no effect on the skin temperature however. Bluefarb, et al (113) stated in a review that menthol was effective as an agent to relieve itching by substituting a cooling sensation.

Itching, its causes and treatment has recently been reviewed by Beare (114). This author mentions menthol as one of the older therapies; the most frequently used and most generally accepted of the antipruritics.

In a review article Gellin and his associates (114a) reviewed the etiology and treatment of allergic contact dermatitis due to rhus dermatitis (poison ivy, poison oak and poison sumac). The authors

noted that most cases of pruritis accompany these conditions are amenable to treatment with lotions containing menthol, camphor and/or phenol.

The combination of menthol with lignocain and benzylalcohol was found to be effective and safe for a teething solution for infants by Seward (115). Menthol was included with methylsalicylate in a cream which also contained adrenalin, methylnicotinate and other components in a study of topical analgesics. This was an open study which showed good results by Bhandeare (116.).

In an objective study, Melton (117) injected histamine to produce itch. He showed that menthol in a variety of vehicles did not appear effective in controlling this itch. However, the author questions whether it was the method or the drug that was responsible. In the same area, Hardy (118) studied the effect on the pain threshold (heat stimulation) of several products; 2% menthol had no effect when tested on the forearm; however, neither did more potent anesthetics. The author concluded that this was not a good site to study. He did obtain good results with benzocaine using the lips and suggested this as a site. However, he did not report any data on the activity of menthol in this site. Freystadtl (119) notes that 10% menthol in alcohol relieves itch, but not by anesthetic action. Salter's textbook of pharmacology (120) described menthol as an antipyretic, analgesic and antiseptic. The efficacy of a menthol-penicillin inhalation in chronic tonsillitis is described by Surkin (121).

Laymon (122) states that menthol has been replaced as an antipruritic by steroids in his review of dermatitis of the hands. Also, in a recent study, menthol as a component of an ointment to control itch from fungal infection along with phenol, coal tar, calomine lotion and Lubriderm ointment was reported by Fromer (123). Harris (124) made a comparative trial of two expectorants used in general practice. Menthol is referenced as a component of Benylin expectorant. Menthol was added to calomine lotion by Singha, et al (125) to make it more soothing for the topical relief of Herpes simplex. Misra (126) conducted an uncontrolled study showing a good effect of a cream containing adrenalin, methylnicotinate, menthol, etc.

Menthol is included by Sulzberger, et al (127) as a component of a prescription for eczematous dermatitis. Menthol is also commonly found in many other dermatological agents, one of the more recent being a lotion indicated for the treatment of acne (127a).

Kasuga (128) showed a slight anesthetic effect of menthol using the guinea pig auditory meatus model. This investigator also reported that menthol enhanced the anesthetic activity of norceine.

Expectorant and Decongestant Activity

The use of menthol in the treatment of symptoms of the common cold or sore throat has been studied or cited in several investigations. Augustin (1) reported that workers exposed to menthol vapors had fewer colds. Watson (129) reviewed the diagnosis and treatment of various types of sore throat. This author advocated menthol as a component for use in a vaporizer for the relief of laryngitis. Greenberg (130) cites menthol as well as eucalyptol and benzoin as still being useful in steam inhalation in tracheitis, sinusitis, and bronchitis to clear the air passages and to lessen bronchial spasm. This author, however, does not believe that these ingredients have any place in asthma therapy. Menthol in a product also containing noscapine, phenieramin, phenylephrine, glyceryl guaiacolate and chloroform, was evaluated in upper respiratory infection by Goldberg, et al (131). In this study, which was double blind, the active drug was much more effective than the placebo. In a recent review, Smith (132) mentions menthol inhalation as a possibility in the treatment of chronic sore throat. Menthol, as well as thymol, are mentioned as components of nose drops and sprays (which are condemned) by Williams (133).

Boyd and Sheppard (134) administered menthol by steam inhalation to urethanized rabbits. The soluble mucous content was augmented and the specific gravity of the respiratory tract fluid lowered. This effect with menthol was produced with

doses of less than 1 mg/kg of body weight of rabbit as added to the vaporizer. The authors calculate that this corresponds to a systemic absorption of around 20 mcg/kg. These workers concluded that the effects were due to the direct stimulation of mucous secreting cells in the respiratory tract. They also noted that inhalation of larger amounts of menthol depressed the volume output and mucous content.

The effect of menthol on the mucous membrane of the nose was studied in two investigations by Fox. In the first of these (135) he used an objective technique to measure decongestant activity in the nose. This technique, which is subject to question, required the patient to compress the soft palate against the pharyngeal wall to close off the nose from the throat. An airstream was induced in one nostril and out the other. In this study, menthol in a concentratio as low as 0.5% decreased the airflow shortly after administration. Airflow was back to normal within 15 minutes. In the second study (136) Fox showed that 1% menthol caused a deleterious change in the mucous membranes of rabbits. In this latter study the drug was applied directly in oil for periods of up to 9 months. The liquid petrolatum used as a carrier also produced changes but the author notes that these were to a lesser degree.

Contrasting data was obtained by Noller (137) using an electronic technique to measure airflow after menthol instillation in human subjects. When the airflow from both nostrils was recorded after direct application of an ointment containing 2.82% menthol to 1 nostril (18 subjects) there was a temporary lumen constriction in 9 subjects in the nostril which had the application. After 30 to 60 minutes however, an increased airflow was observed which lasted several hours. The author notes that the initial swelling of the nostril which was expected based on the Fox studies was not thoroughly confirmed. Such swelling was noted also in 3 cases without menthol application apparently due to irritation of the nostril by the cotton swab.

In 3 children, menthol ointment was applied to the chest and back by Noller with one nostril remaining closed throughout the experiment except during measurement. Increased airflow was noted only in the open nostril up to 4 hours after administration. It was concluded that the effect of menthol was due to the vapor.

In 1908, Young (138) reported that menthol had several unusual internal uses. He reported complete or partial cure for Meniere's syndrome, vomiting during pregnancy, histeria, asthma, enteric fever, lack of appetite, seasickness. An earlier reference to the use of menthol in nasal congestion is that of Atkinson (139). This brief monograph described the use of menthol crystals applied directly to the nostril for the relief of nasal congestion. Somewhat later, Hirsch (140) reviewed animal studies showing synthetic menthol only slightly less toxic than natural menthol and reported a human study showing somewhat ambivalent response in ability to distinguish natural from synthetic menthol. The limited clinical study here showed the equivalence of natural and synthetic menthol in the treatment of diseases of the sinuses.

It was found by Butler, et al (141) that the inhalation of pure menthol increased airway resistance but the subjects reported they were less congested. The author believes this is due to the subject associating coolness with airflc However, he also states, based on other data in his article, "by the judicious use of menthol in small quantities, it has been possible to retain some of the pleasant odor of menthol without sacrificing any of the vasoconstrictor effectiveness of the amines." The inhalers which he studied contained menthol and several amines with decongestant activity. It may appear from this data that menthol at low concentrations has a decongestant effect.

MENTHOL

ANIMAL TOXICOLOGY STUDIES

CARCINOGENICITY STUDIES

1. Menthol was one of 41 food additives from the GRAS list tested for carcinogenicity potential using a mouse pulmonary tumor system developed by Andervont and Shimkin. Thirty/sex/group, A/He, 6 to 8 weeks old mice were administered menthol intraperitoneally, 3 times weekly, at doses of 25 or 100 mg/kg/day for a total of 20 doses. Twenty-four weeks after the first injection, the mice were necropsied and the lungs were examined grossly and microscopically. In addition the liver, kidneys spleen, thymus, intestine, and salivary and endocrine glands were examined for abnormalities. Tumor incidences were compared between treated and control (untreated and water vehicle) groups.

The incidence of pulmonary tumors in mice given menthol was comparable to the controls, therefore, menthol is not regarded as a pulmonary carcinogen in this test. (1)

2. Fifty/sex/group, young adult B6C3F1 mice and Fisher 344 rats, were orally administered dl-menthol by dietary admixture. DL-menthol was given at concentrations of 0 (untreated control), 2000 or 4000 ppm to mice and 0, 3750 or 7500 ppm to rats for 103 weeks. All surviving rats were killed at 105 weeks and all surviving mice at 104 weeks.

MENTHOL CONTINUED

Mean body weights of dosed rats and mice were only slightly lower than those of the corresponding controls. No unusual clinical signs, attributable to the administration of dl-menthol, occurred in either species at any dose level. Survival at the end of the study was at least 62% in all dosed and control groups of each species, therefore, sufficient numbers of animals were at risk for the development of late-appearing tumors.

The incidence of tumors in both mice and rats given dl-menthol was less than or comparable to their respective controls.

Under the conditions of this study, menthol is not regarded as carcinogen. (2)

3. Young female, Sprague-Dawley rats, were orally administered oxygenated [(-)-menthol] and other monoterpenes by dietary admixture at concentrations of 1% (initial study) or 0.5% (final study). In the initial study, the rats were fed the treated or control diets for two weeks prior to induction of mammary tumors by a single gastric intubation of 65 mg/kg of DMBA in 0.5 ml of sesame oil. The rats were maintained on the diets for an additional 18 weeks. In the final study, the diet was fed only for two weeks prior to and one week following DMBA administration. Beginning five weeks post-DMBA administration, the rats were palpated for mammary tumors and weighed at weekly intervals. The tumors were prepared and examined histologically.

More than 95% of the mammary tumors were carcinomas. Menthol-treated groups had a significantly greater latency period (median latency period of 80 days vs. 63 days for controls)

and the total number of tumors was less (average number of tumors/rat was 103 vs. 152 for controls).

Menthol acted as a chemopreventive agent in this model of tumor production. (3)

MUTAGENICITY

4. Menthol was one of 15 chemicals tested with and without metabolic activation by the sister chromatid exchange (SCE) assay and the chromosome aberration assay in Chinese-hamster ovary cells (CHO) to evaluate its mutagenic potential. Menthol was tested at doses ranging from 2.5 to 250 μg/ml in DMSO solvent.

No mutagenic potential was shown in either test for menthol. Therefore, based on this study, menthol was non-mutagenic. (4)

5. The Ames test was conducted in *Salmonella typhimurium* strains TA1537, TA1535, TA100, TA98 and TA97 with and without metabolic activation with rat liver S-9 fraction. Menthol was dissolved in DMSO and tested at concentrations of 6.4, 32, 160 and 800 μg/plate.

No mutagenic potential was demonstrated in the Ames test at any concentration tested, with or without the presence of metabolic activation. (5)

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METHYL SALICYLATE

Methyl salicylate is present in the leaves of several plants but is now usually prepared synthetically. It is also known as wintergreen oil, betula, sweet birch, or teaberry oil. The formula is $C_8H_8O_3$ with a molecular weight of 152.14. It melts at 8.6°C and boils at 220° - 224°C. This chemical is slightly soluble in water and is miscible with alcohol. The food additive status of methyl salicylate is noted in the Code of Federal Regulations.

ANIMAL TOXICOLOGY

Due, in part, to its chemical relationship to the widely used drugs sodium salicylate and aspirin, the toxicity of methyl salicylate has been extensively investigated. Also, up to most recent times, the presence in the home of oil of wintergreen as a flavoring agent and as a home remedy has led to misuse with resultant toxic manifestations. Currently, packaging regulations require items containing more than 5% methyl salicylate to be in "child-proof" packaging.

Acute Toxicity

The acute oral toxicity of over 100 synthetic and natural flavoring materials and related compounds were reported by Jenner, et al (1). The acute LD_{50} of methyl salicylate in the adult Osborne-Mendel Rat was determined to be 887 mg/kg. The LD_{50} obtained in the guinea pig by these investigators was 1060 mg/kg.

In an earlier report, Clarke, et al (2) reported an LD_{50} in mice of 1110 mg/kg. These authors further state that it appears in lower animal that the toxicity of methyl salicylate is essentially identical to salicylic acid. It is in their view, however, conceivable that in man the small proportion of unhydrolyzed ester may have a greater toxic action.

Lacroix (3) reported fairly detailed observational toxicology of methyl salicylate in conscious and anesthetized dogs. Hodge (4) in reporting on approximate oral toxicity in rats of selected household products published an estimated lethal range of wintergreen essence of 8 to 10 ml/kg. Castagnou, et.al. (4A) reported that the lethal dose of methyl salicylate in the rabbit was 1.80 grams/kg.

Subacute and Chronic Toxicity

Hagan, et al (5) reported on the subacute and chronic toxicity of several food flavorings and compounds of related structure. With methyl salicylate, no adverse effects were found in the following studies: 50 mg/kg fed to two male and two female dogs for two years; 500 mg/kg fed to 1 male and 1 female dog for 8 to 9 days; 1,000 parts per million fed to 10 male and 10 female rats for 17 weeks; and 1,000 parts per million fed to 25 male and 25 female rats for 2 years. Deaths were observed when 20,000 ppm were fed to 24 male and 24 female rats for 49 weeks. All animals died by the end of the feeding period. Excess cancellous bone, growth retardation and rough coats were noted. At a feeding level of 1200 mg/kg (to 1 male and 1 female dog) death occurred within 3 days. Weight loss and liver changes were noted. Intermediate findings were obtained at intermediate doses.

In 1962, Webb and his co-workers (6) reported further data on the subacute and chronic toxicity of methyl salicylate in dogs, rats and rabbits. In this study, no adverse effects were produced in rats fed methyl salicylate for two years at levels up to 0.21% of the diet. These workers extended their investigation (7). In this study rats received methyl salicylate at levels of up to 2% of the diet for up to two years. Statistically significant growth retardation occurred on the 1% and 2% diets. All rats on the 2% diets died by the 50th week. Administration to dogs of 500 mg/kg/day of methyl salicylate resulted in weight loss or death in all dogs. The livers of the dogs on the higher levels (1200 and 800 mg/kg/day) had moderate to marked fatty necrosis. Dogs receiving 350

or 150 mg/kg/day for 2 years had enlarged livers and enlargement of hepatic cells. These investigators also reported (8) that topical application, with rubbing, of methyl salicylate to the defurred skin of rabbits resulted in early death and kidney damage at doses from 4 ml/kg/day and higher.

Packman and co-workers (8A) reported no evidence of adverse effects in weanling albino rats after a two year chronic toxicity study using methyl salicylate. The rats were fed methyl salicylate at levels of 0.07% or 0.21% of the diet.

Reproductive Studies

The effect of methyl salicylate on rat reproduction has also been explored. In 1959, Warkany and co-workers (9) showed that congenital malformations could be induced in rats by salicylate poisoning of the mother while the embryos are in the early stages of development. These results were observed with methyl salicylate and with other salicylates as well. Smith, et al (10) injected methyl salicylate into pregnant Long-Evans rats. At doses of 0.1 to 0.2 ml. administered on the 9th, 10th and 11th day of gestation, methyl salicylate caused under development of the maxillary processes and failure of emergence of the nasomedial process in the young.

The effect on rat reproduction has been studied in detail by Collins and his co-workers (11) also. They initially reported possible dose related decreases in the average number of progeny per litter in the 2nd and 3rd generations in rats fed at up to 5000 ppm methyl salicylate for three generations. These results were reportedin greater detail in 1971 (12). There were no fertility decreases at any dose level of methyl salicylate fed. Osborne-Mendel rats were fed for 3 generations. However, at the 3,000 and 5,000 ppm levels, significant decreases were seen in average litter sizes; average number of live born progeny; average number of survivors to day 4 and average number of survivors to weaning. The decrease

seen in the number of live born progeny appeared to be dose related. External examination of the new-born and weanling rats from all the litters disclosed no gross abnormalities. In autopsies of the 3rd generation weanlings, the findings were negative.

The embryotoxic and teratogenic effect of methyl salicylate was confirmed in mice and rabbits by Szabo and co-workers (13). The malformations observed included cleft-palate, exencephaly, hydrocephalus, omphalocele and open eyelid.

Hoar, et al (14) and Woo and Hoar(14A,14B) studied the growth of fetal rat kidneys during late gestation in both normal and methyl salicylate treated rats. An attempt was made to differentiate between the retarded renal growth and hydronephrosis in methyl salicylate treated fetuses.

Gross, Fitzhugh, and Montell discuss studies of the effects of methyl salicylate in rat reproduction as an example of methodology for evaluating safety of food additives (15). Concepts originally developed for the safety testing of carcinogens are modified to apply to other toxic effects in food additives. Results on the interference of methyl salicylate with reproduction in the rat are used for illustrative purposes. The studies encompass three successive generations, two matings per generation, and dietary levels of methyl salicylate of up to 5000 ppm. The suggested procedure provides for making an estimate of the safe level of the additive in the food of the test animal used.

Other Studies

The relationship of salicylates to gastric hemorrhage and other gastrointestinal irritations is well established. In respect to methyl salicylate, Davison,

et al (16) confirmed the capability of methyl salicylate to cause irritation.

The relationship of methyl salicylate to myocardial toxicity and cardiovascular disease has been studied by Ojiambo. He reported (17) that dogs given a lethal dose of methyl salicylate had a several fold increase in body oxygen consumption over the normal. Within two hours after administration of the methyl salicylate, arterial lactate and potassium levels began to increase. These levels increased until death occurred. It is the author's contention that methyl salicylate interferes with the efficiency of oxidative phosphorylation. Ojiambo is of the further opinion (18) that reviews of the literature implicate methyl salicylate in myocardial changes. He found that small doses increase the rate and strength of muscular contraction of the heart whereas large doses decrease the strength of muscular contraction. The author further notes that there was a 59% rate of mortality among 43 cases of oil of wintergreen poisoning. Finally, this investigator reports (19) that a review of environmental factors in cardiovascular disease implicates methyl salicylate as an uncoupler of oxidative phosphorylation.

HUMAN SAFETY

Reviews

There have been several reviews of poisoning by misuse of methyl salicylate. Craig (20) in Scotland reviewed 50 cases out of a total of 74 deaths observed from misuse of volatile oils over a 20 year period. Three of these 74 deaths were attributed to methyl salicylate. He also cited data from England indicating that 54 out of 454 deaths from accidental poisoning

were attributable to volatile oils in this same 20 year period. Thirty-six of these 54 deaths were due to methyl salicylate. Craig further points out that methyl salicylate is a common cause of poisoning in children. Of the three cases reported here in detail, one involved a 25 month old girl receiving less than 1/2 ounce. Symptoms included acidosis and liver damage similar to that obtained in general with salicylates.

Salicylate intoxication has also been reviewed by Pierce (21). He states that salicylates are the most common toxic agent in childhood poisoning. These compounds are rapidly absorbed in the gastrointestinal tract, cause local G.I. irritation, stimulate the respiratory center, increase the metabolic rate and interfere with carbohydrate metabolism and the normal blood coagulation mechanisms. Pierce further states that emergency treatment must prevent further salicylate absorption by means of gastric lavage or induced emesis, correct water and solute deficits, and reduce serum and tissue salicylate levels through increased renal excretion or peritoneal dialysis.

Anderson (22) reviewed cases observed in a Nova Scotia Regional Poison Control Center over a 5-year period, from 1965 to 1969. In this period there were 45 accidental poisonings from methyl salicylate compared to 1,813 for aspirin.

Several cases of methyl salicylate poisoning in children have been reviewed by Cann, et al (23). In each case, accidental ingestion of methyl salicylate was from bottles marked, "Caution, keep out of the reach of children." The author discussed the probable course of events in the blood

plasma and kidney during salicylate intoxication. Several treatments are discussed depending on the degree of intoxication, and the length of time from poisoning. That the stomach should be emptied as quickly as possible is stressed. In more severe cases these authorities recommend hemodialysis. Gross and Greenberg(23A) reviewed cases of methyl salicylate poisoning from 1868-1946. Forty-seven poisonings from methyl salicylate are cited. Nine of these 47 poisonings are further described under individual case reports.

Methyl salicylate myopathy in man has been reviewed by Ojiambo (24). Among the symptoms he observed were tachycardia, elevated respiratory rate, and high blood pressure. He also observed high levels of potassium in the blood. In one of the cases described, the patient suffered extensive damage to muscle tissue which ultimately led to amputation of the lower leg. In the cases where methyl salicylate intoxication was fatal, autopsies showed that death was the result of congestive heart failure from degeneration and fragmentation of muscle tissue in the heart.

Samitz, et al (25) in their review of occupational dermatoses in dentists and allied personnel, cited methyl salicylate as one of the less frequent sensitizers.

Reviews on methyl salicylate toxicity have also been published by Sless (26) Gordon (27), and Pelino (28) who stated that methyl salicylate was more toxic than aspirin. The AMA published a review of the safety of flavored sweetened medications in which they cite methyl salicylate as an example of a flavor in candied sweets leading to abuse of products containing therapeutic levels of these agents (29).

Godfrey (30) presented data on the utility of sodium bicarbonate for treatment of salicylate poisoning. He also expressed the view that methyl salicylate is more toxic than aspirin. Decker, et al (31) stressed the use of activated charcoal in poisonings of this type. Finally, the value

of peritoneal dialysis in acute methyl salicylate intoxication is described in the article by Halle and Collipp (32).

Case Reports

Twenty-eightliterature reports of individual cases of methyl salicylate toxicity are included in this review. These may be summarized as follows:

1. In 1799, Longmore (33) reported that a tea made of herbs (which probably contained methyl salicylate) caused food poisoning in 14 men of the Royal Artillery in Quebec.
2. In 1875, Hamilton (34) reported a single case with non-fatal consequenc of an adult female who drank approximately 1/2 ounce.
3. In 1920, Myers (35) noted a case where 1 ounce was ingested by a 2-year old who recovered but voluntary respiration ceased at one point.
4. In 1922, Legrain and Badonnel (36) reported convulsions and death in an adult female who took 60 grams of oil of wintergreen with suicidal intentions.
5. In 1927, Pincus, et al (37) reported a 22 month old white male swallowe not more than 60 ml of oil of wintergreen with fatal consequences (apparently due to kidney damage).
6. In 1928, Olmsted and Aldrich (38) reported 2 cases with acidosis noted as sequellae. Both recovered. One was a 4-year old female; the other a 2-year old male.
7. In 1930, Meyerhoff (39) reported a single case with fatal consequences in a 22-month old male who consumed 24 cc. Continuous vomiting resulted. The patient was lavaged with bicarbonate but the patient expired. Principal damage was to lung, kidney and stomach.
8. In 1937, Stevenson (40) reported 3 cases; one with fatal consequences. One of the non-fatal cases was a 2-year old male who drank about 15 cc of oil of wintergreen.

9. In 1937, Lawson, et al (41) observed death in a young child with rheumatic fever following application of methyl salicylate oil to the skin.

10. In 1938, Eimas (42) reported a case of a 22-month old boy who ingested 1 teaspoonful of oil of wintergreen with fatal consequences.

11. In 1943, Townsend (43) reported a case in which an adult male died 24 hours after ingesting approximately 4 ounces of a methyl salicylate containing liniment.

12. In 1943, MacCready (44) reported on 5 cases. A 2-year old male consumed 15 cc, was lavaged, and recovered rapidly. A similar occurrence in a 55-year old made is also reported. A 2-year old female ingested less than 30 cc, was lavaged, improved in 15 hours, and was discharged in 3 days. However, in a 3-year old, after about 7.5 cc, death occurred in approximately 2 hours. Finally, a case of a 48-year old female who consumed approximately 4 cc, vomited, was lavaged and recovery is reported.

13. In 1945, Troll and Menten (45) reported on a 2-year old male who ingested 30 cc of oil of wintergreen with fatal consequences.

14. In 1945, Stevens and Kaplan (46) reported on a 17-month old girl who ingested one teaspoonful of oil of wintergreen. This patient recovered fairly rapidly following emergency treatment.

15. In 1947, Laforet, et al (47) cites the case of death due to methyl salicylate misuse in a 38-year old male with a history of alcoholism.

16. In 1947, Cancelmo (48) reviewed the literature and reported on two cases. One of these involved ingestion of 90 cc of oil of wintergreen in a 26-year old male with fatal consequences. Convulsions and cyanosis were noted. In another case, a 22-year old male ingested 30 cc and recovered.

17. In 1948, Crossland (49) reported a suicidal attempt of a woman who ingested 60 to 90 cc of oil of wintergreen. Acidosis was the most prominant problem in this case.

18. In 1949, Howell (50) reported an adult case and reviewed the symptoms, signs, and the treatment. This patient drank a "mouthful" but recovered. The initial blood salicylate was 77 mg %.

19. In 1950, Derobert (51) reported the clinical details of a single case in a 3-year old child.

20. In 1956, Done and Atterness(51A) reported on a two-year old male who ingested an estimated 20 ml. of oil of wintergreen. The patient was treated by exchange transfusion which resulted in prompt clinical improvement.

21. In 1957, Adams, et al (52) reported on a 20-month old boy who ingested approximately 5 ml. of oil of wintergreen and was successfully treated using exchange transfusion with 1200 ml. of whole blood.

22. In 1958, Mendelson, et al (52A) reported on a 60 year old man who ingested various alcohols including methyl salicylate. The patient recovered after treatment.

23. In 1964, Shelley (53) reported on psoriasis in a 6-year old boy. This was apparently involved with sweet birch pollen which induced a reaction because of its methyl salicylate content.

24. In 1967, Kloss and Boeckman (54) reported and discussed a case where intermittent peritoneal dialysis is used for methyl salicylate toxicity.

25. In 1968, Fine, et al (55) also found hemodialysis useful in treating a methyl salicylate overdose. In this case, a 7-month old girl consumed 4 ml. of methyl salicylate.

26. In 1971, Decker, et al (56) cited a case that indicated activated charcoal may aid in dialysis of methyl salicylate.

27. In 1973, Winek, Collom, and Voldeng (57) reported a case of a 52-year old male who consumed 3 ounces of methyl salicylate with fatal consequences. This patient apparently mistook the methyl salicylate bottle for castor oil. The initial blood salicylate level was 134 mg. %.

28. In 1975, Bickers, et al(57A) reported on a 48 year old woman who drank substantial quantities of a mouthwash containing methyl salicylate, eucalyptol, menthol, thymol and ethanol - over a period of months. The woman suffered from acute attacks of hepatic porphyria but completely recovered. Using an experimental system with avian liver, the whole mouthwash was shown to increase hepatic δ-aminolevulinic acid synthetase; however, methyl salicylate had no effect on this enzyme.

ABSORPTION AND METABOLISM

Abbott (58) determined total plasma salicylates in a series of 3 male subjects after the ingestion of methyl salicylate chewing gum and capsules. The methyl salicylate content of the chewing gum was determined to be 43.8 mg/stick or 219 mg/5 sticks. Five sticks were chewed for 1 hour and blood samples were obtained before and at 10, 20, 40 minutes 1, 2, 4 and 8 hours after chewing was initiated. The absence of significant plasma levels from the chewing gum indicated a lack of methyl salicylate absorption by this route. Plasma salicylate levels were obtained after a single 200 mg. encapsulated dose which supports this contention.

As long ago as 1934, Brown and Scott (59) studied the absorption through human skin of methyl salicylate applied in pure form, in aqueous suspension, in oil solution, in alcoholic solution, and in ointment bases. The surfaces of both hands were used, the material being applied then followed by immersion in a bath at 43 to 44°C. for one hour. The quantity absorbed was measured in 10 male subjects by determination of salicylate in the urine. Maximum absorption resulted from application of an aqueous suspension containing 11.8% methyl

salicylate and minimal absorption from the pure ester. Temperature proved to be an important factor. A rise from 26 - 28°C to 43 - 44°C in the immersion bath in the test with the aqueous suspension resulted in an increased absorption of 190 to 237%.

The percutaneous absorption of the salicylates has been studied more recently in rabbits by Cotty, et al (60). These workers found that the rate of absorption of methyl salicylate in mineral oil applied to the skin of rabbits was directly proportional to concentration. Vehicles that were highly volatile affected the rate of absorption of methyl salicylate negatively by evaporating and thus reducing the effective region of the skin from where absorption could take place.

Clarke, et al (2) reported that plasma values for methyl salicylate and free salicylate after oral administration of methyl salicylate to rats and dogs demonstrated that complete hydrolysis occurs shortly after administration. Somewhat less was hydrolyzed by human subjects. The major site of hydrolysis of methyl salicylate in the rat, rabbit, dog and monkey was the liver.

These major sites of hydrolysis were confirmed by Davison, et al (61). However, this investigator noted that other organs may play a minor role, and somewhat less was hydrolyzed in man than in the animals tested. A smaller proportion of unhydrolyzed ester in man may indicate a more toxic action than for free salicylate.

Ojiambo reported on two studies of methyl salicylate metabolism. In the first (62), healthy mongrel dogs were challenged with methyl salicylate intragastrically. Absorption was delayed initially but increased steadily for up to 4 hours as determined by plasma salicylate levels. Hydrolysis appeared to be maximal after 2 hours. A mechanism of hydrolysis and detoxification for methly salicylate is postulated in the dog. Ojiambo (63) further subjected dogs to 700 mg/kg of methyl salicylate. Using the hind limb for measurement, plasma

blood flow was shown to increase from a baseline of 1.2 ml/minute to 9.6 within 4 hours after methyl salicylate ingestion. Potassium levels in the arteries increased; however, a net efflux of potassium from the muscle occurred. Arterial lactate concentration was increased. Oxygen extraction by the muscles increased markedly for up to 2 hours after which it began to drop reaching a low level at the time of death.

Hruban (64) found an increase in number and in size of microbodies of hepatocytes in renal tubular cells of rats fed methyl salicylate as well as other salicylates.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of methyl salicylate has been reported in several studies over a number of years. In 1928, Rideal, et al (65) studied antibacteria activity and surface tension. He reported that the phenol coefficient of methyl salicylate against S. typhosa was 0.4. Miller (66) reported the phenol coefficient by the Tanner method as 1.76. She also reported that a 1:100 dilution of methyl salicylate killed S. typhosa in 2.5 minutes and a 1:250 dilution in 15 minutes.

Methyl salicylate was found by Mashimo, et al (67) to kill Staphylococcus, B. gartner, B. cyocyaneus, and M. tuberculosis in the range of concentrations of 1:100 and 1:1000.

The antibacterial activity of many essential oil vapors against various bacteria were studied by Kellner, et al (68). Methyl salicylate vapors were active against E. coli, S. typhosa, Neisseria, S. Fecalis, Strep. pyogenes, S. aureus, B. megatherium, C. diphtheriae, and O. albicans. In a further report Kellner and Kober (69) reported on the activity of many essential oils. Methyl salicylate oil was effective against the same species as the vapor.

Using a filter paper disc method, Maruzzella, et al (70) reported that methyl salicylate was active against four of the 11 species they tested. These were

B. mesentericus, S. aureus, Ps. aeruginosa, and S. marcescens. This group further reported (71) on the antifungal activities of essential oils. Methyl salicylat was active against 5 of the 18 species tested. The antibacterial activity of combinations of essential oils was also evaluated (72). It appears from this study that the combination of eucalyptus and methyl salicylate was less active than either of the single components. Finally, this group (73) reported on the activity of the vapors of several essential oils. The wintergreen oil vapors tested in this study was active only against M. avium.

In 1891, Miller (74) reported on a study of antibacterial activity in the human mouth using a rinse technique. In this study, methyl salicylate showed antibacterial activity but was generally "slow" in action. Colditz studied the effect of methyl salicylate on bacteria pathogenic to the eye (75). It did not appear to be effective for this use. Maruzzella, et al, also reported on the effect of combinations of antibiotics and essential oils on S. aureus (76). Addition of essential oils appeared to enhance antibiotic activity.

In a study of the correlation between peroxide number, water solubility and antibacterial efficacy, Schurmann, et al (77) showed that methyl salicylate possessed antibacterial activity. The antibacterial efficacy of volatile oils was correlated with peroxide number as well as solubility.

ANALGESIC EFFICACY

The effectiveness of methyl salicylate as an analgesic has been reviewed in the U. S. Dispensatory (78) and in the APhA Handbook of Non-Prescription Drugs by Dickison (79). The U. S. Dispensatory reports the long use of methyl salicylate in the treatment of various forms of rheumatism. It is noted to be more prone to cause stomach upset when administered orally and probably less efficient than sodium salicylate when administered by this route. It is capable of being absorbed through the skin and has been applied externally for systemic effects.

Methyl salicylate is widely employed in liniments and ointments for its counter-irritant effect. The APhA handbook (79) lists products containing this ingredient and points out, in the case of methyl salicylate, that the systemic analgesic value from percutaneous absorption is questionable.

Von Czetch - Lindenwald (80) found that methyl salicylate produced a cooling sensation but had no effect in lowering the skin temperature. In a recent controlled study, White, et al (81) compared the effect of an ointment containing menthol and methyl salicylate with a placebo on induced muscular pain and subjective response. In this case, neither the active drug nor the placebo altered skin resistance. However, the ointment decreased the muscle action potential whereas the placebo did not. The subjects noted that compared with the placebo, the product produced a feeling of warmth with reduced pain. In a more recent controlled study, White(81A) showed that the application of a cream containing menthol and methyl salicylate resulted in a significantly greater increase in range of motion and digital dexterity compared to placebo. The active product also reduced perceived pain while the placebo did not.

Methyl salicylate was shown to enhance the anesthetic activity of narceine by Kasuga (82). Guinea pig auditory and human skin models were used. Pain was induced by capillary wire stimulation. Peterson, et al (83) studied the response of skin to rubefacients using temperature readings. No particular activity of methyl salicylate was demonstrated using his method.

A liniment containing salicylate, nicotinic acid and benzoic acid was studied versus a perfumed control. In this case both the placebo and the active ointments were found to be useful. However, the medicated ointment succeeded in 1/2 more patients than the control. These authors (84) advocate use of liniment as a local treatment. Results of studies on an analgesic cream containing menthol,

methyl salicylate, adrenalin, methyl nicotinate, phenisin and chlorpheniramine maleate have been reported recently. Bhandare (85) reported its efficacy as a topical analgesic using an open study. Misra (86) reported an uncontrolled study showing good effects of this medication.

METHYL SALICYLATE

ANIMAL TOXICOLOGY STUDIES

CARCINOGENICITY STUDIES

1. Methyl salicylate was one of 41 food additives from the GRAS list tested for carcinogenicity potential using a mouse pulmonary tumor system developed by Andervont and Shimkin. Fifteen/sex/group, A/He, 6 to 8 weeks old mice were administered methyl salicylate intraperitoneally, 3 times weekly, at doses of 100 or 500 mg/kg/day for a total of 24 doses. Twenty-four weeks after the first injection, the mice were necropsied and the lungs were examined grossly and microscopically. In addition the liver, kidneys spleen, thymus, intestine, and salivary and endocrine glands were examined for abnormalities. Tumor incidences were compared between treated and control (untreated and water vehicle) groups.

The incidence of pulmonary tumors in mice given methyl salicylate was comparable to the controls, therefore, methyl salicylate is not regarded as a pulmonary carcinogen in this test. (1)

REPRODUCTIVE STUDIES

2. The reproductive toxicity of methyl salicylate (MS) was evaluated according to the Fertility Assessment by Continuous Breeding (FACB) protocol. Doses for the definitive study (Task 2) were selected from a range-finding study (Task 1). Male and female CD-1 mice were allocated to three treatment groups (20/sex/group) and a vehicle (corn oil) control group (40/sex). Methyl

salicylate was administered by gavage at doses of 100, 250 and 500 mg/kg/day during a 7-day premating period and for an additional 100 days during cohabitation as mating pairs.

There was no effect upon body weight changes and fertility index of any of the treated groups. At the high dose level (500 mg/kg/day), there was a decrease in: mean number of litters, average number of pups/litter, number of live pups and the mean live pup weight. The data of these parameters were comparable to the controls at the mid- and low-dose levels.

A third study was conducted (Task 3) to determine which sex was responsible for the effects seen at the high dose level in the definitive study. Due to reduced fertility of all the groups, it was not possible to determine if there was a sex-related reason for the changes described at the 500 mg/kg/day dose level.

The data indicate that oral doses of ≤250 mg/kg/day of methyl salicylate do not affect the fertility of CD-1 mice under the conditions of this study. Under the conditions of this study, five hundred mg/kg/day of methyl salicylate decreased the litter size, number of live born and pup weights of CD-1 mice. (2)

3. The reproductive toxicity of methyl salicylate (MS) was evaluated according to the Fertility Assessment by Continuous Breeding (FACB) protocol. Doses for the definitive study (Task 2) were selected from an range-finding study (Task 1). Male and female CD-1 mice were allocated to three treatment groups (20/sex/group) and a vehicle (corn oil) control group (40/sex). Methyl

salicylate was administered by gavage at doses of 25, 50 and 100 mg/kg/day during a 7-day premating period and for an additional 100 days during cohabitation as mating pairs and a 21-day segregation period.

There was no effect of any dose level on: 1) the number of pairs able to produce at least one litter, 2) number of litters/pair, 3) number of live pups/litter, and 4) the sex distribution. The adjusted female and combined live pup weights were significantly greater for the breeding pairs given 100 mg/kg than the other dose groups. This was regardeo as a chance finding in the absence of other effects.

The fertility and reproductive performance of the F_1 generation of the final litters from the control and 100 mg/kg groups (one or two male and female pups/litter) were assessed. Each weanling was maintained on the same treatment as their parents. At 90 ± 10 days of age, a male and a female from different litters within a treatment group were cohabited from 1 to 7 days. The females were allowed to deliver their litters. No significant effects were noted on mating behavior, fertility rate or reproductive performance. In addition, there were no toxicologically significant differences in the sperm assessments and the organ weights of these F_1 generation animals.

Under the conditions of this study, methyl salicylate was not a reproductive toxicant in the F_0 and F_1 generations of mice administered daily doses from 25 to 100 mg/kg. (3)

METHYL SALICYLATE CONTINUED

4. Methyl salicylate was administered orally or topically to pregnant LVG strain hamsters on the 7th day, 9th hour of gestation. The doses were 175 mg/100 gms body weight by the oral route and 350 or 525 mg/100 gms body weight by the topical route. The animals receiving methyl salicylate topically were anesthetized with Nembutal™ to prevent ingestion of the test substance and the test substance was wash off 2 hours following application. The study also included oral, topical and Nembutal™ controls. Most embryos were recovered at the 9th gestation day and some were allowed to continue development; however, few survived to day 12 of gestation. The embryos were observed for morphological changes. Plasma levels and whole embryo tissue levels of methyl salicylate were determined periodically.

Plasma levels peaked (125 mg/100 ml) approximately two hours after oral administration. Plasma levels reached 50 mg and 120 mg/100 ml following topical administration of 350 and 525 mg/kg, respectively. Comparison of maternal and fetal salicylate levels in older fetuses showed that salicylate levels reached the fetus at some fraction of the concentration found in the mother and the maximum level was reached more quickly in the mother than in the fetus.

The percent of fetuses with neural tube defects produced by the oral administration of 175 mg/100 gms body weight was comparable to the percent of fetuses from mothers treated topically with 525 mg/kg of body weight. Fetuses from mothers given 350 mg/kg of methyl salicylate topically were comparable to oral controls.

High doses of methyl salicylate given topically or orally are teratogenic to hamsters and may be related to achievement of comparable plasma levels. (4)

5. This study was undertaken to characterize the immediate implications of fetotoxicity in terms of the growth and biochemical differentiation of key organ systems in late gestation rat fetuses. The biochemical endpoints were selected because of their relevance to perinatal organ function and as such represented reliable and sensitive indicators of fetal maturation. They examined the brain for DNA and protein content (indicators of cell number and cell size), the lung for phosphatidylcholine and sphingomyelin content (the primary surface active materials), the liver for glycogen deposition (a vital energy source for the newborn pup) and the kidney for protein content and alkaline phosphatase activity (a marker of renal tubular development).

Three groups (5 litters/group) of 90-day old pregnant CD rats were administered methyl salicylate at doses of 0, 200 or 400 mg/kg/day on gestation days 9 and 10 (presence of sperm in vaginal smear was day 1 of gestation). The dams from each group were killed on gestation day 21 and the fetuses and organs were processed according to protocol for the parameters listed above. Fetuses with readily identifiable external malformations were excluded from biochemical analyses. All fetuses not used in the biochemical analysis were fixed and examined for external and internal abnormalities.

The following occurred in the high dose group: embryo lethality (50%-high dose vs 11%-controls), reduction of fetal weight (2.71 g-high dose vs 4.14 g-controls) and teratogenic responses in one or two fetuses in a single litter which included: cleft palate, encephalocele, gastroschisis, hydrocephaly, and spina bifida. At 200 mg/kg. one fetus had a diaphragmatic hernia and one fetus in each of two litters had an encephalocele. Based on biochemical

parameters, there were dose-related reductions in the development of brain, lung, liver and kidneys.

The fetal changes described above occurred in dams given high doses of methyl salicylate. Further study is required to determine if the biochemical effects affect the perinatal development and maturation of the offspring. (5)

6. This study evaluated a number of chemicals, including methyl salicylate, using a continuous breeding model. COBS Crl:CD-1 (ICR)Br mice were allocated into control (40/sex) and treated (20/sex/group) groups. The mice were administered methyl salicylate in the feed or in the drinking water during a 7-day premating period and continuously for 98 days, as cohabited mating pairs at doses of 20 to 500 mg/kg/day. Thereafter, the pairs were separated and treatment continued for up to 42 days (until weaning of any remaining litters. Body weights were measured periodically for the parent generation and the newborns. The litters/offspring were examined for sex ratio, number of litters/pair, number of live and dead pups within 12 hours of birth, then the litters were discarded.

At 500 mg/kg/day the following occurred: reduction of litter size, number of live pups and live pup body weight. At ≥50 mg/kg/day, the only finding was a reduction of the adjusted mean body weight of the offspring.

Under the conditions of this study, except for a reduction of the adjusted mean body weight of

the offspring, methyl salicylate was well-tolerated at a doses ≤ 250 mg/kg/day. (6)

7. Pregnant female CD rats from Charles River were administered 0.05 or 0.1 ml methyl salicylate intraperitoneally on days 10 and 11 of gestation and controls received no treatment. Maternal body weights were measured weekly. The young were obtained by cesarean section on gestation day 21 or postnatally at 1, 6, 12, or 24 days of age. They were counted , weighed and examined for viability and external malformations. The kidneys of the offspring were weighed, sectioned transversely through the hilum, and the length of the renal papilla was graded according to an arbitrary scheme.

Females given 0.1 ml of methyl salicylate gained less weight, had fewer and smaller offspring, and had more resorptions and malformed young than the controls. The fetal kidneys in this treated group weighed significantly less than those of the controls. The development of the renal papilla was retarded in the offspring from the dams given 0.1 ml of methyl salicylate. By post-natal day 6, the renal papillary growth and kidney weights were comparable to the controls. However, a small number of kidneys (11/138) from the treated groups had gross dilation of the renal pelvis and reduction of the renal parenchyma at weaning.

Based on the results of this study, "apparent" hydronephrosis in neonatal offspring may be simply due to delayed renal development associated with reduced growth of the maternal and fetal generations and not a permanent malformation. (7)

8. This study was conducted to determine whether substances known or suspected to cause subtle or transient anatomical alterations in renal development were capable of altering renal functional development. Methyl salicylate was one of three chemicals evaluated in this study. Sprague-Dawley rats were administered methyl salicylate i.p. at doses of 200, 250 and 300 mg/kg/day on gestation days 11 and 12. Due to a high incidence of embryotoxicity at the 300 mg/kg/day dose, there were not enough pups in this group for all renal function tests. Renal function (maximal urine concentrating ability, proximal tubule transport and urine flow, osmolality, pH, and chloride content) was measured following birth through weaning.

Compared to controls , after desmopressin acetate (DDAVP, a vasopressin analog) challenge, urine osmolality was decreased at 250 mg/kg/day on post- delivery day 6, and urine volume was increased in this group after DDAVP injection on post-delivery day 14. Urine flow was also increased in this group on post-delivery day 2 after 4 hours of isolation from the dam, a significant period of water deprivation to neonates. By post-delivery day 30. urine concentrating ability was comparable to controls.

The data indicate that there is a transient delay in the maturation of urine concentrating ability in rats transplacentally exposed to methyl salicylate at high doses (≥ 250 mg/kg/day given i.p. on gestation days 11 and 12). (8)

MUTAGENICITY

9. Using the protocol approved by the National Toxicology Program (NTP), methyl salicylate was tested in the Salmonella/microsome (Ames) test. *Salmonella typhimurium* strains TA 1535, TA 1537, TA 97, TA 98 and TA 100 were incubated with and without rat and hamster liver S-9, at doses of 1,000, 3,300, 10,000, 33,300, 100,000 and 333,300 μg of methyl salicylate/plate.

Methyl salicylate did not show mutagenic potential at any of the concentrations (1,000 - 333,300 μg/plate) tested. (9)

THYMOL

Thymol, also known as thyme camphor; 5-methyl-2-isopropyl-1-phenol; 1-methyl-3-hydroxy-4-isopropylbenzene; 3-p-cymenol; 3-hydroxy-p-cymine; and m-thymol has the formula $C_{10}H_{14}O$ with a molecular weight of 150.21. It is obtained from the essential oil of thymus vulgaris and other plants or is produced synthetically. The melting point is 51°C and it boils at about 233°C (with appreciable volatility in water vapors). One gram dissolves in about 1000 ml. of water; highly soluble in alcohol and oils. The food additive status is described in CFR as an additive to dietary foods and as a preservative. (21 CFR 121.2520)

ANIMAL TOXICOLOGY

Acute and Subacute Studies

Edwards and Hall (1) reported the LD_{50} in mice of thymol by I.V. injection as 74 mg/kg. The toxicity of thymol and isothymol were compared by Livingston (2). In this relatively early study the data is mainly narrative and on individual animals. Of 16 rabbits receiving 0.5 g/kg, all survived at least 16 days. Of six rabbits receiving 0.75 g/kg one died in 9 days, the rest survived longer. Six rabbits were administered 1.0 g/kg of thymol, 3 died within 6 days, the rest survived beyond the normal 10 day acute period. Data at higher doses showed the same trends.

Izeki (3) compared the toxicity of thymol with a related compound. He reported that the intravenous injection of 5 mg/kg of thymol to the rabbit resulted in a transient fall of blood pressure and inhibition of respiration. This paper also reports that thymol inhibits an intestinal parasite in mice *in vitro* and *in vivo*.

In his study of the acute oral toxicity of 107 synthetic and natural flavoring materials and related compounds, Jenner (4) administered thymol by

intubation to the rat and guinea pig. Animals were observed for two weeks for toxic signs at time of death. The acute LD_{50} of each compound was determined in rats using the Osborne-Mendell strain. For thymol the LD_{50} in rat was cited as 980 mg/kg and in the guinea pig at 880 mg/kg.

Hagan, et al. (5) reported the subacute and chronic toxicity of a number of food flavoring components. Thymol was fed at a level of 10,000 parts per million to 5 female and 5 male rats for 19 weeks with no untoward effects.

Special Studies

Adrien, Caujolle, and Franck (6) found that thymol affected the blood pressure when injected intravenously. It also increased respiration when administered by this method. This author also reported that thymol showed good antibacterial activity against several organisms.

HUMAN SAFETY

Reviews

Thymol has been widely used in the past, particularly in the Far East to control worm infestations in humans. Barnes (7) stated that over a million doses of thymol had been safety administered for this purpose. His report notes 20 fatalities as having been reported at these gram range doses

in debilitated patients. The author advocates 2.6 grams in divided doses as perfectly safe for adults. Two specific cases of fatality are included in this review. In the first case, 2.6 grams was given in a single dose to a 34 year old female with a history of probable heart disease. She died within 3 hours. In the second case, a 1.3 gram initial dose plus a 0.65 gram second dose was administered to a 40-year old female. This individual developed pneumonia and died in 9 days. The author felt that thymol was implicated as a contributing factor in this second case.

Lane (8) also reviewed his experience with thymol as an anthelminic. He reports millions of 4 gram doses being given with minimal risk. He also reviewed reported deaths due to thymol and concludes that the deaths were not the result of the thymol administration.

Hewlett reviewed 12 cases of lipoid pneumonia and reported (9) that these were alledgedly due to the misuse of an oil base medication containing menthol and thymol.

Samitz (10) cites thymol as being one of the less frequent sensitizers in occupational dermatoses in dentists and other allied personnel.

Studies

Smeenk, et al. (11) reported on an ointment containing 0.1% thymol which caused contact allergy. There were at least 8 other components in this product. The individual components were tested in 27 subjects allergic to the ointment and thymol was determined not to be the causative agent. One positive reaction to thymol was noted in these 27 subjects.

Lachelin (12) reports that a paste with soap, iodine, potassium iodide, thymol and astringents had been used successfully for therapeutic abortion. The role of thymol in this product is unclear.

No untoward effects of nasal drops with essential oils including thymol were found in a survey of around 124,000 infants receiving these drops (Breuninger, et al. 13). The author concluded that while there was no indication for nasal application of these essential oils in infants they were not unsafe.

In the dental area, Perrault, et al. (14) studied several agents of potential value as antibacterials for the use in cavity filling. He evaluated these compounds for their effects on the odontoblast. Thymol had no deleterious effect when applied for 10 minutes or less whereas alcohol had a slight harmful effect.

Case Reports

Zimmerman (15) cites a single case of thyrotoxicosis allegedly resulting from the use of a toothpaste containing 1/4% thymol. Edens (16) from the same clinic, reports 6 cases of thyrotoxicosis allegedly due to thymol. One of these used a mouthwash containing thymol, 1 a cough syrup and 4 a toothpaste. These results must be considered in light of the probability that some of these products cited may have contained the iodide salt of thymol, rather than thymol itself. There were no later reports of thyrotoxic activity obtained in this review. In relation to these reports it should be noted that Mittler and Benham (17) studied the nutritional availability of iodine from thymol-iodide. This was done in albino rats fed iodine deficient diets. In the case of the thymol-iodide compound, it was shown that it was partially effective in reducing thyroid enlargement and that 25 to 50% of the available iodine became concentrated in the thyroid gland. This confirmed the availability of iodine from thymol-iodide. Bickers, et al. (17A) reported a case in which a patient allegedly consumed substantial quantities of an essential oil-based mouthwash containing eucalyptol, menthol, thymol, methyl salicylate and alcohol over a period of months; the mouthwash appeared to precipitate acute attacks of hepatic porphyria.

00-000110

An experimental study utilizing an avin liver preparation showed that the mouthwash was a potent inducer of hepatic δ-aminolevulinic acid synthetase. However, thymol, one of the components, showed minor activity in this respect.

Pharmaceutical and Chemical Studies

The chemical and physical properties of thymol are described in USP XVIII (18). Taha and Gomaa (19) describe a specific method for the determination of micro quantities of thymol in pharmaceutical preparations. This method involved the oxidative coupling of para-phenylenediamine with thymol. The reaction product highly colored indaniline dye is measured colorimetrically. Maximum absorption is 550 nm. Zwaving (20) describes the gas chromatographic method for determining thymol in vegetable matter.

ABSORBTION, METABOLISM, AND RELATED STUDIES

Absorbtion and Metabolism

In an early paper, Schroder and Vollmer (21) described several chemical tests for phenolic compounds including thymol. In using this test to study the metabolism of thymol, they found that thymol administered orally to rabbits is 25 to 95% absorbed in 24 hours. This absorption is followed by urinary excretion. Only in the first few hours after administration were noticeable quantities of thymol found in the blood, kidney or liver. Thymol was not detected in the lung at all after oral ingestion. They also measured the respiratory gases of rabbits injected with thymol eliminated through respiration. It was noted also that the administration of thymol caused concentration of water in the lung and the liver. At an even earlier date, in 1915, Seidell (22) described a chemical method for isolating and analyzing thymol in the urine, where it was recovered as thymol glucuronate. At most about 50% of the ingested thymol was recovered. There was practically no thymol recovered from the feces.

In 1934, Robbins (23) administered 1 gram of thymol to each of 4 dogs. 346 mg. of this dose was excreted in 48 hours in the urine. 90% of this was

higher dose of 3 grams, 23% of the administered dose was excreted in the urine within 48 hours; 95% in the next 24 hours. No thymol was found in the feces at this higher dose.

Thymol was administered rectally to rats by Grisk and Fischer (24) and detected subsequently in the lungs. The author discusses whether the levels found are pharmacologically active but draws no conclusions.

Meyer, et al. (25) showed fairly rapid absorption following oral ingestion of thymol.

Special Studies

Matsumoto, et al. studied convulsive mechanisms of phenol derivatives including thymol. Although a number of derivatives were shown in this study (26) to cause convulsive reactions when injected into rats, thymol did not cause convulsions even when 3.0 ml/100 grams body weight were injected.

Seeman and several co-workers have reported a series of studies on erythrocyte membrane stabilization by various materials (27, 28, 29, 30). It was found that the effect of thymol on hypotonic hemolysis of erythrocytes is independent of pH in the range of 4 to 8 (30). The temperature dependence of this phenomena and further detail on erythrocyte expansion was also reported (31). It is stated that there is a relationship between the ability of a drug to protect erythrocytes against hypotonic hemolysis and anesthetic efficacy. In a control experiment, the greater susceptibility of erythrocytes to sodium chloride hemolysis at lower temperatures was verified. Low concentrations of thymol in the range of 10.3 to 10-3 molar to 10-4 molar protected erythrocytes against hypotonic hemolysis. However, high concentrations of alcohol anesthetics have a direct and immediate lytic effect. Thymol was less effective at reducing hemolysis at 37°C than at either 0 or 21°C.

membrane from beef thyroid and enhanced adenyl cyclase activity stimulated by flouride ion.

Greaser, et al. (33) found that concentrations of thymol that abolished Ca^{++} accumulating ability, but did not reduce the Ca^{++} activated ATPase activity, caused the formation of transparent patches on the surface of negatively stained vesicles. In 1971, Thyrum and his co-workers (34) suggested that local anesthetics displaced Ca^{++} from a Ca^{++} phospholipid complex while narcotizing agents such as thymol exert their action by increasing the amount of Ca^{++} bound to phospholipids and tissue membrane.

It was shown by Freytag (35) that thymol increased the activity of oral cilia from the frog at relatively low concentrations. A frog esophagus model was used by Das, et al. (36) to measure the effect of various drugs on ciliary motility. Thymol at 0.1% concentration depressed activity in this study.

Heathcote (37) found that thymol depressed the isolated heart of the frog and rabbit by directly acting on the cardiac muscle. Thymol was also shown to dilate coronary vessels. With thymol there was a fall in blood pressure.

Thymol was shown by Kato, et al. (38) to be only weakly active in induction of phagocytic activity of endothelial cells in the skin capillaries of white mice.

Jensen and Dyrud (39) showed that extracts of thyme inhibited acetylcholine initiated contractions in smooth muscle tissues. This effect was also present, through to a lesser degree, with serotonin induced contractions. The thyme extract itself was found to have a spasmolytic effect. The relaxing effect of bradykinin was also found to be potentiated by thyme extract.

The relationship between solubility, capillary and hemolytic effects of several essential oils was studied by Rhode (40). No particular relationship was shown.

A new series of potential central nervous system depressant drugs which are ethers of thymol were described by Ashford and his co-workers (41). These were shown to reduce hypermotor activity, prolong sleeping time, and lower body temperature and alter the effects of adrenaline and strychnine in rats. In a recent study by Dewhirst and Goodson (41A), thymol along with eugenol, guiacol, cresol and capsaicine inhibited prostaglandin (PG) synthesis by 50% at concentrations to 5 to 30μM. In this study the addition of arachidonate to PG synethetase from a macrosomal preparation of sheep seminal vesicles was used to initiate the following reaction: 1 mole of arachidonate plus 2 moles of O_2 form 1 mole of PG. A Clark oxygen electrode was used to monitor oxygen decrease in a closed reaction chamber. Prostaglandins (PG) are potent mediators c inflammation and drugs that block PG synthesis are anti-inflammatory.

Sensory Effects

In a review of the effect of essential oils on taste and smell, Bournot (42) noted that to be smelled a substance must reach the olfactory nerve endings. He noted that with thymol and other essential oils, inhalation not only affects sense organs but the oils are also absorbed through the respiratory system and can exert their pharmacological effects on various parts of the body. He also observed that thymol was bactericidal and acted as an irritant to the mucosa.

Van Skramlik studied the sensory effects of several essential oils (43). These effects were measured after inhalation, oral administration, and application to the skin. This author pointed out that all essential oils have an effect on the sense of smell but to a varying degree. However, most of these do not have any specific effect on taste other than producing sensations of bitterness or sweetness.

Antimicrobial Activity

Thymol has antibacterial, antifungal, antiyeast, as well as antihelminic activity. The latter will not be covered in this review.

It was noted in 1930 by Collier and Nitta (44) that essential oils had been used by Egyptians for the preservation of mummies. These workers tested essential oils for bactericidal activity against various species by a use dilution method. Thymol was bactericidal towards Streptococcus at a 1:1600 to 1:4000 dilution; against Staphylococcus at a 1:1600 dilution, against E. Coli at a 1:400 dilution, and against Vibrio at a 1:8000 dilution.

The phenol coefficient tests versus S. typhosa was used by Miller (45) to study the bacteriocidal efficiency of essential oils. She reported that a 1:2250 dilution of thymol killed this organism in 2.5 minutes and a 1:3000 dilution in 15 minutes. The phenol coefficient obtained by the Tanner method was 27.6.

Silberstein (46) demonstrated the greatly superior disinfectant activity of phenol as compared to phenyl alcohol, ethanol, and antiformin.

Saturated solutions of thymol and nutrient broth were tested by Edwards and Hall (1). The saturated solutions were diluted to find the lowest killing dilution. S. aureus was killed by a 7% or higher dilution which represented a 1:8570 concentration of pure thymol. Comparative values for killing E. coli were 1:6667 and for C. hofman, 1:10,000.

Katayama and Nagai (47) used an agar streak method to find the effective dilution of thymol required to kill several species of organisms. The effective dilution to kill B. subtilis was 1:1000, E. coli approximately 1:2000; S. enteritidis 1:2000, S. aureus 1:1000, Proteus morganaii 1:1000, and Ps. auruginosa around 1:2000.

Mashimo, et al. (48) found that the concentration of thyme oil required to kill organisms including Staphylococcus and M. tuberculosis was in the range of 1:1000 dilution.

Day (49) isolated bacteria from decayed teeth (primarily lactobacilli) and tested thymol for activity against them. The phenol coefficient found

for thymol against these organisms was 23.4 This worker also tested the penetration of thymol into decay by melting crystals and applying them directly to the decay area. According to his data, definite sterilization was shown in the presence of gross decay. In an early study, Goodrich (50) treated a film of bacteria with various test solutions for various periods of time followed by incubation. In this study, thymol as the saturated aqueous solution was a very good antiseptic for mouth bacteria. It gave the best results of the chemicals and mouthwashes tested.

In his study of the antibacterial activity of essential oil vapors, Maruzzella and Sicurella (51) reported that oil of thyme, red NF and oil of thyme, white NF were active against all of the test organisms studied. These included E. coli, S. aureaus, B. subtilis, Strep faecalis, S. typhosa, and Microbacterium avium. The method used was to inoculate solidified agar in petri dishes with the test organisms. Filter paper discs were saturated with each volatile oil and placed in the center of the dish covers. Dishes were inverted and incubated at 37°C for 24 hours (72 hours in the case of M. avium). The clear zones on the surface of agar above the filter paper discs were measured.

The antibacterial efficacy of vapors of several volatile materials was also studied by Grubb (52). Solidified agar was inoculated with the test organism and then inverted. The material to be tested was placed in a cup inside the cover and the whole dish was incubated. Thymol showed a 75% inhibiting activity versus S. aureus.

The utility of several essential oils for room disinfection were studied by Kellner and Kober. In one study (53) the antibacterial activity of the

vapors of these essential oils versus various species was studied. Thymol was found to be highly effective against all the organisms studied, which were E. coli, S. typhosa, Neissieria, S. faecalis, S. pyogenes, S. aureus, B. megatherium, E. diptheriaie, and O. albicans. These workers also showed that the oil itself was effective against the same organisms (54). Further substantiation is reported by these workers in a later article (55).

As noted above, Maruzzella and co-workers have also studied the in vitro antibacterial activity of various oils. In one study (56) thymol was found active against 4 species which included N. perflava, B. subtilis, S. Marcescens, and E. coli. They also investigated the antifungal activity of these essential oils (57) and reported that thymol was active against all 18 species tested.

Scheurman, et al. (58) correlated antibacterial effect with peroxide number and solubility. In this study thymol was shown to possess antibacterial activity.

Booth and Sefton (59) tested a number of compounds as possible agents for the destruction of therobacilli and thiobacilli. Among those found effective was a mixture of crystalline thymol with dicyclohexylamine nitrate.

In their study of 73 different phenolic compounds Weuffen and Richter (60) reported data on thymol which appears moreor less in line with results reported by others. This particular study was concerned with bacteria contaminating X-ray film materials.

Shibasaki and his co-workers (61) studied thymol, benzoic acid and several other compounds against several bacteria and molds. Their results generally confirm the activity of thymol reported in other studies.

Dunham and MacNeal (62) studied several volatile oils and other materials for antiviral activity. These were tested by inoculating Vaccinia virus onto corioallantoic membranes of developing chick embryos. The virus suspension was mixed with a test solution then inoculated into the egg preparation. While ethanol was essentially inactive, thymol, as well as other essential oils showed efficacy. Thymol appeared somewhat more effective than menthol or eucalyptol in this study.

Several alcohols, ketones and phenols were tested for their activity against baker's yeast. Thymol showed the greatest activity of the series tested against S. cerevisiae as well as S. typhosa. Alcohol was much less active against these two organisms. It was noted in this paper by Lindenberg and Massin (63) that the more active compounds had greater lipid solubility than water solubility.

Wilson (64) reported that 2 to 4% thymol in chloroform was recommended for Candida infections. In an earlier study, (in 1926) Myers (65) studied several volatile oils. This study indicated that thymol was effective in destroying yeast and showed fungicidal activity many times greater than that of several other volatile oils tested.

The following report data in studies on the antifungal activity of thymol. Blum and Fabian in 1943 (66) reported on a study of spice oils and their components for controlling microbial surface growth. Thymol (as thyme) was tested against organisms which infect fermented food products. It was found to be not the best but not the worst of those tested for that purpose.

Moving into human efficacy, Devoe (67) studied the effect of thymol on keratomycosis. He points out incidentally, that Herpes simplex is the "most preceeding" eye disease to keratomycosis. Thymol is listed as an

antifungal agent for this disease but no particular data is given.

Myers (68) reported as far back as 1927 that thymol was the most effective among 23 volatile oils tested in destroying the mold found on the hands of workers in the fruit cannery. He also reported that thymol was effective against actinomycosis. In a further report this author (69) reports a number of cases where actinomycosis infections were cured in a matter of weeks by thymol taken internally and also applied externally to the lesions.

Lerner and Lerner's text on dermatological medications (70) describes several fungicidal preparations for use by humans, particularly against mycotic infections of the feet. Thymol is used as an active ingredient for this purpose. More (71) reported on the use of thymol preparations for the treatment of seborrhea, psoriasis, pityriasis rosea, dermatitis venenata and ring worm. In a more esoteric use, Richards and Hawley (72) reported from a marine biology laboratory that thymol is effective as a 0.1% solution against most mold growths.

Thymol was tested as an antifungal agent against Monilia albicans, M. candida, and M. parapsilosis by Stoval, and his co-workers (73). A saturated solution (1:1000) of thymol at 25°C was capable of killing each of these in less than 5 minutes. At a concentration of 1:5000 in glucose broth, thymol inhibited the growth of most fungus cultures for two weeks but did not kill them. At the same concentration in rabbit serum, thymol showed no tendency to inhibit the growth of three species of Monilia. In studies where thymol was administered in olive oil through a stomach tube, rabbits could tolerate .04 to 0.5 gram of thymol per kg. of body weight daily without serious ill effect. However, it was found that thymol delivered through the stomach

catheter had no inhibiting effect on the growth on M. albicans in vivo.

In a brief monograph, Kim and Chang (74) described the use of thymol to protect leather from molds. It was reported by Bomar (75) that Aspergillus niger spores were inhibited from growth while exposed to thymol vapors. This appeared to be a bacteriostatic effect since the spores resumed growth when removed from contact with thymol. Finally, in the area of antifungal activity, thymol was reported useful in control of Tinea infections by Gastard (76).

Thymol as a component of a topical antifungal medication is mentioned in the most recent addition of Krantz and Carr (77). Wilson (78) states that thymol, based on his clinical experience, is effective in the treatment of fungus infections of the nails. Clemens (79) successfully treated a case of actinomycosis with orally administered thymol.

In a United States Patent (79A), Harvey and Greenspan disclosed the use of thymol in treating influenza virus infection. An elixir of thymol (1% w/v) was prepared with alcohol, polyethylene glycol and excipients and used in animal tissue culture experiments. Recommended dosage was 0.5 to 100 mg. per kilogram of body weight of thymol in a unit dosage form.

Shively and Hartsell (79B) studied the lytic spectrum of ten species of pseudomonads. They showed that Thymol when tested with lysozyme gave increased lysis.

The action of thymol on bacteria and bacteriophages was studied by Wahl and Blum-Emerique (79C). Thymol was bacteriostatic and bactericidal and it did not inactivate free bacteriophages. They also found that thymol does not prevent the attachment of phage to bacteria, and it stops the multiplication of phages without inactivating them.

Preservative Efficacy

Kuroda reported that thymol was more active at acid pH (80). Wahl and Blum-Emerique (81) found that thymol was both bacteriostatic and bactericidal. At 37°C it sterilized a culture of Y6R, which contained over 10^7 organisms, within five hours. 98% of the organisms were killed in 1/2 hour. However, in this study it was reported that thymol did not inactivate the bacteriophage liberated nor did it impede the fixation of phage by the bacteria. These authors suggest thymol as a way of preparing phage by killing the organism and not the phage.

A number of species of pseudomonads were tested for bacteriolysis in lysozyme solutions with one of several chemical agents including thymol by Shively and Hartsell (82). Several of the combinations studied including thymol-lyzozyme showed synergistic effects in the lysis of all the pseudomonads studied. However, it is of interest to note that Hughes (83) had reported that a 25% solution of "glycerin of thymol" was found to be contaminated with Pseudomonas. Thoma, et al. (84) found that the polyoxyethylene stearates inhibit the antibacterial activity of thymol and the polyethylene glycols had no effect.

Bohm (85) showed that thymol was active as a preservative agent. Tetumoto (86) showed that thymol sterilized S. aureus, P. vulgaris, S. typhosa and V. cholera. Bullock and Lightbown (87) studied the effect of thymol and other preservatives on the preservation of concentrated infusions of quassia. Thymol was not particularly effective for this use. Reddish in his textbook (88) describes the activity of thymol against S. typhosa and M. pyogenes in terms of the phenol coefficients.

Mold preventing action of several phenolic compounds was studied by Fujikawa, et al (88A). Thymol in concentration of 0.007% was shown to prevent molding in soy sauce during a five day test period.

This activity was comparable to the mold preventing activity of propyl p-hydroxybenzoate in 0.01% concentration used as control.

In Vivo Studies

Graff (89) and Hunkirchen (90) studied a product containing thymol, eucalyptol, menthol and other essential oils. This product showed good antibacterial activity; data on the individual components was not given. Also this product was evaluated clinically (Hunkirchen) and was found effective in inflammatory changes in the mouth.

As far back as 1891, Miller (91) studied several essential oils and acids in killing bacteria in the mouth using a rinse technique. Thymol was shown to have activity in this study. Henderson (92) recommended thymol as well as menthol and eucalyptol as components of nasal sprays and drops to be used as antiseptics, while Prinz and Greenbaum (93) believed that acid mouthwashes were relatively undesirable and noted that thymol was effective as a component of these. Eichorn, more recently in 1968 (94) reviewed antibacterial agents for use in the mouth and throat and pointed out that essential oils including thymol were highly suitable for disinfection of the mouth and throat because of the volatility and lipid solubility. This author also noted that the vapors were endowed with bactericidal action. Good therapeutic effects were achieved with aerosols in the treatment of laryngitis, pharyngitis, tracheitis, bronchitis, etc.

A mouthwash containing thymol and menthol, phenol, sodium phenelate, sodium tetraborate, glycerine and chlorophyll was evaluated by Novick and Sodhi (95). In this study, the mouthwash sprayed in the throat was compared with penicillin and placebo. Throat cultures were taken and counts of _S. hemolyticus_ made. The mouthwash reduced the count 40 to 70% in 24 hours, the placebo 0% and penicillin 17%. Using a slight modification of the same formula, Jones, _et al._ (96) compared the mouthwash with saline and no treatment in the control of bacteremias associated with tooth extraction.

The mouthwash was significantly superior to saline and the authors recommended its routine use. In a further study of Cutcher, et al. (97) the mouthwash was shown to affect a 30.7% reduction in the number of bacteremias compared to the control.

EFFICACY

Reviews

Several general sources cite the efficacy of thymol. In 1879, Fluckiger, et al. (98) reviewed the botany and chemistry of essential oils to that date. These authors stated that thymol was first "noticed" in 1725 and recommended in place of phenol in 1868. They also state that it is an efficient external stimulant and has been proposed as a disinfectant in place of phenol. A few years later, in 1882, Wood (99) stated that thymol was powerfully antiseptic but that its fragrant odor was a disadvantage. This author also notes that it has been administered in doses up to 30 grains per day with a few instances of nausea and vomiting. Some negative data in regard to efficacy is also cited. In 1891, Potter (100) stated that thymol was a powerful antiseptic and disinfectant, a local irritant, and anesthetic for skin and mucous membranes. He went on to point out that it is much more permanent and powerful than phenol and much less toxic. It has been used as an antiseptic and as a gargle, spray or inhalation in laryngitis.

In somewhat more recent times, Currier (101) cited thymol as well as other essential oils and other materials as valuable antiseptics. This author stressed prevention of sickness by mouthwash and nasal irrigation. Grosicki (102) lists thymol as an antiseptic component in hemorrhoidal products. In his review for the APhA Handbook of O.T.C. Drugs on external analgesics, Dickinson (103) notes that thymol has irritant action and some antiseptic value.

Buckley (104) in 1926 reviewed available drugs for use in the oral cavity. He stated that thymol has local analgesic properties, is a superior antiseptic to phenol and is less toxic than phenol. Thymol is advocated as a component

of gargles for treatment of sore throat by Watson (105). This opinion is also reflected by Cutter (106).

In a review of the common cold, Gellenthien (107) divided the cold into three stages. For the first and third stages he recommended the instillation of an antiseptic douche in the nose. For the second stage (which he defined as a profuse watery secretion), he recommended thymol along with menthol and eucalyptol in mineral oil as a soothing agent. Beckman (108) described thymol for use in a mouthwash for relief of stomatitis. Williams (109) in discussing acute infection of the upper respiratory tract, which includes a good description of nasal anatomy and physiology, divided cold into two stages; the viral invasion and secondary infection. Thymol is mentioned as a component of nose drops and sprays which this author condemns.

In a 1958 review of mouthwashes, gargles, paints and lozenges (110) a negative view of efficacy is expressed in regard to thymol. This paper points out that thymol and similar volatile oils are protoplasmic poisons and thus, have antiseptic activity. However, they are deemed to be irritant, rubefacient and locally anesthetic. Consequently, low concentration of volatile oils are used to avoid local reactions. This results in poor antibacterial effect.

Efficacy Studies (Respiratory Disease)

Carissimi, et al. (111) described the synthesis and pharmacological properties (principally antitussive activity, local anesthesia, narcosis potentiation, hypertensive and respiratory depressing activities) of a series of thymol and guaicol derivatives. It was noted in this study that the pharmacological activities are generally greater in the thymol series.

Dastugue and Brun (112) found that thymol facilitates the narcosis of larvae by aiding penetration of narcotizing substance. The expectorant activity of thymol and other essential oils has been studied by Boyd and Sheppard (113). Urethanized rabbits were administered thymol by steam

inhalation. This treatment augmented soluble mucous content and lowered the specific gravity of the respiratory tract fluid. The dose of thymol required to produce this effect was around 100 mg/kg as added to a vaporizer. This was calculated to correspond to 2 mg/kg of the drug as absorbed. These authors concluded that the effects were due to direct stimulation of mucous secreting cells in the respiratory tract.

A cat model was used by DeKeuning (114) to study antitussive activity. Thymol was not active as an antitussive in this model but was secretolytic. He did note that thymol was capable of potentiating water soluble fractions from a thyme oil that showed antitussive activity.

Turletti (115) reviewed the use of thymol in diseases of the respiratory tract. He points out that thymol was well tolerated and mainly effective against tuberculosis by lysing the bacteria. Thymol as the aqueous solution, was advocated as a routine gargle to prevent throat and mouth infection by Goodrich and Way (116).

In 1933, Freytag studied the effect of thyme oil on ciliary activity in the frog (117). This author found that thyme oil was less effective than either thymol or carvacrol. These latter compounds increased the rate of ciliary movement by as much as 100% relative to Ringer's solution even at dilutions of 1:50,000.

Efficacy Studies/Other

In 1967, Tyson, et al. reported the use of a topical preparation for the treatment of dandruff (118). In addition to thymol this product contains trichlorocarbanilid, salicylic acid and allantoin. This was compared with selenium sulphide and lorothidol. The product containing thymol was effective and well tolerated by the patients.

Buccellato has studied thymol in a number of areas principally related to its cellolytic activity. In 1964 (119) he reported that thymol at doses

of 1 to 4 grams over 2 cycles of 64 and 69 days with a 35 day interval cured a case of Kapori's disease. The author hypothesized the direct action of thymol on proliferated spindle cells and generally on tissue of mesodermal origin. The following year this author reported a case of dermatomyositis cured in 8 months by oral administration by thymol (120). The author at this point further hypothesized that thymol was active on diseases of mesodermal tissues. Also, in this same year, Buccellato (121) reported a case of progressive scleroderma cured with oral and parental administration of thymol. Buccellato also reported that 5 out of 6 cases of lupis erythematosus were favorably treated with thymol. The fact that the toxicity of this drug was slight was confirmed by its use in pregnant women. The author further states that thy- appeared to arrest other mesodermal diseases and cancers. The author feels there is greater cellolytic activity on neoplastic tissue. This appears inversely proportional to degree of cell proliferation (122). He also reported (123) some more specific studies on the cellolytic activity of thymol. Freystadtl (124) found that 0.1% thymol in alcohol relieved itching but not because of its anesthetic activity.

THYMOL

ANIMAL TOXICOLOGY STUDIES

CARCINOGENICITY STUDIES

Thymol was one of 41 food additives from the GRAS list tested for carcinogenicity potential using a mouse pulmonary tumor system developed by Andervont and Shimkin. Fifteen/sex/group, A/He, 6 to 8 weeks old mice were administered thymol intraperitoneally, 3 times weekly, at doses of 50 or 250 mg/kg/day for a total of 24 doses. Twenty-four weeks after the first injection, the mice were necropsied and the lungs were examined grossly and microscopically. In addition the liver, kidneys spleen, thymus, intestine, and salivary and endocrine glands were examined for abnormalities. Tumor incidences were compared between treated and control (untreated and water vehicle) groups.

The incidence of pulmonary tumors in mice given thymol was comparable to the controls, therefore, thymol is not regarded as a pulmonary carcinogen in this test. (1)

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LISTERINE

MUTAGENICITY STUDIES

Listerine was evaluated for mutagenic potential [gene mutation, chromosome damage and DNA repair (UDS)] in the following bacterial, *in vitro* and *in vivo* tests: Ames Salmonella typimurium, rat hepatocyte primary culture/Unscheluled DNA Synthesis (UDS) and mouse micronucleus tests.

1. Salmonella typimurium strains TA1535, TA1537, TA1538, TA98 and TA 100 were incubated with and without rat liver S-9, at doses of 50, 167, 500, 1667 and 5000 μg of Listerine/plate. Appropriate positive and negative controls were used.

Listerine did not show mutagenic potential in the Ames test, with or without S-9 metabolic activation. (1)

2. The Unscheduled DNA Synthesis (UDS) test was conducted to evaluate the potential of Listerine to interact with DNA by exposing rat liver cells *in vitro* to Listerine and tritiated thymidine. The extent of DNA repair was determined by measuring the amount of radioactivity incorporated into the liver cell nuclei and comparing the results to unexposed cells. Liver cell cultures were treated with 20 μl of Listerine concentrations ranging from 0.16 to 5000 μg/ml. Appropriate positive and negative controls were used.

Listerine did not show mutagenic potential at any of the concentrations tested in the Unscheduled DNA Synthesis assay. (2)

3. The Micronucleus test was conducted to evaluate the potential of Listerine to cause chromosome damage using an *in vivo* test. Chromosome damage is determined by measuring the number of micronuclei in the bone marrow polychromatic erythrocytes of mice. Listerine was administered intraperitoneally as a single dose of 5 ml/kg to 15 mice/sex. Five mice/sex were sacrificed 30, 48 and 72 hours after dosing. Positive control (triethylenemelamine) animals and the negative control (28.65% ethanol) animals were sacrificed 30 and 48 hours after dosing, respectively.

Listerine did not show mutagenic potential at any of the periods of sacrifice in the Mouse Micronucleus test. (3)

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The Effect of Listerine Antiseptic® on Reduction of Existing Plaque and Gingivitis

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In short-term clinical trials, Listerine Antiseptic® has been shown to be an effective antiplaque and antigingivitis agent when used either in conjunction with or in the absence of normal oral hygiene measures. To evaluate the effect of Listerine Antiseptic on existing plaque and gingivitis over a 6-month period, 145 subjects were entered in a double-blind, supervised clinical trial. Minimum requirements for entering into the study were a preexisting plaque score of ≥ 1.8 using the Turesky modification of the Quigley-Hein Index and a preexisting gingival index of ≥ 2.0 using a noninvasive modification of the Loe-Silness Index. Selection by random code determined use of one of three products: Listerine Antiseptic, a true vehicle control, or sterile colored water. Participation required twice daily supervised rinses of 20 ml for 30 seconds. At the conclusion of the study, Listerine Antiseptic displayed a reduction of plaque scores by 20.8% compared to the vehicle and 22.2% compared to the water control. The reduction in gingivitis scores observed with Listerine Antiseptic was 27.7% compared to the vehicle and 28.2% compared to the water control.

It is firmly established that dental plaque is the main etiological agent of inflammatory periodontal disease.[1-3] Consequently, prevention of dental plaque accumulation has been shown to correlate with control of disease progression.[4,5]

Patients contribute to plaque removal by performing various cleansing procedures. While a large variety of techniques and aids are available, and organized dental care has stressed preventive measures in the control of periodontal disease, these disorders remain widespread.[*] The prevalence of these disorders may be traced in part to a lack of consistently applied oral hygiene measures. This lack of consistency, in turn, may relate to difficulty in modifying human behavior.[6,7]

As a result, interest has turned to cost-effective, patient-compliant oral hygiene measures. In this category, mouthrinses with antimicrobial activity have been widely investigated as a possible aid in plaque control. Chlorhexidine, in particular, has been shown to significantly reduce plaque and gingivitis[8,9] but is not currently available for patient use in the United States. Previous short-term clinical studies have shown the efficacy of Listerine Antiseptic in inhibiting plaque and gingivitis.[10-14] These studies, ranging in length from 7 to 60 days, have indicated that this over-the-counter mouthrinse is effective in reducing plaque accumulation and gingivitis development when used in conjunction with, or in the absence of, normal oral hygiene procedures. The purpose of this study was to evaluate the effect of Listerine Antiseptic on existing dental plaque and gingivitis over the course of a 6-month trial.

Materials and Methods

Approximately 200 individuals were screened for entry into the study. The screening exam consisted of evaluation of teeth #3, 9, 12, 19, 25, and 28 for gingivitis and

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plaque by the indices described below. One hundred and forty-five systemically healthy male and female students or employees of Fairleigh Dickinson University satisfied the criteria and were entered in the study. Subjects ranged in age from 18 to 54 years.

Criteria for entry included a minimum of 20 natural, scorable teeth, and the absence of large carious lesions. Orthodontically banded, fully crowned, abutment and third molar teeth were excluded. Subjects receiving medications that could affect the oral soft tissues (*i.e.*, systemic antibiotics or anti-inflammatory drugs) were not included. The use of oral contraceptives was permitted throughout the course of the study; its use was noted but did not constitute exclusion. A total of five subjects were receiving these drugs.

Baseline examination consisted of an oral examination to determine the presence of oral soft or hard tissue pathology. A minimum gingival score of 2.0 using a noninvasive modification of the Loe-Silness Index[15] was required. The labial and lingual gingival margins and interdental papillae of all scorable teeth were evaluated as follows:

0—normal (absence of inflammation)
1—mild inflammation (*e.g.* slight changes in color) of any portion of the marginal gingival unit
2—mild inflammation (but no edema) of the entire gingival unit
3—moderate inflammation, (moderate glazing, redness, edema and/or hypertrophy)
4—severe inflammation (marked redness and edema/hypertrophy, spontaneous bleeding, or ulceration).

A minimum plaque score of 1.8 using the Turesky modification of the Quigley-Hein Index[16] was required. This index emphasizes plaque at the gingival one-third of the tooth surface. Assessment of plaque was made by staining the teeth with FD + C Red #3 dye. Each tooth surface was divided into mesial, central, and distal thirds. The criteria for scoring were as follows:

0—no plaque
1—separate flecks or a discontinuous band of plaque at the gingival margin
2—thin (up to 1 mm) continuous band of plaque at the gingival margin
3—band of plaque wider than 1 mm but covering less than 1/3 of the gingival third of the tooth surface
4—plaque covering more than 1/3, but less than 2/3 of the gingival third of the tooth surface
5—plaque covering 2/3 or more of the gingival third of the tooth surface.

All scorable teeth were evaluated and the average score for each subject was then calculated. Extrinsic tooth stain was measured using a modification of the Lobene Index.[17] The 12 anterior teeth were scored. Area and intensity of stain were evaluated on a 3-mm wide, crescent-shaped band of tooth structure adjacent to the gingival margin. The area on each surface was noted as follows:

0—no stain
1—stain over 1/3 the gingival region
2—stain over 1/3 to 2/3 the gingival region
3—stain over more than 2/3 the gingival region.

The intensity of the stain on each surface was noted as follows:

0—no stain
1—light stain (tan)
2—moderate stain (brown)
3—heavy stain (dark brown).

The area and intensity scores were multiplied for a single tooth score; single tooth scores were added for the total score.

Having met minimal criteria, participants were randomly assigned to one of three groups: Listerine Antiseptic, vehicle control (which consisted of the 26.9% hydroalcoholic vehicle containing all ingredients in Listerine except its essential oils), or water control. Subjects participated in twice-daily supervised rinsing on Monday through Friday, once in the morning and once in the afternoon, with a minimum of 2 hours between rinse periods. Each participant rinsed with 20 ml of his or her assigned product for 30 seconds. No eating or drinking was permitted for 15 minutes after each rinse of mouthwash. Coded, 3-ounce bottles and graduated plastic cups were distributed for the twice daily unsupervised rinses on weekends; coded 16-ounce bottles were distributed for holidays and recesses. Subjects were required to maintain a diary of unsupervised rinse use.

Lactona M39 soft bristle toothbrushes (Lactona Corp., Hatfield, PA) and a nonfluoridated, low-abrasive toothpaste were periodically distributed for unsupervised use. Subjects were instructed to follow their usual oral hygiene regimen, and use of dental floss was permitted to remove trapped food. Subjects were requested not to use commercial mouthrinses, oral irrigators, and other oral hygiene devices. No toothbrushing or rinsing was permitted on the mornings of each examination (screening, baseline, 1 month, 3 months, and 6 months). Upon completion of the 6-month clinical trial, subjects were offered a complete scaling and prophylaxis. Restorative dental services were permitted during the course of the study.

The study was conducted double-blind. All intraoral examinations were done by one investigator.

TABLE 1. *Demographic Variables of the 129 Participants Who Completed the 6-month Trial*

	Listerine Group		Vehicle Control Group		Water Control Group	
	Number	%	Number	%	Number	%
Sex						
Men	28	62	26	57	30	70
Women	17	38	20	44	13	30
Smokers	6	13	7	15	9	21
Nonsmokers	39	87	39	85	34	79
Age (years)						
Average	26.1		27.9		24.7	
Standard error	1.0		1.5		0.9	
Range	19–50		18–54		19–48	

TABLE 2. *Adjusted Mean Plaque Index Scores* (± SEM) *at 1, 3, and 6 Months After Initiation of the Clinical Trial*

Group	1 Month	3 Months	6 Months
Listerine Antiseptic	2.212 ± 0.059	1.908 ± 0.060	1.929 ± 0.062
Vehicle control	2.572 ± 0.059	2.391 ± 0.060	2.436 ± 0.065
Water control	2.651 ± 0.061	2.486 ± 0.061	2.480 ± 0.066

Listerine Antiseptic ($p < 0.001$) at 1, 3, and 6 months *versus* vehicle and water controls.

Approval for this study was received from the Institutional Review Board; informed consent of all subjects was obtained. Statistical interpretation was by analysis of covariance. Data for plaque and gingivitis were adjusted for differences in baseline means. This adjustment was not done for stain.

Results

Of the 145 subjects who entered the study, 48 were assigned to the Listerine Antiseptic (LA) group, 48 were assigned to the vehicle (V) control group, and 49 were assigned to the water (W) control group. One hundred and twenty-nine of the participants completed the 6-month clinical trial. Of these 129 participants, 45 were in the LA group, 43 in the V group, and 41 in the W group. Fourteen subjects were discontinued due to a failure to participate or inability to attend twice-daily supervised rinses; two subjects were unable to complete the study due to illness. Table 1 provides the demographic characteristics of the participants who completed the study.

As compared to the control groups, the LA group displayed significant decreases in plaque and gingivitis scores at 1, 3, and 6 months. Adjusted mean plaque and gingivitis scores over the course of the trial are provided in Tables 2 and 3. When converted to percentage reductions, the reductions in dental plaque scores observed with LA as compared to V were 14.0%, 20.2%, and 20.8% at 1, 3, and 6 months, respectively. LA also reduced gingivitis scores by 5.4%, 18.8%, and 27.7% at 1-, 3-, and 6-month intervals when compared to the V control. Slightly greater reductions were noted for the LA group when compared to the W group. For plaque, the reductions were 16.5%, 23.2%, and 22.2% at 1, 3, and 6 months, respectively. Over the course of this trial, the reductions in gingivitis seen with LA compared to W were 10.2%, 21.0%, and 28.2%. There were no significant differences between the V and W control groups for plaque at any examination interval. For gingivitis, the V group had significantly less gingivitis than the W group ($p \leq 0.002$) at the 1-month examination period, but this difference was not detected at the 3- or 6-month intervals. No statistically significant differences in stain index scores were noted between any of the groups at any of the examination periods (Table 4).

Discussion

The results obtained in this clinical trial indicate that Listerine Antiseptic was effective in reducing existing plaque and gingivitis over a 6-month period. The effectiveness of this agent in reducing plaque and gingivitis had previously been demonstrated in a number of short-term studies.[10–14] Kennedy and Kravets[10] demonstrated that Listerine Antiseptic, when used with normal oral hygiene measures was effective in reducing plaque accumulation over a 7-day trial. A 50% reduction in dry plaque weight was observed in the Listerine Antiseptic group as compared to baseline. The reduction observed in the group that rinsed with 0.9% saline and maintained normal oral hygiene procedures was only 10%. Gomer *et al.*[11] conducted 7-day trials evaluating the effect of Listerine Antiseptic on developing plaque. Following prophylaxis, subjects were allowed to follow normal oral hygiene measures. Listerine Antiseptic was observed to cause statistically significant plaque reductions of from 39% to 55% as compared to baseline. Under the same protocol, the other test rinses (0.9% saline, benzethonium chloride, hexedine, and zinc chloride) showed no statistically significant plaque reduction. In a 3-week study employing 79 adults, Menaker *et al.*[12] evaluated Listerine Antiseptic as an aid to normal

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toothbrushing in removal of dental plaque. Using individuals who were screened for a tendency to form dental plaque, a double-blind clinical trial was initiated after a thorough dental prophylaxis. Participants rinsed with Listerine Antiseptic twice daily over the course of the trial. At the completion of the trial, the group that used the Listerine Antiseptic displayed a statistically significant 43% reduction in accumulation of plaque as compared to the group that used toothbrushing and a control mouthrinse.

In a study designed to test the effectiveness of Listerine Antiseptic in the absence of normal oral hygiene measures, Lusk *et al.*[13] evaluated gingivitis and plaque accumulation in a group of 13 periodontists over 12-day periods. Rinsing three times per day for 1 minute with Listerine Antiseptic reduced gingivitis by 79% ($p < 0.01$) as compared to a water rinse. Rinsing three times per day for 5 seconds, Listerine Antiseptic reduced gingivitis 31% (not statistically significant) as compared to a water rinse. Statistically significant reduction in plaque surface area and new plaque accumulation as measured by wet weight were also observed when Listerine Antiseptic was used three times per day for 1 minute and three times per day for 5 seconds. Fornell, Sundin, and Lindhe[14] also evaluated the effect of Listerine Antiseptic on plaque accumulation and gingivitis in the absence of normal oral hygiene measures. This investigation used a crossover study design in which participants received repeated prophylaxes and followed oral hygiene measures to improve gingival health. All oral hygiene measures then ceased, and rinsing began. Over a 2-week evaluation, participants using Listerine Antiseptic three times per day for 60 seconds displayed 53% less plaque surface area accumulation, 93% less plaque wet weight accumulation, and 47% lower gingivitis scores than the group using the water rinse control.

TABLE 3. *Adjusted Mean Gingival Index Scores (± SEM) at 1, 3, and 6 Months After Initiation of the Clinical Trial*

Group	1 Month	3 Months	6 Months
Listerine Antiseptic	2.082 ± 0.027	1.575 ± 0.034	1.197 ± 0.038
Vehicle control	2.200 ± 0.026	1.939 ± 0.033	1.655 ± 0.039
Water control	2.317 ± 0.027	1.933 ± 0.034	1.668 ± 0.040

Listerine Antiseptic ($p \leq 0.002$) at 1 month *versus* vehicle and water controls.
Listerine Antiseptic ($p < 0.001$) at 3 and 6 months *versus* vehicle and water controls.
Vehicle control ($p \leq 0.002$) at 1 month *versus* water control.

TABLE 4. *Mean Stain Index Scores (± SEM) at Baseline and 1, 3, and 6 Months After Initiation of the Clinical Trial*

Group	Baseline	1 Month	3 Months	6 Months
Listerine Antiseptic	0.46 ± 0.056	0.36 ± 0.061	0.46 ± 0.083	0.53 ± 0.076
Vehicle control	0.50 ± 0.077	0.41 ± 0.069	0.44 ± 0.090	0.60 ± 0.107
Water control	0.42 ± 0.074	0.32 ± 0.055	0.37 ± 0.072	0.55 ± 0.073

No statistically significant differences between groups was detected.

In the present study, participants were selected for existing plaque and gingivitis, and then randomly assigned to either treatment (Listerine Antiseptic) or one of two control (hydroalcoholic vehicle, water) groups. When used in conjunction with the participants' normal oral hygiene procedures, Listerine Antiseptic produced a clinically statistically significant reduction in accumulated dental plaque ($p < 0.001$) and existing gingivitis ($p < 0.001$) over a 6-month period. At 6 months, existing plaque scores were reduced in the Listerine Antiseptic group by 28.6% from baseline, 20.8% compared to the vehicle control scores, and 22.2% compared to the water control scores. Gingivitis reductions observed in the Listerine antiseptic group at 6 months were 55.7% as compared to baseline, and 28% compared to either the vehicle or water controls. These results were achieved in a population of dentally conscious individuals. As a result, with participation in a monitored 6-month study, an improvement in the oral health of the total population would be expected. This expected reduction was observed.

The greatest reductions in clinical parameters of gingival disease were observed with the treatment group. While the data is nonparametric, the observed reduction in gingivitis scores was even more pronounced than the reduction in plaque scores. In their report on the effect of Listerine Antiseptic on developing plaque and gingivitis, Fornell, Sundin, and Lindhe[14] observed that the treatment group displayed significantly less development of gingivitis than the water control. They noted, however, that the gingivitis that developed in their control group was mild, and they suggested that a greater effect of Listerine Antiseptic would be observed in a population with a greater tendency to develop gingivitis. By beginning our clinical trial with a population that did not receive any treatment to reduce existing gingival inflammation, we confirmed the suggestions of Fornell, Sundin, and Lindhe[14] regarding the ability of Listerine Antiseptic to modify the development of gingival inflammation.

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An *in vitro* study of the supragingival 6-month plaque collected from the patients in this study was conducted by Fine and Mandell.[18] They analyzed the wet plaque collected from buccal and lingual surfaces of the available first molars from the subjects. Analysis was for total plaque weight, total protein, Limulus lysate activity as a measure of gram negative organism activity, and fluorescent antibody as a measure of gram-positive organism activity. Their results detected no differences in Limulus lysate or fluorescent antibody activity. This suggested that the percentages of gram-negative and gram-positive organisms in the plaque were the same regardless of control or treatment regimen, and that use of Listerine did not upset the balance of the normal flora. Analysis of protein per unit weight, however, detected a decrease in protein content in the plaque harvested from the Listerine Antiseptic group. This suggested a decrease in the overall numbers of microorganisms within the plaque matrix of this group. Therefore, one can suggest that the mechanism of action of Listerine Antiseptic is a nonspecific reduction in the total number of microorganisms per plaque volume, with a resulting decrease in pathogenicity of the plaque challenge and a decrease in clinically detectable gingivitis.

This clinical trial suggests that Listerine Antiseptic, when used twice daily for 30 seconds, is effective in controlling plaque accumulation and gingivitis in a population that did not receive a prophylaxis. This report supports previous studies regarding the efficacy of this agent. Consideration of the clinical data, as well as the over-the-counter availability of Listerine Antiseptic, suggests that it should be considered as an aid to standard oral hygiene procedures for control of dental plaque and gingivitis.

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Efficacy of Listerine antiseptic in inhibiting the development of plaque and gingivitis

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Abstract. A 9-month double-blind controlled clinical study was conducted on adult subjects using either Listerine antiseptic, its vehicle control, or a water control in order to determine the efficacy of the antiseptic mouthrinse in inhibiting the development of plaque and gingivitis. Following screening examinations for minimal entry levels of plaque and gingivitis, all subjects received a complete prophylaxis. Subjects then continued their usual oral hygiene habits for a 3-week normalization period and were examined for soft tissue abnormalities and baseline measurements of plaque, gingivitis, and tooth stain. 2 additional prophylaxes were then performed, followed by a second baseline gingival examination. Zero plaque was re-established by rubber cup polishing and twice daily rinsing was begun. Soft tissue, plaque, gingivitis, and extrinsic tooth stain were evaluated after 1, 3, 6 and 9 months of rinsing with the randomly assigned mouthrinses.

Results demonstrated that Listerine antiseptic significantly reduced the development of plaque at 1, 3, 6 and 9 months and the development of gingivitis at 9 months, as compared to its vehicle control or water control.

During the past 25 years, it has become generally accepted that dental plaque is the main etiological agent of most forms of periodontal disease. Considerable emphasis by the dental profession has been placed on the prevention of the early form of these diseases, which is usually gingivitis, and on the prevention of its progression to the more destructive forms of periodontitis. Approaches to the prevention of gingivitis have centered upon increasing the public's awareness of the need for maintaining adequate levels of oral hygiene and research directed at evaluating mechanical and chemical methods of reducing and inhibiting plaque accumulation. Unfortunately, the widespread incidence of gingivitis indicates that current methods of educating and motivating patients to practice effective levels of oral hygiene have not been overly successful on a large-scale basis. The use of safe, effective, and convenient antimicrobial agents offers one approach to significantly reducing the world-wide incidence of periodontal diseases.

Listerine antiseptic has been shown to be an effective antiplaque agent. Previous short-term clinical studies ranging in duration from 7 to 60 days have shown that it retards the accumulation of dental plaque and decreases the severity of gingivitis when used either as a supplement to or in the absence of normal oral hygiene procedures (Kennedy & Kravets 1970, Gomer et al. 1972, Lusk et al. 1974, Fornell et al. 1975, Menaker et al. 1979). A longer term clinical study by Lamster et al. (1983) has shown that this agent significantly reduced existing plaque accumulations and severity of existing gingivitis in a 6-month experimental period. Unlike this previous 6-month study, the present study investigates the ability of the antiseptic mouthrinse to inhibit the develop-

ment of plaque and gingivitis in gingivitis-prone subjects after multiple prophylaxes were performed to maximize initial gingival health.

Material and Methods

144 medically healthy subjects consisting mostly of dental students and staff of Fairleigh Dickinson University Dental School, ranging in age from 18–54 years, were entered into the study. Criteria for entry were a minimum of 20 sound natural teeth, a gingivitis score of ≥2.0 using a non-invasive modification of the Loe-Silness Index (Loe & Silness 1963), and a plaque score of ≥1.8 using the Turesky modification (Turesky et al. 1970) of the Quigley-Hein Index (Quigley & Hein 1962). Grossly carious, fully crowned or restored, or orthodontically bonded teeth were excluded. Subjects with destructive periodontal disease or those on antibiotic or anti-inflammatory drugs were excluded from the study.

Upon entering the study, all subjects were given a prophylaxis (scaling and rubber cup polishing) to remove all plaque, calculus and extrinsic tooth stain. Subjects then followed their usual oral hygiene habits for a 3-week period. On the 22nd day following prophylaxis, all subjects had baseline 1 examinations of their soft tissue, plaque, gingivitis and extrinsic tooth stain. 2 additional prophylaxes were performed on each subject 4 to 7 days apart to minimize existing gingivitis prior to the initiation of rinsing. A second gingivitis examination (baseline 2) was performed 3 to 4 days following these additional prophylaxes. A rubber cup polishing was then performed prior to the first rinse to re-establish zero measurements for plaque, as confirmed by erythrosine disclosing solution, and for extrinsic tooth stain.

Subjects were assigned by a random code to one of 3 groups: Listerine antiseptic, a vehicle control which was identical to the active rinse less the active ingredients of eucalyptol, thymol, methyl salicylate and menthol, or a sterile colored water control.

Subjects began a regimen of rinsing with 20 ml of the assigned product for 30 s, twice daily, starting the day following the baseline 2 examination and rubber cup polishing. For the first 6 months, the rinsing was supervised by a hygienist on weekdays, except for holidays and recesses. The appropriate products were supplied for weekend and holiday use and the subjects were required to maintain a record of these unsupervised rinsings. Rinsings were unsupervised during the final 3-month experimental period. During this study, subjects followed their usual routine oral hygiene habits but were instructed to refrain from using other mouthrinses and to advise the investigator of the use of any medications. Oral contraceptive use did not constitute an exclusion from the study. All subjects were periodically given a non-therapeutic, low abrasive dentifrice and soft nylon toothbrushes.

Subjects were examined at a screening examination for entry into the study, baseline 1, baseline 2 (gingivitis only), and at 1, 3, 6, and 9 months following the initiation of rinsing. Subjects were instructed to refrain from brushing or rinsing on the mornings of examination days so that more reproducible plaque scores could be obtained.

Gingivitis was scored using a modification of the Loe-Silness Index (Loe & Silness 1963). The buccal and lingual gingival margins and interdental papillae of all acceptable teeth were scored as follows:

0 normal (absence of inflammation)
1 mild inflammation (e.g., slight changes in color) of any portion of the marginal gingival unit
2 mild inflammation (but no edema) of the entire gingival unit
3 moderate inflammation (moderate glazing, redness, edema and/or hypertrophy)
4 severe inflammation (marked redness and

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edema/hypertrophy, spontaneous bleeding or ulceration)

Plaque was scored using the Turesky modification (Turesky et al. 1970) of the Quigley-Hein Index (Quigley & Hein 1962) which emphasized plaque accumulations in approximation to the gingival margin. Buccal and lingual surfaces were scored using erythrosine disclosing solution as follows:

0 no plaque
1 separate flecks or a discontinuous band of plaque at the gingival margin
2 thin (up to 1 mm) continuous band of plaque at the gingival margin
3 band of plaque wider than 1 mm but covering less than 1/3 of the tooth surface
4 plaque covering more than 1/3 but less than 2/3 of the tooth surface
5 plaque covering 2/3 or more of the tooth surface

Extrinsic tooth stain on the labial surfaces of the 12 anterior teeth was scored by a modification of the Lobene index (Lobene 1968) described by Lamster et al. (1983). The order of each examination was soft tissue, stain index, gingivitis index, and plaque index. Following the 9-month examination, wet plaque was harvested supragingivally from the buccal and lingual surfaces of 20 teeth, weighed and frozen for biochemical analysis (Fine et al. 1985).

The study was conducted double-blind and all scoring was performed by one examiner. The study was designed to provide a minimal power of 0.8 for detecting a clinically important difference at the 0.05 probability level. Average indices or scores were determined for each subject and these averages were analyzed by analysis of covariance. In the analyses, plaque and gingivitis scores were adjusted for differences in baseline means. The 3 treatment groups were well balanced with respect to age and sex and no stratification was required for these or other population variables. Separate analyses were performed for the group of 127 volunteers who completed 6 months of the study and for the subject group of 85 volunteers who completed the entire 9-month study period.

Results

Of the 144 subjects accepted after screening, 136 subjects remained in the study after the 3-week normalization period. 127 subjects completed 6 months of the study; 44 in the experimental group, 38 in the vehicle control group, and 45 in the water control group. 85 subjects continued in the study for an additional 3 months of unsupervised rinsings; 27 in the experimental group, 28 in the vehicle control group and 30 in the water control group. Most of the subjects who did not participate for the additional 3 months of the study were dental students who had recently graduated and were not available for the 9-month examination.

The mean plaque index scores for the 127 subjects who completed 6 months and for 85 subjects who completed 9 months are given in Tables 1 and 2, respectively. Analysis of covariance using the baseline 1 scores as the covariate were performed at each post-treatment time. Using the 127 subjects who completed 6 months, the differences between the experimental group and the control groups were significant at 1, 3, and 6 months. When converted to % reduction of dental plaque scores, adjusted for pre-treatment differences, the experimental group showed a 12.1%, 18.3%, and 18.0% reduction as compared to the water control at 1, 3, and 6 months, respectively. When compared with its vehicle control, it showed an 8.1, 16.0, and 14.9% reduction in plaque at 1, 3, and 6 months, respectively. No significant differences were found between the water and vehicle control groups at any time period.

Using the 85 subjects that completed 9 months, the differences in plaque index scores

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Table 1. Mean plaque scores for experimental, vehicle and water controls for 127 subjects who completed 6-months

Mittlere Plaque-Scores der Versuchs-, Vehikel- und Wasser-Kontrollgruppen, nach 6 Monaten bei 127 Probanden

Scores moyens de la plaque chez 127 sujets ayant participé à l'étude pendant les 6 mois premiers. Valeurs suivant le groupe: expérimental, témoin utilisant l'excipient (vehicle) ou l'eau (water)

Group	Baseline 1	1 month	3 months	6 months
experimental	2.06±0.05*	1.77±0.05	1.76±0.06	1.84±0.06
water	2.09±0.06	2.03±0.06	2.18±0.07	2.27±0.07
vehicle	2.13±0.05	1.97±0.06	2.15±0.08	2.22±0.07

* Standard error of the mean.

Analysis of covariance using baseline 1 scores as the covariate showed differences between adjusted scores of experimental and water control significant at the $p<0.01$ level at 1, 3, and 6 months; differences between experimental and vehicle control were significant at 1 month at the $p<0.05$ level and at 3 and 6 months at the $p<0.01$ level.

* *Standardirrtum des Mittelwertes.*

Die Analyse de Co-Varianz, bei der die Scores der Ausgangsuntersuchung 1 als Co-Variat gewählt worden sind, zeigte, dass die Unterschiede zwischen den justierten Scores der Experiment- und Wasser-Kontrollgruppen sich auf einem Signifikanzniveau von $p<0.01$ (nach 1, 3 und 6 Monaten) befanden: die Unterschiede zwischen der Versuchs- und der Vehikel-Kontrollgruppe hatten nach 1 Monat eine Signifikanz von $p<0.05$ erreicht und befanden sich nach 3 und 6 Monaten auf dem Niveau von $p<0.01$.

* *Erreur-type de la moyenne.*

L'analyse de covariance considérant la covariation des scores de l'examen baseline 1 mettait en évidence entre les scores ajustés du groupe expérimental et du groupe témoin utilisant l'eau des différences significatives a un niveau de $p<0.01$ à 1, 3 et 6 mois; les différences entre le groupe expérimental et le groupe témoin utilisant l'excipient étaient significatives a un niveau de $p<0.05$ à 1 mois et $p<0.01$ à 3 et 6 mois.

Table 2. Mean plaque scores for experimental, vehicle and water controls for 85 subjects who completed 9 months

Mittlere Plaque-Scores der Versuchs-, Vehikel- und Wasser-Kontrollgruppen, nach 9 Monaten bei 85 Probanden

Scores moyens de la plaque chez 85 sujets ayant participé à l'étude pendant les 9 mois; valeurs suivant le groupe: expérimental, témoin utilisant l'excipient ou l'eau

Group	Baseline 1	1 month	3 months	6 months	9 months
experimental	1.99±0.07*	1.68±0.06	1.68±0.07	1.75±0.09	1.93±0.08
water	2.13±0.07	2.07±0.08	2.22±0.09	2.38±0.09	2.49±0.07
vehicle	2.20±0.06	2.03±0.08	2.28±0.09	2.26±0.08	2.36±0.08

* Standard error of the mean.

Analysis of covariance using baseline 1 scores as the covariate showed differences between adjusted scores of experimental and water control significant at the $p<0.01$ level at 1, 3, 6, and 9 months; difference between experimental and vehicle control were significant at 1 month at the $p<0.05$ level and at 3, 6, and 9 months at the $p<0.01$ level.

* *Standardirrtum des Mittelwertes.*

Die Analyse de Co-Varianz, bei der die Scores der Ausgangsuntersuchung 1 als Co-Variat gewählt worden sind, zeigte, dass die Unterschiede zwischen den justierten Scores der Experiment- und Wasser-Kontrollgruppen sich auf einem Signifikanzniveau von $p<0.01$ (nach 1, 3, 6 und 9 Monaten) befanden: die Unterschiede zwischen der Versuchs- und der Vehikel-Kontrollgruppe hatten nach 1 Monat eine Signifikanz von $p<0.05$ erreicht und befanden sich nach 3, 6 und 9 Monaten auf dem Niveau von $p<0.01$.

* *Erreur-type de la moyenne.*

L'analyse de covariance considérant la covariation des scores de l'examen baseline 1 mettait en évidence entre les scores ajustés du groupe expérimental et du groupe témoin utilisant l'eau des différences significatives au niveau de $p<0.01$ à 1, 3, 6 et 9 mois; la différence entre le groupe expérimental et le groupe témoin utilisant l'excipient était significative au niveau de $p<0.05$ à 1 mois et $p<0.01$ à 3, 6 et 9 mois.

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Table 3. Mean gingivitis scores for experimental, vehicle and water controls for 127 subjects who completed 6 months

Mittlere Gingivitis-Scores der Versuchs-, Vehikel- und Wasser-Kontrollgruppen, nach 6 Monaten bei 127 Probanden

Scores moyens de la gingivite chez 127 sujets ayant participé à l'étude pendant les 6 premiers mois: valeurs suivant le groupe: experimental, temoin utilisant l'excipient ou l'eau

Group	Baseline 1	Baseline 2	1 month	3 months	6 months
experimental	1.60 ± 0.04*	1.39 ± 0.05	1.54 ± 0.04	1.27 ± 0.05	1.31 ± 0.07
water	1.60 ± 0.03	1.38 ± 0.04	1.55 ± 0.04	1.38 ± 0.05	1.46 ± 0.06
vehicle	1.59 ± 0.05	1.33 ± 0.05	1.49 ± 0.05	1.25 ± 0.05	1.37 ± 0.07

* Standard error of the mean.

There were no significant differences between any groups at any time period using baseline 2 as the covariate.

* *Standardirrtum des Mittelwertes.*

Die Analyse der Co-Varianz, bei der die Scores der Ausgangsuntersuchung 2 als Co-Variat gewählt worden sind, zeigten in keinem Zeitabschnitt bei und zwischen keiner Gruppe signifikante Unterschiede.

* *Erreur-type de la moyenne.*

Il n'existait à aucun moment de différence significative entre aucuns des groupes quand on considérait la covariation des scores de l'examen baseline 2.

between the experimental group and the control groups were significant at 1, 3, 6, and 9 months. When converted to % reductions of dental plaque scores, adjusted for pre-treatment differences, the experimental group showed a 15.5, 20.9, 23.7, and 19.5% reduction as compared to the water control at 1, 3, 6, and 9 months, respectively. When compared to vehicle control, the experimental rinse showed a 11.7, 21.6, 18.6, and 13.8% reduction in plaque at 1, 3, 6, and 9 months, respectively. No significant differences were found between the water and vehicle control groups at any time.

The mean scores for gingivitis for the 127 subjects who completed 6 months and 85 subjects who completed 9 months are presented in Tables 3 and 4, respectively. Following an initial prophylaxis on the 127 subjects who completed the 6 months, all three groups had a mean modified gingival index score of 1.60. After additional prophylaxes, their gingival index was reduced to a baseline 2 adjusted mean of 1.36. When converted to % reduction in gingivitis scores, adjusted for pre-treatment differences at baseline 2, the experimental mouthrinse reduced gingivitis scores by 0.9%, 7.9% and 10.4% compared to water control at 1, 3, and 6 months, respectively. Compared to the vehicle control, gingivitis scores were reduced −1.6%, −0.5% and 6.5%, at 1, 3, and 6 months, respectively. No significant differences were found between the treatment groups at any time period, and mean gingivitis scores were still at or near baseline 2 scores.

Following an initial prophylaxis on only the 85 subjects that completed 9 months, all 3 groups had a modified gingival index score of 1.60. After additional prophylaxes, their gingivitis index was reduced to a baseline 2 adjusted mean of 1.39. After 9 months of rinsing, only the experimental group showed a significant decrease in gingivitis scores (mean of 1.13) compared to baseline 2 scores. Both the vehicle control and water control, with means of 1.43 and 1.52 at 9 months, respectively, now demonstrated a trend towards a return to baseline 1 scores.

When converted to % reductions in gingivitis scores, adjusted for pre-treatment differences at baseline 2, the experimental mouthrinse reduced gingivitis scores by 5.1, 9.0, 20.8, and 23.9% compared to the water control at 1, 3, 6, and 9 months, respectively. Compared

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Table 4. Mean gingivitis scores for experimental, vehicle and water controls for 85 subjects who completed 9 months

Mittlere Gingivitis-Scores der Versuchs-, Vehikel- und Wasser-Kontrollgruppen, nach 9 Monaten bei 85 Probanden.

Scores moyens de la gingivite chez 85 sujets ayant participé à l'étude pendant les 9 mois: valeurs suivant le groupe: experimental, temoin utilisant l'excipient ou l'eau

Group	Baseline 1	Baseline 2	1 month
experimental	1.59±0.04*	1.39±0.07	1.51±0.04
water	1.60±0.04	1.42±0.05	1.61±0.04
vehicle	1.61±0.07	1.36±0.06	1.50±0.06
Group	**3 months**	**6 months**	**9 months**
experimental	1.23±0.06	1.23±0.09**	1.13±0.08***
water	1.37±0.06	1.57±0.07	1.52±0.10
vehicle	1.25±0.07	1.42±0.09	1.43±0.10

* Standard error of the mean.

** Experimental group had significantly less gingivitis than water control at 6 months ($p<0.05$) using baseline 2 as the covariate.

*** Experimental group had significantly less gingivitis than water control and vehicle control at 9 months ($p<0.05$) using baseline 2 scores as the covariate.

* *Standardirrtum des Mittelwertes.*

** *Bei der Wahl der Ausgangsuntersuchung 2 als Co-Variat zeigte es sich, dass die Versuchsgruppe bei der Kontrolle nach 6 Monaten signifikant weniger häufig Gingivitis hatte ($p<0.05$) als die Wasser-Kontrollgruppe.*

*** *Bei der Wahl der Ausgangsuntersuchung 2 als Co-Variat zeigte es sich, dass die Versuchsgruppe nach 9 Monaten signifikant weniger häufig Gingivitis hatte ($p<0.05$) als die Wasser-Kontroll- und die Vehikel-Kontrollgruppe.*

* *Erreur-type de la moyenne.*

** *Le groupe expérimental avait significativement moins de gingivite que le groupe témoin utilisant l'eau à 6 mois ($p<0.05$) quand on considérait la covariation des valeurs de l'examen baseline 2.*

*** *Le groupe expérimental avait significativement moins de gingivite que le groupe témoin utilisant l'eau et le groupe témoin utilisant l'excipient à 9 mois ($p<0.05$), quand on considérait la covariation des scores de l'examen baseline 2.*

to its vehicle control, gingivitis scores were reduced by 0.7, 3.2, 14.1, and 22.1% at 1, 3, 6, and 9 months, respectively. The differences between the experimental and water control group were statistically significant ($p<0.05$) at 6 months; differences between the experimental and both control groups were statistically significant ($p<0.05$) at 9 months.

No significant extrinsic staining or soft tissue effects were observed in any of the groups.

Discussion

The results of this study indicate that Listerine antiseptic was effective in reducing the accumulation of plaque and the development of gingivitis in a 9-month experimental period following several prophylaxes. At 9 months, the experimental group showed a mean plaque score 3.0% less than baseline 1; in the water control group there was a 16.9% increase in plaque, and in the vehicle control group a 7.3% increase. Gingivitis scores for the experimental group were 18.7% less at 9 months than at the baseline 2 examination, as compared to an increase in the gingival index of 7.0% for water and 5.1% for vehicle controls.

The effectiveness of this agent in reducing plaque accumulation and gingivitis scores has been documented in several short-term clinical

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studies and in a 6-month study by our group. The latter study by Lamster et al. (1983) demonstrated that Listerine was effective in reducing existing plaque and gingivitis scores. Subjects in that study showed a statistically significant reduction in plaque and gingivitis scores at 1, 3, and 6 months for Listerine versus vehicle and water controls. However, that population, unlike the one that participated in the present study, was not given repeated prophylaxes prior to rinsing with the experimental or control agents. In this study, plaque scores were significantly reduced in the experimental group at all examination times as compared to the control groups; however, % reductions were lower in this study than in those reported by Lamster et al. (1983). The lower % reduction probably reflects the lower initial plaque scores in this study due to the multiple prophylaxes received by all participants.

Unlike the study by Lamster et al. (1983), no significant difference for gingivitis was observed between groups in the first 6 months. This is probably due to the improvement in gingival health resulting from 4 prophylaxes initially, followed by continuation of usual oral hygiene. At 6 months, the control groups did show a trend toward higher gingival scores than at baseline 2, whereas the active agent group showed lower gingivitis scores than after the multiple prophylaxes. Only at 9 months, however, did this group demonstrate a statistically significant reduction in the gingival index, compared to controls. This delay in demonstrating a gingivitis reduction is undoubtedly related to the lower initial gingival scores achieved by multiple prophylaxes prior to rinsing in this study (adjusted mean 1.36), as compared to the prior study (adjusted mean > 2.0).

The present study also suggests that since it may require 9 months to observe a significant decrease in the development of gingivitis in patients with an initial lower level of gingivitis, patients with minimal gingivitis and those who are recalled more frequently than every 6 months may not experience additional significant reductions in gingivitis with the use of Listerine antiseptic despite significantly reduced plaque scores. Further research is needed to determine whether a significant reduction in plaque scores without a concomitant decrease in gingivitis scores are of clinical importance.

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Zusammenfassung

Die Wirksamkeit antiseptischen Listerins bei der Inhibition der Plaqueanlagerung und der Entwicklung von Gingivitis

Eine 9 Monate andauernde, kontrollierte Doppelblindstudie wurde mit erwachsenen Probanden durchgeführt. Sie hatte zur Aufgabe, die Wirksamkeit von Mundspülungen mit sowohl antiseptischem Listerin, dessen Vehikel, als auch von Kontrollspülungen mit Wasser hinsichtlich der Einwirkung auf Plaquebildung und auf vorhandene Gingivitis festzustellen. Nach der Probandenauswahl, die Minimalniveaus oraler Gesundheit voraussetzte, wurden alle Probanden mit vollständiger Prophylaxetherapie behandelt. Sie wurden dann während einer 3-wöchentlichen sog. Normalisierungsperiode ihren normalen Hygienegewohnheiten überlassen. Dann wurden Veränderungen an Weichgeweben, Ausgangswerte des Plaquevorkommens, der Gingivitus und das Vorkommen von Zahnverfärbungen registriert. Daran schlossen sich 2 weitere Prophylaxebehandlungen an und eine 2. Ausgangsuntersuchung bewertete die dann vorhandene Situation der Gingiva. Plaquefreiheit wurde durch Polieren mit Gumminäpfchen erreicht. Danach wurden die zweimal täglich vorgenommenen Mundspülungen eingesetzt. Die Weichgewebe, die Plaque, die Gingivitis und auch die von aussen her (extrinsic) induzierte Zahnverfärbung wurden, nach 1, 3, 6 und 9 Monaten der Mundspülung mit zufällig eingesetzten Mundspülungslösungen, überprüft.

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Die Ergebnisse zeigen, dass antiseptisches Listerin die Plaqueentwicklung nach 1, 3, 6 und 9 Monaten signifikant reduzierte. Das gleiche gilt für die nach 9 Monaten registrierte Entwicklung der Gingivitis im Vergleich zu der Kontrollgruppe mit den Vehikelspülungen und der Kontrolle mit Wasser als Spülflüssigkeit.

Résumé

Efficacité de Listerine Antiseptic, utilisé pour inhiber l'apparition de plaque et de gingivite

Chez des sujets adultes utilisant soit Listerine Antiseptic soit, à titre de témoins, l'excipient de ce produit ou l'eau, une étude clinique sur 9 mois a été effectuée en double aveugle, afin de déterminer l'efficacité des rinçages antiseptiques destinés à inhiber l'apparition de plaque et de gingivite. Après des examens de dépistage préliminaire visant à assurer que la plaque et la gingivite des sujets participant à l'étude dépassaient une limite inférieure, tous les sujets ont reçu un détartrage-polissage. Les sujets ont ensuite continué pendant 3 semaines (période de normalisation) leurs soins habituels d'hygiène buccodentaire. Ils ont alors subi un examen pour constater les anomalies des tissus mous et déterminer les valeurs initiales de la plaque, de la gingivite et des colorations dentaires externes (baseline 1). On a ensuite pratiqué deux nettoyages supplémentaires, puis un deuxième examen gingival initial (baseline 2). La plaque a été ramenée à zéro par un polissage à la cupule de caoutchouc et les rinçages bi-quotidiens ont alors commencé. Les tissus mous, la plaque, la gingivite et les colorations dentaires externes ont été évalués après 1, 3, 6 et 9 mois de rinçages avec le produit désigné par tirage au sort.

Les résultats ont mis en évidence que, par comparaison avec son excipient seul ou avec l'eau, utilisés comme témoins, Listerine Antiseptic réduisait significativement la formation de la plaque à 1, 3, 6 et 9 mois et l'établissement d'une gingivite à 9 mois.

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Chemotherapeutic inhibition of supragingival dental plaque and gingivitis development

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Abstract. A 6-month double-blind, controlled clinical study was conducted on 107 healthy adult subjects to determine the efficacy of a mouthrinse used as a supplement to regular oral hygiene measures on supragingival dental plaque and gingivitis. 115 healthy adult patients were recruited for the study. Following screening examinations for minimal entry levels of existing gingivitis and plaque in patients with a minimum of 20 sound natural teeth, extrinsic tooth stain, gingivitis and plaque index scores were recorded. Soft tissues were evaluated. All subjects then received a complete dental prophylaxis, removing plaque, calculus and extrinsic stain. Utilizing their normal oral hygiene, subjects began a regimen of rinsing with 20 ml of the randomly assigned rinse, twice daily for 30 s for 6 months. 7 days after prophylaxis, gingivitis was again scored (baseline 2). Soft tissue, gingivitis, plaque area and extrinsic stain were evaluated again at 3 and 6 months. Results demonstrated that after 6 months, listerine produced a 34% inhibition of both plaque and of gingivitis compared to a hydroalcohol control ($p<0.001$).

Key words: plaque; gingivitis; mouthrinse; listerine; clinical trial.

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Gingivitis, which is prevalent in a large proportion of the adult population (Stamm 1986), can be considered an infectious disease. Clinical signs of gingivitis include increased gingival crevicular fluid flow, erythema, edema, bleeding, loss of normal gingival contour and/or stippling and, in advanced cases, ulceration. Numerous clinical studies have substantiated the role of microbial plaque as the principal etiologic agent in the development of gingivitis. When inadequately controlled, plaque may mature and extend subgingivally, leading to the development of periodontitis. Progression to periodontitis can be retarded or halted by elimination or reduction of dental plaque by either mechanical or chemical means, or both (Suomi et al. 1971, Axelsson & Lindhe 1978, Lobene 1979). Mechanical elimination is currently the most widely accepted means to control plaque. However, mechanical plaque removal is time intensive, requires patient motivation and cooperation, and requires adequate manual dexterity. Thus inherent problems exist in all attempts to educate, train and motivate patients to achieve reduction of plaque solely by mechanical means. For this reason, safe, effective chemotherapeutic agents as adjuncts to mechanical plaque removal would be desirable.

At least three mouthrinses have shown promise as plaque inhibitors. The antiplaque properties of 0.2% concentration of chlorhexidine are well documented. Initial short term studies were followed by long term human trials in which chlorhexidine was administered in different concentrations and delivery modes such as dentifrices and mouthrinses. The overall summation of the results of these investigations suggested the plaque inhibiting potential of this agent. (Löe & Schiott 1970a, Löe 1973, Löe et al. 1976). A longitudinal trial with a mouthrinse containing 0.12% chlorhexidine gluconate was successful in reducing supragingival plaque accumulation and gingivitis in adults compared to a placebo (Grossman et al. 1986). Adverse microbiological changes reportedly did not occur in volunteers participating in that same study (Briner et al. 1986). A sanguinarine mouthrinse showed significant plaque and gingivitis reduction versus a placebo control in a 6-month trial (Palcanis et al. 1986). Microbiological assessment in this study showed a reduction in plaque colony forming units but no adverse effects on the flora from the saliva, tongue or buccal mucosa. Listerine antiseptic (LA), which contains thymol, menthol, eucalyptol and methyl salicylate as active ingredients, has been shown to be an effective antiplaque agent. Clinical studies have shown that LA retarded the formation of supragingival dental plaque (Kennedy & Kravets 1970, Gomer et al. 1972, Lusk et al. 1974, Fornell et al. 1975, Gordon et al. 1975, Menaker et al. 1979, Axelsson & Lindhe 1987, Mankodi et al. 1987) and decreased the degree and severity of gingivitis when used either as a supplement to or in the absence of mechanical oral hygiene in studies ranging from 7 days to 9 months (Lusk et al. 1974, Fornell et al. 1975, Lamster et al. 1985, Axelsson & Lindhe 1987, Mankodi et al. 1987).

This study evaluated the effect of LA and a control mouthrinse on supragingival plaque and on gingivitis. The effect on the plaque flora was determined

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and is reported separately (Minah et al. 1988).

Material and methods

115 healthy male and female adult volunteers, aged 18–60 years, with pre-existing plaque and gingivitis, but without clinical evidence of periodontitis, were screened prior to entry for a minimum of 20 sound, natural teeth. Grossly carious, fully crowned, orthodontically banded, abutment and third molar teeth were not included in the tooth count. Subjects with gross oral pathology or on antibiotic, antibacterial or antiinflammatory therapy were excluded from the study. For each of the 115 subjects meeting entry requirements, a complete intraoral soft tissue examination was performed to record the condition of the oral mucosae. All aberrations were recorded.

Extrinsic tooth stain was scored at baseline by the Lobene extrinsic tooth stain index (Lobene 1968) (Table 1) on the labial surfaces of the 12 anterior teeth. Each tooth was divided visually into the gingival and the body regions. Only the gingival region was scored, both for area and severity (intensity). The area and intensity scores were multiplied by each other for a tooth score; tooth scores were added for an individual total score.

Pre-existing plaque was assessed at baseline 1 by the Turesky modification of the Quigley-Hein Index (Turesky et al. 1970) (Table 2) on the buccal and lingual surfaces of all scorable teeth, using erythrosine disclosing solution. Gingivitis was scored at baseline 1 by the modified gingival index (MGI) (Lobene et al. 1986) (Table 3) on the buccal and lingual surfaces and interdental papillae of all scorable teeth. This non-invasive index is a modification of the gingival index (Lõe & Silness 1963) and was developed to increase sensitivity in the low-region of the scoring scale. In a recent three week clinical study, the MGI correlated significantly with the GI (Lobene et al. 1988). Each subject had minimum plaque and minimum gingivitis means of ≥1.95. One examiner performed all clinical indices; a series of index calibration sessions was held prior to initiation of the study.

Following the baseline 1 exam, subjects were given a complete prophylaxis to remove all plaque, calculus and extrinsic stain. 7 days later, gingivitis scores were again recorded (baseline 2) to correct for the gingival response to the prophylaxis. Baseline 1 and baseline 2 scores were used as covariates in the final analyses.

Subjects were assigned either to LA or control (5% hydroalcohol) group according to a computer-generated random code, by which double-blinding was maintained. Product group code was not disclosed to the examiner or recorded on case report forms.

On the same day as the prophylaxis, subjects began rinsing with 20 ml of the assigned rinse for 30 s, twice daily for 6 months. Rinsings were under supervision on weekdays, unsupervised on weekends and holidays. Subjects were instructed not to rinse, eat or drink for 30 min following use of the test rinses. Personnel dispensing the test mouthwashes did not participate in any other aspect of the protocol. During the study, subjects followed their usual oral hygiene and dietary habits, but were instructed to refrain from using commercial mouthrinses and to advise the investigator of the use of antibiotic or antiinflammatory drugs. A soft nylon toothbrush and a fluoridated toothpaste were provided for all subjects throughout the study.

Subjects refrained from all oral hygiene and use of experimental products on examination days until after the examinations. Individual case report forms were used for soft tissue, stain, plaque and gingivitis indices: separate forms were used for each index at each examination interval. Findings were called out to a recording assistant. The examination procedures were repeated at 3 and 6 months. The examiner did not have access to case report forms until the completion of the examination.

The study was designed to provide a minimal power of 0.80 for detecting a clinically important difference to be statistically significant at the 0.05 probability level. A mean index or score was determined for each subject and the averages were analyzed by analysis of covariance.

Results

Initially, 115 subjects were entered; 107 completed the 6-month study. The treatment groups were well-balanced with respect to age, sex and smoking status obviating the need for stratification by these variables (Table 4).

Table 1. Tooth stain index (Lobene)

Area
- 0 – no stain detected
- 1 – stain up to 1/3 of the gingival region
- 2 – stain over 1/3 to 2/3 of the gingival region
- 3 – stain over more than 2/3 of the gingiva region

Severity or intensity
- 0 – no stain
- 1 – light stain (can be seen with close examination)
- 2 – moderate stain (obvious but not aesthetically unacceptable)
- 3 – heavy stain (obvious and aesthetically unacceptabel)

Table 2. Quigley-Hein plaque index (Turesky modification)

- 0 – no plaque
- 1 – separate flecks or discontinuous band of plaque at the gingival (cervical) margin of the surface
- 2 – thin (up to 1 mm), continuous band of plaque at the gingival margin of the surface
- 3 – band of plaque wider than 1 mm but less than 1/3 of surface
- 4 – plaque covering 2/3 or more, but less than 2/3, of surface
- 5 – plaque covering 2/3 or more of surface

Table 3. Modified gingival index (Lobene)

- 0 – absence of inflammation
- 1 – mild inflammation (slight change in color, little change in texture) of *any portion* of but not the entire gingival unit
- 2 – mild inflammation of the *entire* gingival unit
- 3 – moderate inflammation (glazing, redness, edema and/or hypertrophy) of the entire gingival unit
- 4 – severe inflammation (marked redness, edema and/or hypertrophy, spontaneous bleeding, congestion, or ulceration) of the entire gingival unit

Table 4. Demographic variables

	Listerine antiseptic		*Hydroalcohol control*	
	No.	%	No.	%
Sex				
male	32	59.3	30	56.6
female	22	40.7	23	43.4
total	54		53	
Smokers	9	16.7	7	13.5
Non-smokers	45	83.3	45	86.5
Age				
average	28.48		27.64	
std. error	0.87		0.67	
range	22–60		20–45	

Table 5. Adjusted mean plaque index scores

Group	3 months	S.E.*	6 months	S.E.
listerine antiseptic	1.600	0.068	1.150	0.062
5% hydroalcohol control	1.805	0.069	1.753	0.062

*Standard error.

Table 6. % of tooth surfaces which received a plaque index of 0, 1, 2, 3, 4, 5 at baseline and after 6-months of trial

Plaque index (Quigley-Hein) Score	0	1	2	3	4	5
Listerine						
baseline	0	5	45	40	8	2
6 months	35	25	29	10	1	0
Control						
baseline	0	4	48	40	7	1
6 months	15	23	39	21	2	0

Plaque

Adjusted mean plaque index scores at 3 and 6 months are presented in Table 5. Adjusted treatment means are estimates of what the treatment means would be if all the group means at baseline were equal to the mean for all subjects at baseline (Steel & Torrie, 1980). Analyses of covariance, using the pre-prophylaxis baseline scores as covariate, showed that LA inhibited the accumulation of supragingival dental plaque by 11.4% ($p<0.05$) at 3 months and by 34.4% ($p<0.001$) at 6 months compared to its hydroalcohol control. Measurements performed at the 6-month examination when compared to baseline revealed at 54.6% reduction of plaque in the LA group compared to 31.1% decrease in the control population. Tooth surfaces with plaque index scores of 0 or 1 increased 55% in the LA group, from 5% at baseline to 60% at 6 months, and 34% in the control group from 4% at baseline to 38% at 6 months. Surfaces with plaque index scores of 2 to 5 were reduced from 95% at baseline to 40% at 6 months in the experimental group, while in the control group these were decreased from 96% at baseline to 62% at 6 months (Table 6).

Gingivitis

Adjusted mean gingivitis index scores are presented in Table 7. Using baseline 1 scores as covariate, gingivitis development was inhibited in the LA group by 3.4% at 3 months and by 33.7% ($p<0.001$) at 6 months, compared to the control. Using baseline 2 scores as covariate, LA inhibited the development of gingivitis by 4.2% at 3 months and by 34.0% ($p<0.001$) at 6 months, compared to the control.

The distribution of gingivitis index scores at baseline 1, baseline 2 and after 6 months are shown in Table 8. The effect of the prophylaxis following baseline 1 exams was demonstrated most markedly by the decrease in the percent of gingival units which were scored 3 at baseline 2, from 31% at baseline 1 to 17% in the LA group and from 24% at baseline 1 to 12% at baseline 2 in the control group.

The % of clinically healthy gingival units (score of 0) increased from 0% at baseline 2 to 38% at 6 months in the LA group, and from 0% at baseline 2 to 19% in the control group at 6 months. In total, 76% of the gingival scores in the LA group improved between baseline 2 and 6 months, while 52% of these scores in the control group improved during the course of the study.

Extrinsic tooth stain; soft tissue

Extrinsic tooth stain was not observed in either the experimental or control group in this study, as shown in Table 9. A stain index score of 1.0 indicates light stain. Average stain index scores for both the LA and control groups were well under 1.0.

Soft tissue condition was evaluated at each examination interval; no soft tissue aberrations attributable to either rinse were noted.

Table 8. % of gingival units which received a gingival index score of 0, 1, 2 and 3 at the baseline 1 and 2 after 6 months of trial

Score	Modified gingival index (Lobene) 0	1	2	3
Listerine				
baseline 1	0	3	65	31
baseline 2	0	12	71	17
6 months	38	31	29	2
Control				
baseline 1	0	4	72	24
baseline 2	0	14	74	12
6 months	19	31	47	4

Discussion

Interest in preparations formulated to reduce supragingival plaque accumulation and thereby inhibit gingivitis is a recent development. The antiseptic rinse (LA) under study has been utilized for many years and has been shown to possess antiplaque capabilities (Lusk et al. 1974, Fornell et al. 1975).

The primary objective of this investigation was to determine the effect of the long term use of LA. The results indicate that although both rinses showed significant improvement from their respective baselines, LA significantly inhibited the development of plaque and gingivitis compared to the hydroalcohol control. These results were comparable to the reduction of plaque and gingivitis obtained by other investigators using LA as a supplement to normal oral hygiene (e.g., Gomer et al. 1972, Lusk et al. 1974, Gordon et al. 1985, Axelsson & Lindhe, 1987). Furthermore, oral soft tissues were not adversely affected by twice daily use of listerine antiseptic for 6 months nor was there an increase in extrinsic tooth stain as compared to baseline or control.

Table 7. Adjusted mean gingivitis index scores

Adjusted for baseline 1 group	3 months	S.E.*	6 months	S.E.
listerine antiseptic	1.522	0.069	0.918	0.059
5% hydroalcohol control	1.576	0.069	1.385	0.060

*Standard error

Table 9. Extrinsic tooth stain index

Group	Baseline 1 mean	Baseline 1 S.E.*	3 months mean	3 months S.E.	6 months mean	6 months S.E.
listerine	0.23	0.094	0.07	0.032	0.06	0.037
5% control	0.17	0.050	0.10	0.013	0.02	0.010

*Standard error

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A number of possibilities can be offered to explain the antiplaque/antigingivitis activity of this agent. It might inhibit: the proliferation of newly developed or established plaque; the colonization or adherence of plaque organisms; or selected plaque inhabitants with a concommitant decrease in endotoxin content (Fine et al. 1985). Each of these events could lead to plaque reduction after a long exposure to the agent and still not produce pronounced microbiological shifts.

In the case of mouthrinses such as chlorhexidine, the marked plaque reduction has been primarily attributed to the strong antimicrobial action of chlorhexidine (Löe et al. 1970). A 6-month trial with a mouthrinse containing 0.12% chlorhexidine gluconate led to significant plaque reduction at 3 and 6 months versus a placebo in adult volunteers who rinsed twice daily with the product. Adverse side-effects of chlorhexidine were increased accumulation of dental calculus and extrinsic tooth stain (Grossman et al. 1986).

There was an overall reduction of plaque and gingivitis in the both the LA and control groups. In part this may be attributed to the professional dental prophylaxis each subject received at baseline (Table 5). Additionally, the logistics, design and structure of clinical studies of this type can have a profound effect on an individual subjects' overall willingness to improve their oral hygiene. This so called "placebo effect" has been reported previously (Axelsson & Lindhe, 1987) and must be recognized when one interprets results of clinical trials.

In conclusion, a comparison of LA to a 5% hydroalcohol control, when used as a supplement to normal oral hygiene following a prophylaxis, revealed that LA significantly reduced plaque and gingivitis over a six month period, without an increase in extrinsic tooth stain.

Zusammenfassung

Chemotherapeutische Inhibition der Entwicklung von supragingivaler Zahnplaque und Gingivitis

An 107 gesunden erwachsenen Probanden wurde eine 6 Monate andauernde, doppelblinde, kontrollierte klinische Studie durchgeführt um die Wirksamkeit einer Mundspülungsflüssigkeit festzustellen, die zur Verhinderung supragingivaler Plaquebildung als zusätzliches Moment bei regelmässig vorgenommenen oralen Hygienemassnahmen angewandt wurde. 115 gesunde erwachsene Patienten nahmen an dieser Studie teil. Nach Auswahluntersuchungen von Patienten mit mindestens 20 gesunden natürlichen Zähnen zur Feststellung der geringsten, zur Versuchsteilnahme berechtigenden vorliegenden Gingivitis- und Plaqueniveaus, wurden von aussen verursachte (extrinsic) Zahnverfärbungen, wie auch Gingivitis- und Plaqueindex-Scores registriert. Die Weichgewebe wurden beurteilt. Alle Versuchspersonen wurden dann vollständig prophylaktisch behandelt, wobei Plaque, Zahnstein und Verfärbungen entfernt wurden. Im Rahmen der Durchführung ihrer normalen oralen Hygienemassnahmen begannen die Versuchspersonen mit einer zusätzlichen, 30 Sekunden langen Mundspülung mit 20 ml einer zufällig zugeteilten Spülungsflüssigkeit, die dann 6 Monate lang, 2 mal täglich vorgenommen wurde. 7 Tage nach der Prophylaxebehandlung wurde die Gingivitis erneut registriert (Ausgangsuntersuchung 2). Weichgewebe, Gingivitis, mit Plaque bedeckte Regionen und Verfärbungen wurden nach 3 und 6 Monaten wiederum beurteilt. Die Resultate zeigten, dass das Listerin, im Vergleich mit einer mit Hydroalkohol spülenden Kontrollgruppe, eine 34%-ige Inhibition sowohl der Plaqueanlagerung als auch der Gingivitis ($p<0.001$) erreichen konnte.

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Résumé

Inhibition chimiothérapeutique du développement de la plaque dentaire sus-gingivale et de la gingivite

Une étude clinique contrôlée en double aveugle d'une durée de six mois a été menée chez 107 adultes sains afin de déterminer l'efficacité d'un bain de bouche utilisé en tant que supplément des mesures d'hygiène buccale régulières sur la plaque sus-gingivale et la gingivite. 115 adultes sains ayant au moins 15 dents saines ont été recrutés pour cette étude. A la suite des examens de dépistage pour de faibles niveaux d'entrée de plaque et de gingivite, la coloration dentaire extrinsèque ainsi que les scores de plaque et de gingivite ont été enregistrés. Les tissus mous ont été évalués. Tous les sujets ont alors reçu un nettoyage dentaire complet consistant à éliminer la plaque, le tartre et la coloration extrinsèque. Poursuivant leur hygiène buccale normale, les sujets ont débuté un régime consistant en un bain de bouche avec 20 ml d'une solution répartie au hasard, deux fois par jour pendant 30 secondes et durant une période de six mois. Sept jours après le nettoyage, le score de gingivite a de nouveau été noté. Les tissus mous, la gingivite, la plaque et la coloration extrinsèque ont encore été évalués aux mois 3 et 6. Les résultats ont démontré qu'après six mois d'utilisation la listérine avait produit une inhibition de 34% de la plaque et de la gingivite vis-à-vis d'un contrôle eau-alcool ($p < 0{,}001$).

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Effects of 6 months use of an antiseptic mouthrinse on supragingival dental plaque microflora

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Abstract. This study was undertaken to determine whether long-term use (6-months) of an antiseptic mouthrinse (Listerine Antiseptic, Warner Lambert Co., Morris Plains, NJ, USA) led to an undesirable succession of oral pathogens or the emergence of resistant microbial forms. Supragingival plaque was collected from 83 subjects before treatment and after either 3 or 6 months use of either the active antiseptic or a 5% hydroalcohol control. Subjects rinsed with their assigned mouthrinse twice daily under supervision. The plaque samples were analyzed for microbial content by darkfield microscopy, culture on a series of nonselective and selective bacterial media, and by recognition of microbial forms by recognition of distinct colony on a nonselective medium. Statistical analysis of the results revealed no significant microbial shifts including no significant increases in presumptive oral pathogens, spirochetes, black-pigmented *Bacteroides*, *Streptococcus mutans*, or *Candida albicans*. Additionally, no detectable rise in either staphylococci or enteric bacteria, potential opportunistic pathogens, was observed.

Key words: supragingival plaque microflora; antiseptic mouthrinse.

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Numerous reports on the in vitro antimicrobial activities of commercial antimicrobial mouthrinses and the active ingredients contained in them have been published (Lim et al. 1982, Dzink & Socransky 1985, Salem et al. 1987, Roberts & Addy 1981). However, the in vivo antibacterial activities of these mouthrinses have not been extensively examined. With the introduction of antiplaque and antigingivitis claims made by some new as well as established manufacturers of commercial mouthrinses, the need for critical evaluation of the antimicrobial activities of these products against plaque microflora has been recognized. One concern has been that their long-term usage could possibly lead to an undesirable succession of opportunistic species, or species which develop resistance to the antimicrobial mouthrinse.

The Council on Dental Therapeutics of the American Dental Association recently provided guidelines for acceptance of chemotherapeutic products for the control of supragingival dental plaque and gingivitis (*JADA* (1986) *112* 529). These guidelines include testing provisions of clinical efficacy, toxicological profiles, and microbiological assessment of supragingival dental plaque. Concerning the latter, the guidelines state that the "microbiological profile should demonstrate that pathogenic or opportunistic micro-organisms do not develop over the course of the study".

The present investigation was designed to test a commercial antiseptic mouthrinse, Listerine Antiseptic (Warner Lambert Co., Morris Plains, NJ), to meet provisions approved by the Council on Dental Therapeutics. The microbiological assessments are presented in this report. Clinical results will be reported separately.

Material and Methods

Subject selection criteria

Subjects were healthy male and female adult volunteers, aged 18–20 years, with pre-existing plaque and gingivitis, but without periodontal disease (ADA Type III or IV). Subjects with gross oral pathology or on antibiotic, antibacterial or anti-inflammatory therapy were excluded from the study.

Study design

115 volunteers were entered into the study. Subjects were assigned either to a treatment or control group according to a computer-generated random code, by which double-blinding was maintained. Of the 115 subjects entered into the study, 83 (42 in the active group and 41 in the control group) were selected by random code for characterization of the microbial content of their dental plaque. Immediately before the baseline clinical exams, supragingival plaque was harvested from the buccal and lingual surfaces of four designated teeth. Subjects were then given a complete prophylaxis to remove all plaque, calcu-

lus, and extrinsic tooth stain and began supervised rinsing with their assigned rinse while continuing their normal oral hygiene. Subjects were instructed to refrain from using commercial mouthrinses and to advise the investigator of the use of antibiotic or anti-inflammatory drugs.

Supragingival plaque was again collected after 3 months' treatment from 40 subjects (21 from the active group and 19 from the control group) for microbiological examination. As at the baseline exam, plaque was harvested before the clinical examinations. Plaque from these subjects was not harvested at 6 months since it was not known if the 3-month plaque harvest itself would alter the subsequent microbial composition. Supragingival plaque was collected from the remaining 43 subjects in the microbiology subset (21 from the active group and 22 from the control group) immediately before the 6-month clinical examinations.

Treatments

Listerine Antiseptic (Warner Lambert Co., Morris Plains, NJ), a combination of four essential oils (thymol, menthol, eucalyptol, and methyl salicylate) in an alcohol/surfactant base, was the active antiseptic in this study. The control was a similarly colored, flavored 5% ethanol (v/v) in water mouthwash.

Plaque collection

The 4 first molar teeth (or, if missing or unacceptable, the adjacent anterior tooth) were isolated with cotton rolls to prevent contamination with saliva. Supragingival plaque was collected with a curette from the buccal and lingual surfaces of each tooth. No effort was made to collect total plaque, nor was the harvested plaque weighed. Individual tooth plaque samples were placed immediately in vials containing 0.5 mls of reduced transport fluid (Loesche et al. 1972). The plaque specimens and vials were flushed with 85% N_2, 5% CO_2, 10% H_2 during collection. They were then placed in an anaerobic chamber (atmosphere 85% N_2, 5% CO_2, 10% H_2) for all further procedures. The plaque sample was dispersed initially with a Vortex mixer for 20 s, following which a pooled (by individual) aliquot was removed for microscopic examination. The remaining individual tooth plaque samples were diluted to 4.0 ml with reduced transport fluid, sonically dispersed for 20 s with a Kontes sonifier, then serially diluted in reduced transport fluid. Aliquots, 50 or 25 μl of each dilution, were used to inoculate selective and nonselective solid agar media.

Microscopic examination

Equal aliquots of plaque suspensions harvested from each of the four teeth from one subject were anaerobically pooled and mixed by vortexing. An aliquot of the mixed suspension was placed on a microscopic slide under a cover slip, and viewed by darkfield microscopy. Individual cells were identified by cell morphology within the following groups: spirochetes, motile rods, non-motile rods, and cocci. Sufficient numbers of fields were counted to assure statistical reliability. Results were recorded as the number of organisms within each cell morphological type per 100 cells observed (6).

Culture media and conditions

The nonselective and selective culture media used in this study are listed in Table 1. Presumptive identification of colonies on selective media was usually made by colonial morphology and Gram stain. When these measures were not adequate colony identities were confirmed using a battery of biochemical tests including Gram-stain, catalase and oxidase production, and end product analysis. Two colonies of the micro-organism in question were identified when additional tests were necessary. The API Identification Systems (Analytab Products, Plainview, NY), API20A for anaerobic bacteria, API20C for yeasts and API20E for Enterobacteriaceae were also employed for colony identification.

Approximate facultative anaerobe to obligate anaerobe ratios were calculated by dividing the MM10 + O_2 count by the obligate anaerobe count. The obligate anaerobe count was obtained by subtracting the MM10 + O_2 count from the MM10 count. Several bacterial genera and species were enumerated by recognition of characteristic colony morphology on the nonselective medium. These groups are listed in Table 2. This method provided a means of enumerating some bacterial genera for which selective media are not available and for confirmation of counts on selective media.

Table 1. Nonselective and selective culture media used for the cultivation of supragingival plaque bacteria

Culture medium	Incubation conditions	Incubation period (days)	Selected organisms
MM10 sucrose blood agar, MM10 (Loesche et al. 1972)	anaerobic 37°C	7	Nonselective: anaerobes and facultatives
MM10 sucrose blood agar, MM10 + O_2	aerobic 37°C	7	nonselective: aerobes and facultatives
Actinomyces agar, AC, (Zylber & Jordan 1982)	anaerobic 37°C	4	*Actinomyces* sp.
Desoxycholate agar, DC (BBL)	10% CO_2 in air, 37°C	4	enteric bacteria
Laked blood agar with kanamycin and vancomycin, LKBV (Finegold & Citron 1985)	anaerobic 37°C		*Bacteroides* sp.
Mannitol salt agar MS (BBL)	10% CO_2 in air	4	staphylococci
Mitis-salivarius bacitracin agar, (Gold et al. 1972)	anaerobic aerobic, RT	3 2	*Streptococcus mutans*
Neisseria agar N (Ritz 1967)	aerobic 37°C	4	*Neisseria* sp.
Rogosa SL agar RSL (Difco)	10% CO_2 in air, 37°C	4	*Lactobacillus* sp.
Sabouraud's agar SAB (BBL)	aerobic, 30°C	7	yeast
Veillonella agar, V (Difco)	anaerobic, 37°C	4	*Veillonella* sp.

Statistical analysis

Cellular morphological groups enumer-

Table 2. Recognizable bacterial groups on MM10 by colony and gram stain morphology

Group
Actinomyces sp.
Fusobacteria
Black Pigmented *Bacteroides* sp.
Streptococcus sp.
Streptococcus salivarius
Streptococci other (species other than *S. mutans*, *S. salivarius*, or *S. sanguis*)
Veillonella sp.
Streptococcus sanguis
Rods other (gram-negative anaerobic rods, probably *Bacteroides*)
Rods spreading vibriotypes *Selenomonas* and *Camphylobacter*
Yeast
Cocci other – not *Veillonella* gram-negative cocci
Cocci-
Neisseria (confirmed)

ated by darkfield microscopy were analyzed as the percentage for each type. Since plaque samples were pooled from each of the four teeth in each subject, the subject served as the observation unit.

Counts (colony forming units) from the selective and nonselective media and counts by colony morphology (described above) were analyzed using both the tooth site and the individual as the observation units. This analysis showed that there was much greater variation between subjects than between tooth sites within a subject. Therefore all analyses reported below are based on using the individual as the observation unit. Counts from the individual teeth were pooled by the most probable number technique to form individual estimates for each subject.

The observed counts or colony forming units observed at each dilution counted were statistically analyzed to determine variances, ranges, and significant differences. The data was transformed to the log $base_{10}$ for analysis. Various transformations of the data failed to yield any approximation to the normal distribution. All analyses were therefore performed using the nonparametric Wilcoxon Rank Sum Test and the Wilcoxon Signed Rank Test. These data were analyzed for intergroup differences between treatment groups and for intragroup differences between baseline and posttreatment values. To examine intragroup changes from pretreatment, signed ratios of larger to smaller were constructed such that ratios with the pretreatment larger were (−) and those with the posttreatment larger were (+). In cases where the denominator was zero, a very large number of equal size was assigned to all.

Results

The objectives of the microbial characterization of supragingival plaque were to determine if long-term use of the antiseptic mouthrinse led to shifts in the composition or balance of the plaque microbial flora or to the overgrowth of pathogenic or potential opportunistic pathogens. Supragingival plaque samples collected before treatment and following either 3 or 6 months use were examined for microbial content using three microbiological approaches. Cellular morphological groups, including motile rods, nonmotile rods, spirochetes and cocci were enumerated by darkfield microscopy (Fig. 1). Several nonselective and selective bacterial culture media were used to recover potentially opportunistic pathogens and oral pathogens (Table 1). Finally, several bacterial groups (Table 2) were enumerated by a differential count of recovered colonies of MM10.

Intergroup treatment differences

Cell morphology – darkfield microscopic examination

The only significant differences between the treatment groups occurred at 3 months, when significantly fewer spirochetes and more cocci were observed in the Listerine treatment group than in the control group (Fig. 1). No differences in spirochete percentages were observed at 6 months. The change in the incidence of spirochetes is probably due to the extremely low variation in the sample rather than to a meaningful flora shift. The shift in the incidence of cocci in the Listerine group at 3 months was not supported by colony counts on nonselective and selective media (see below) and may be within the normal population variation of the oral flora. No significant differences in coccal forms were observed microscopically at 6 months.

Recovery on nonselective and selective culture media

The media counts were evaluated for intergroup differences as the observed colony forming units relative to the total count observed on MM10. These ratios are presented in Table 3. In cases in which no counts were observed on the selective media, a small number corresponding to $\log_{10} = -8.00$ was assigned to facilitate the statistical analyses.

No significant intergroup treatment differences in counts on either the nonselective or selective culture media were observed. Examination of the data for treatment effects relative to pretreatment levels also failed to reveal any significant differences. Facultative/anaerobe ratios determined as described in *Material and Methods* were also evaluated for intergroup differences. As ob-

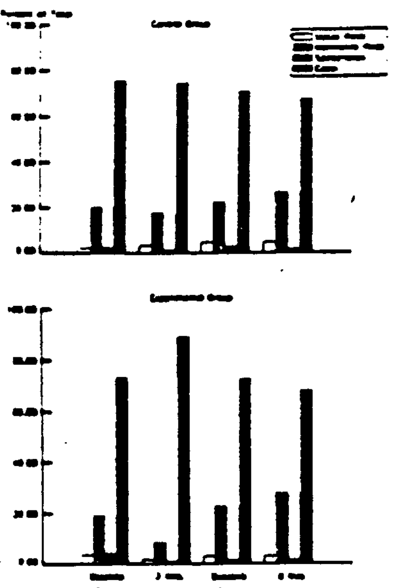

Fig. 1. Distribution of plaque bacteria, darkfield microscopy. Examinations were carried out by methods described by Listgarten & Hellden (1978). Baseline results for the 3- and 6-month treatment periods are presented separately for each treatment group.

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Table 3. Median colony forming units on nonselective and selective culture media relative to colony forming units recovered on MM10

	3-month treatment period			
	antiseptic group		control group	
culture media	pre-treatment	post-treatment	pre-treatment	post-treatment
MM10	1.0000	1.0000	1.0000	1.0000
MM10+O_2	0.5572	0.6026	0.6109	0.5943
	(1.0691)[1]	(1.0715)	(1.0520)	(1.0495)
AC	0.0036	0.0398	0.0109	0.0525
	(2.8840)	(1.2764)	(2.1627)	(1.3274)
DC	0.0000	0.0000	0.0000	0.0000
	(1.3428)	(4.9204)	(1.6711)	(1.5776)
LBKV	0.0061	0.0000	0.0009	0.0003
	(2.7227)	(2.6853)	(3.6058)	(2.7669)
MSB	0.0000	0.0000	0.0000	0.0000
	(1.0000)	(2.1878)	(1.0000)	(3.0200)
N	0.0022	0.0016	0.0017	0.0049
	(1.7783)	(1.6106)	(1.7989)	(1.6866)
RSL	0.0000	0.0000	0.0000	0.0000
	(1.3459)	(1.0000)	(2.0045)	(1.0000)
SAB	0.0000	0.0000	0.0000	0.0000
	(1.3521)	(1.0000)	(1.8197)	(1.4256)
V	0.0964	0.0316	0.0577	0.0413
	(1.5205)	(1.6255)	(1.3740)	(1.8365)
MS	0.0000	0.0000	0.0000	0.0000
	(1.5524)	(2.8576)	(1.6106)	(3.0409)
F:A ratio	1.2618	1.5136	1.5668	1.4655
	(1.1614)	(1.2106)	(1.1324)	(1.1169)

[1] sef = standard error factor: multiply and divide median by sef to obtain 68% confidence limits.

Table 3 (cont'd)

	6-month treatment period			
	antiseptic group		control group	
culture media[1]	pre-treatment	post-treatment	pre-treatment	post-treatment
MM10	7.938[2]	7.177	7.945	7.306
	(0.046)[3]	(0.130)	(0.074)	(0.089)
MM10+O_2	7.771	6.827	7.735	6.957
	(0.054)	(0.105)	(0.080)	(0.139)
AC	0.0165	0.342	0.0107	0.0412
	(1.4566)[4]	(2.0417)	(2.9242)	(1.5596)
DC	0.0000	0.0000	0.0000	0.0000
	(1.4555)	1.3709)	(1.4388)	(1.4622)
LBKV	0.0012	0.0007	0.0002	0.0005
	(17579)	(1.5885)	(2.5293)	(1.5346)
MSB	0.0000	0.0000	0.0000	0.0000
	(1.0000)	(2.5823)	(1.0000)	(1.8281)
N	0.0022	0.0015	0.0018	0.0017
	(1.8707)	(2.3714)	(1.9409)	(1.6596)
RSL	0.0000	0.0000	0.0000	0.0000
	(1.9907)	(1.0000)	(1.4791)	(1.0000)
SAB	0.0000	0.0000	0.0000	0.0000
	(2.2491)	(1.0000)	(1.0000)	(1.0000)
V	0.0318	0.0262	0.0366	0.0832
	(1.4723)	(1.4859)	(1.4421)	(1.2677)
MS	0.0000	0.0000	0.0000	0.0000
	(1.8030)	(2.6485)	(1.6749)	(2.1777)
F:A ratio	1.3122	1.4322	1.2503	1.0046
	(1.1482)	(1.2246)	(1.0839)	(1.1695)

[1] Culture media abbreviations, see Table 1.
[2] Colony forming units and ratios presented as $\log_{10}$ for cells in the harvested cell suspension.
[3] Numbers in parentheses are standard errors (equal to the standard deviations of calculated medians).
[4] sef = standard error factor: multiply and divide median by sef to obtain 68% confidence limits.

served with the nonselective and selective plate counts described above, no significant difference was detected between treatment groups or relative to pretreatment levels.

Colony morphology differential counts
Median counts of bacterial groups enumerated by colony morphology are listed in Table 4. Only one significant intergroup difference between the active and control treatments was observed. The other gram-negative anaerobic rod counts (isolates which did not fall within other categories, most of which appeared to be nonpigmented *Bacteroides sp.*) were significantly less in the active group than in the control group at 6 months. No significant difference or trend toward the difference described above was observed in the 3 month sample results. The colony morphology data was also examined for intergroup differences relative to pretreatment counts; two instances of significant differences between the treatment groups were observed. At 3 months, *Veillonella* counts in the active group were significantly lower than in the control group, and at 6 months the *Fusobacteria* count was lower in the active group than in the control group. The difference in recovered *Veillonella* was not confirmed on the selective media for this organism nor was a difference by colony morphology observed at the 6-month sampling period. The Fusobacteria difference observed at 6 months was not seen at 3 months nor was there an indication of a trend towards this difference.

Intragroup differences

The data presented in Table 3 was also examined for differences between changes from baseline to each sampling period within each group. Many significant reductions and a few significant increases in counts/relative counts of observations on nonselective and selective media and by colony morphology were observed. However, other than the few cases already mentioned, the intragroup differences occurred in both treatment and control groups and are probably not the result of use of either treatment. It is possible that factors common to each treatment group, such as an increased awareness of oral hygiene or normal variation, may have contributed to these intragroup differences.

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Table 4 Median counts by colony morphology recovered on MM10[1]

Treatment group	Sampling period	Fuso.[2]	S. sang.	Actino.	Veillo.	Rod O	Strep. O
antiseptic	baseline/ 3 months	6.556	7.107	7.473	7.025	7.031	6.906
control	baseline/ 3 months	6.407	6.987	7.350	6.862	6.756	7.009
antiseptic	3 months	4.956	5.607	6.629	6.178	6.097	6.431
control	3 months	5.649	6.076	6.534	6.544	5.928	6.592
antiseptic	baseline/ 6 months	6.656	7.023	7.491	6.903	6.690	7.033
control	baseline/ 6 months	6.582	6.869	7.284	7.192	6.974	7.106
antiseptic	6 months	5.472	5.934	6.765	6.192	6.220	6.365
control	6 months	5.928	6.324	6.836	6.594	6.342	6.775

[1] Values presented as $\log_{10}$ of enumerated colony forming units.
[2] Abbreviations: Fuso., Fusobacteria; S. sang., *S. sanguis*; Actino., *Actinomyces*; Veillo., *Veillonella*; Rod O., Rods other; Strep. O., Streptococci other than viridans streptococci.

Discussion

Only 2 reports of studies similar to the one described here in which the long-term use effects of an oral antiseptic on the oral microflora have been assessed have appeared in the literature (Palcanis et al. 1987, Briner et al. 1986). Viadent*, a sanguinaria extract/zinc chloride-containing mouthrinse, was evaluated in a 6-month clinical trial with 12 subjects each in the treatment and control groups. Samples harvested from buccal mucosal sites were analyzed for recovery of enteric bacteria, staphylococci, viridans streptococci, yeast, and total bacteria recovered on a nonselective blood agar medium. No significant microbial shifts were detected nor was increased resistance to the active agents by organisms isolated observed over the treatment period. This study however was not comparable to the present one since mucosal rather than plaque bacteria were evaluated.

In another study, a 0.12% chlorhexidine gluconate-containing mouthrinse was evaluated for its effects on the microbial composition of dental plaque over a 6-month treatment period (Briner et al. 1986). Plaque samples were analyzed on a series of nonselective and selective culture media for total aerobes, total anaerobes, streptococci, *Actinomyces*, *Nisseria*, fusobacteria, gram-negatives, and yeast. Total plaque was harvested from selected teeth from approximately 33 subjects in the active group and 38 in the placebo group. Only total aerobes, total anaerobes, streptococci, and *Actinomyces* were recovered in sufficient levels for statistical analyses. No significant differences between the treatment groups were observed at baseline. *Actinomyces* reductions from 80 to 97% were observed after 3 and 6 months chlorhexidine use. Total streptococci, aerobes, and anaerobes were reduced from 42 to 85% in the active group over the same treatment periods. No detectable shifts in proportions of the microbial groups evaluated were observed.

The present study was more extensive than the works cited above in that several different microbiological approaches were used to evaluate the composition of the supragingival dental plaque following either 3 or 6 months use of the antiseptic mouthrinse. Darkfield microscopy was used to enumerate bacterial groups that cannot be quantitatively cultured or are destroyed by methods used to disperse plaque samples. To date, this has been the only method for enumerating Spirochetes. Plaque samples were also plated on a series of nonselective and selective culture media (Table 1), allowing quantitation of presumptive oral and opportunistic pathogens as well as normal inhabitants of dental plaque.

Several groups of organisms were also enumerated by recognition of their distinctive growth on the nonselective medium (MM10). No attempt was made to harvest the total plaque from the selected teeth, nor were the harvested plaque samples weighed to provide weight analyses. It was believed that total plaque could not be efficiently recovered without the use of a disclosant dye and approved disclosant dyes have been shown to possess antimicrobial activity (Baab et al. 1983). Similarly, plaque samples recovered from individual teeth cannot be accurately weighed without affecting the viability of the plaque bacteria. Therefore, only representative plaque samples were collected and recovery was analyzed on a relative basis to the total number of organisms recovered on the nonselective medium (MM10). In this manner, significant shifts in the microbial composition of dental plaque would be discernible. Previous work with this antiseptic mouthrinse has, however, shown that a single rinse significantly reduces recoverable levels of odorigenic bacteria on the tongue and in the gingival crevice through 3 hours post use (Pianotti & Pitts 1978, Pitts et al. 1983).

Before beginning treatment, all subjects were given a complete prophylaxis and were instructed to continue their normal oral hygiene practices except for the twice daily supervised rinses with their assigned mouthrinse. It is therefore not surprising that the composition of the microbial flora at all harvest periods was characteristic of a young supragingival plaque (Laird & Grant 1983). Our experiments did show that the oral supragingival microflora was not adversely affected after volunteers used the oral antiseptic twice daily for 6 months. Specifically, opportunistic pathogens did not emerge and proportions of indigenous oral bacteria remained largely unchanged. A number of possibilities can be offered to explain antiplaque mechanisms by a chemotherapeutic agent. (1) The agent might inhibit proliferation of established or new plaque. (2) Colonization interference or adherence reduction might occur. (3) Broad spectrum inhibition of the plaque inhabitants might occur to a limited extent. Each of these events would lead to plaque reduction after long-term use of the agent without producing pronounced microbial shifts. The operable mechanism for the antiseptic mouthrinse evaluated in this study have not been elucidated, however, it is possible that more than one mechanisms is functional simultaneously.

In summary, the microbiological evaluations of supragingival dental plaque demonstrated that long-term use of the antiseptic studied did not cause a meaningful shift in its microbial composition or emergence of presumptive oral pathogens. Additionally, it can be de-

* Viadent: Vipont Laboratories, Inc., Fort Collins, CO, USA.

duced from the results of this study that bacterial susceptibility to the antiseptic did not decrease since this would have led to a detectable microbial flora shift.

Zusammenfassung

Die Wirkung des sechsmonatigen Gebrauchs eines antiseptischen Mundspülmittels auf die supragingivale dentale Plaquemikroflora

Die Studie wurde durchgeführt, um zu bestimmen, ob der Langzeitgebrauch (6 Monate) eines antiseptischen Mundspülmittels (Listerine Antiseptic, Warner Lambert Co., Morris Plains, NJ, USA) zu einer unerwünschten Verschiebung in Richtung oraler Pathogene oder dem Auftauchen von resistenten mikrobiellen Formen führt. Supragingivale Plaque wurde vor der Behandlung sowie nach 3 oder 6 Monaten des Gebrauchs entweder der aktiven antiseptischen Lösung oder einer 5%igen Hydroalkohol-Kontrollösung von Probanten gewonnen. Die Probanten spülten mit ihrem zugewiesenen Mundspülmittel zweimal täglich unter Überwachung. Der mikrobielle Inhalt der Plaqueproben wurde mittels Dunkelfeld, Kulturen auf einer Reihe von nichtselektiven und selektiven Nährmedien und durch das Erkennen der mikrobiellen Formen mittels Identifizierung von bestimmten Kolonien auf einem nichtselektiven Medium analysiert. Die statistische Auswertung der Erbegnisse zeigte keine signifikante mikrobielle Verschiebung einschließlich keiner signifikanten Zunahme der mutmaßlichen oralen Pathogene: Spirochäten, schwarz pigmentierte Bacteroides, Streptococcus mutans oder Candida albicans. Zusätzlich wurde bei den potentiell opportunistischen Pathogenen, wie Staphylokokken oder Enterobakterien, kein nachweisbarer Anstieg beobachtet.

Résumé

Effets de l'utilisation pendant 6 mois d'un produit antiseptique pour bains de bouche sur la flore microbienne de la plaque supra-gingivale

Cette étude a été entreprise pour examiner si l'utilisation prolongée (6 mois) d'un produit antiseptique pour bains de bouche (Listerine Antiseptic, Warner Lambert Co., Morris Plains, NJ, USA) avait pour effet indésirable l'établissement d'organismes pathogènes buccaux ou de formes microbiennes résistantes. On a recueilli la plaque supra-gingivale de 83 sujets avant traitement et après utilisation soit du produit antiseptique soit d'une solution hydroalcoolique témoin à 5%, pendant une durée soit de 3 mois soit de 6 mois. Les sujets faisaient les rinçages sous surveillance 2 fois par jour avec le produit qui leur était assigné. Pour analyser la flore microbienne de la plaque, on examinait les échantillons par microscopie en fond noir, par culture sur une série de milieux sélectifs et non sélectifs et par reconnaissance de formes microbiennes distinctes lors de la croissance des colonies sur un milieu non sélectif. L'analyse statistique des résultats n'a pas mis en évidence de changements microbiens significatifs, en particulier pas d'augmentation significative des éléments présumés pathogènes, spirochètes, *Bacteroides* pigmenté en noir, *Streptococcus mutans* ou *Candida albicans*. De plus, aucune augmentation notable n'a été observée, ni pour les staphylocoques, ni pour les bactéries entériques, pathogènes opportunistes potentiels.

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Comparative effects of 2 chemotherapeutic mouthrinses on the development of supragingival dental plaque and gingivitis

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Overholser CD, Meiller TF, DePaola LG, Minah GE and Niehaus C: Comparative effects of 2 chemotherapeutic mouthrinses on the development of supragingival dental plaque and gingivitis. J Clin Periodontol 1990: 17: 575–579.

Abstract. A 6-month double-blind, controlled clinical study was completed with 124 healthy adult subjects to determine the efficacy of 2 mouthrinses, Listerine (LA) and Peridex (PX), used as supplements to regular oral hygiene measures in reducing supragingival dental plaque and gingivitis. Following screening examinations for entry levels of existing gingivitis and plaque, baseline gingival and plaque area indices, extrinsic tooth stain, supragingival calculus, bleeding and soft tissue condition were recorded. All subjects then received a complete dental porphylaxis to remove plaque, calculus and extrinsic stain. Subjects were randomly assigned to 1 of 3 groups and performed supervised rinses twice daily for 30 s in addition to their normal oral hygiene, for 6 months. All indices were again evaluated at 3 and 6 months. After 6 months, LA and PX significantly ($p < 0.001$) inhibited development of plaque by 36.1% and 50.3%, respectively, and the development of gingivitis by 35.9% and 30.5%, respectively, compared to a hydroalcohol control. PX was more effective in inhibiting plaque and both mouthrinses appeared to be equally effective in inhibiting gingivitis. LA patients did not develop significant levels of stain or supragingival calculus at 6 months, compared to baseline or control. PX patients developed significant levels of extrinsic stain and supragingival calculus compared to baseline and control. Though PX was more effective than LA in the control of plaque, this study indicates that both LA and PX were effective agents in a regimen for the control of plaque and gingivitis.

Key words: plaque; gingivitis; stain; Listerine; Peridex; clinical trial.

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The use of chemotherapeutic mouthrinses as adjuncts in the management of gingivitis has received increased attention in recent years. Numerous studies using a variety of short- and long-term experimental designs have been reported in an attempt to demonstrate mouthrinse efficacy against supragingival plaque and gingivitis. Since it is difficult to design experimental protocols that reproduce conditions under which the mouthrinses are to be used, it has been difficult to assess which formulations are truly useful in therapeutic or preventive regimens. This problem was addessed by the establishment of the American Dental Association Council on Dental Therapeutics' (CDT) acceptance program for chemotherapeutic mouthrinses, which resulted in guidelines for the conduct of clinical studies to demonstrate efficacy (Council on Dental Therapeutics 1986). These guidelines require, in part, that clinical studies: be controlled; of at least 6 months duration; demonstrate significant efficacy against both gingivitis and supragingival plaque; and test the agent in a normal use situation using a study population representative of typical product users.

To date, 2 chemotherapeutic mouthrinses, Listerine (LA) and Peridex (PX) have been approved by the Council on Dental Therapeutics as effective agents in the control of supragingival plaque and gingivitis. Published short-term comparative studies between the products have reported conflicting results (Axelsson & Lindhe 1987, Siegrist et al. 1986). The purpose of this current study was to compare the antiplaque/antigingivitis efficacy of (LA) and (PX) using a protocol satisfying CDT Guidelines. This paper reports the clinical findings; microbiologic results will be reported separately.

Material and Methods

This was a double-blind, controlled, parallel design study. 128 healthy male and female adults, aged 21–62 years, with pre-existing plaque and gingivitis

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but without clinical evidence of periodontitis, were screened prior to entry for a minimum of 20 sound natural teeth, and plaque and gingival index means ≥ 1.95 as determined by the Turesky modification of the Quigley-Hein index (Turesky et al. 1970) and the modified gingival index (Lobene et al. 1986), respectively. Grossly carious, fully crowned, orthodontically banded, abutment and third molar teeth were not included. Subjects with gross oral pathology or on antibiotic, antibacterial or anti-inflammatory therapy were excluded from the study.

Examinations were performed at baseline, 3 months and 6 months. Examiners were standardized in a series of sessions in which the examiners reviewed the clinical criteria in each index prior to the initiation of the study. To assure maximum objectivity and reproducibility, one examiner was used for gingivitis, bleeding and plaque indices and another for the stain and calculus indices. The stain index, calculus index and plaque collection for the microbiologic study were performed prior and blind to the other examiner. Teeth used for plaque collection were eliminated from statistical analysis for gingival, bleeding, and plaque indices.

A complete intraoral soft tissue examination was performed to record the condition of the oral mucosae. *Extrinsic tooth stain* was scored using the Lobene extrinsic tooth stain index (Lobene 1968) on the labial surfaces of the 12 anterior teeth. The gingival and body regions were scored for both area and severity (intensity). The area and intensity scores were multiplied by each other for a tooth score: tooth scores were added for an individual total score. *Supragingival calculus* was measured using a flat calibrated periodontal probe in three constant planes on the lingual surface of the six mandibular anterior teeth according to the Volpe-Manhold method (Volpe et al. 1965, Manhold et al. 1965, Volpe et al. 1967).

Gingivitis was scored using the modified gingival index (MGI) on the buccal and lingual surfaces and interdental papillae of all scorable teeth. The MGI has been shown to correlate significantly with the GI (Lobene et al. 1988). *Bleeding* was scored using the interdental bleeding index (Caton & Polson 1985). *Plaque* was scored using the Turesky modification of the Quigley-Hein index on the buccal and lingual surfaces of all scorable teeth.

Baseline scores were used as covariates in the final analyses. Following the baseline examination, subjects were given a complete prophylaxis to remove all plaque, calculus and extrinsic stain.

Subjects were assigned to LA (active ingredients: menthol, thymol, methyl salicylate and eucalyptol), PX (active ingredient: chlorhexidine gluconate, 0.12%) or control (flavored, colored 5% hydroalcohol solution) group according to a computer-generated random code, by which double-blinding was maintained. Product was dispensed in coded amber bottles. Product code was not disclosed to the examiners or recorded on case report forms.

Starting the day of the prophylaxis, subjects began rinsing with 15 ml of PX or 20 ml of LA or control for 30 s, twice daily for 6 months. Rinsings were supervised twice daily on weekdays. This process ensured compliance with the rinsing protocol and minimized proximity of rinsing to toothbrushing. Subjects maintained a diary to document unsupervised compliance with the regimen on weekends and holidays. Subjects were instructed not to rinse, eat or drink for 30 min following use of the test rinses. Personnel dispensing the rinses did not participate in any other aspect of the study. During the study, subjects followed their usual oral hygiene and dietary habits, but were instructed to refrain from using other mouthrinses and to advise the investigator of the use of antibiotic or anti-inflammatory drugs. Soft nylon toothbrushes and fluoridated toothpaste were provided for all subjects throughout the study.

Subjects refrained from all oral hygiene and use of experimental products on examination days until after the examinations were completed in order to eliminate possible bias due to product odor. Individual case report forms were used for the soft tissue examination, plaque, gingival and bleeding indices, and for ixtrinsic tooth stain and supragingival calculus. Separate forms were used at each examination interval. The examiner did not have access to case report forms during the study.

The study was designed to provide a minimal power of 0.80 for detecting a statistically significant difference in plaque and gingivitis scores at the 0.05 probability level. Mean plaque and gingivitis indices determined for each subject were analyzed by analyses of covariance and least significant difference tests. Nonparametric methods (Wilcoxon signed rank test and Kruskal-Wallis test) were used to analyze stain, calculus and bleeding data when distributions were non-normal (skewed). The distribution of subjects with stain greater than or equal to one were compared by chi-square analyses and pairwise comparisons by Fisher exact probability tests.

Table 1. Demographic variables

	Listerine antiseptic		Peridex		Control	
Sex	no.	(%)	no.	(%)	no.	(%)
male	10	24.4	22	53.7	12	28.6
female	31	75.6	19	46.3	30	71.4
total	41		41		42	
smokers	2	4.9	5	12.5	6	14.3
nonsmokers	39	95.1	35	87.5	36	85.7
Age						
average	29.17		29.24		28.62	
std. error	1.02		1.16		0.84	
range	21–47		23–62		22–42	

Table 2. Adjusted mean plaque index scores

Group	Baseline	3 months	6 months
Listerine antiseptic	2.492±0.042	1.530±0.077*	1.048±0.081
Peridex	2.378±0.036	0.975±0.076	0.815±0.080
control	2.350±0.038	1.749±0.076	1.639±0.079

* Standard error.

Results

Of the 128 subjects who entered, 124 completed the 6-month study, 3 subjects

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dropped out from the study because they were unable to comply with the supervised rinsing schedule. 1 subject in the PX group dropped out when stain began to form. The treatment groups were well-balanced with respect to age and smoking status, obviating the need for stratification by these variables. However, there was a statistically significant imbalance in the distribution of sexes within groups. To assess the effect of this on treatment outcome, analyses were performed to test the treatment-by-sex interaction (analyses of covariance for normally distributed data and analyses of covariance on ranks for nonparametric data). For all analyses, the treatment-by-sex interaction was not significant, obviating the need to stratify by sex (Table 1).

Plaque

Adjusted mean plaque index scores at 3 and 6 months are presented in Table 2. Adjusted treatment means are calculated to compensate for difference in baseline group means, and indicate what the treatment means would be if each of the group means at baseline equaled the mean for all subjects (Steel & Torrie 1980). Analyses of covariance, using the pre-prophylaxis baseline scores as covariate, showed that LA inhibited the accumulation of supragingival plaque by 12.5% ($p<0.05$) at 3 months and by 36.1% ($p<0.001$) at 6 months compared to the hydroalcohol control. PX inhibited the accumulation of supragingival plaque by 44.3% ($p<0.001$) and 50.3% ($p<0.001$) at 3 and 6 months, respectively, compared to control. PX also showed a significant ($p<0.05$) reduction in plaque accumulation versus LA at 3 and 6 months. Tooth surfaces with plaque index scores of 0 or 1 increased 60% in the LA group, from 5% at baseline to 65% at 6 months; 69% in the PX group, from 8% at baseline to 44% at 6 months. Surfaces with plaque index scores of 2 to 5 were reduced from 94% at baseline to 35% at 6 months in the LA group; from 92% at baseline to 23% at 6 months in the PX group, and from 92% at baseline to 56% at 6 months in the control group (Table 3). While the microbiological data will be reported separately, it should be noted that the development of resistance or emergence of opportunistic or potentially pathogenic organisms did not occur in either group.

Table 3. % of tooth surfaces which received a plaque index score of 0, 1, 2, 3, 4 and 5 at baseline and after 6 months of trial

Plaque index score	0	1	2	3	4	5
Listerine						
baseline	0	5	54	34	5	1
6 months	38	27	23	10	1	0
Peridex						
baseline	2	6	52	35	4	1
6 months	52	25	15	7	0	0
Control						
baseline	1	7	52	36	3	1
6 months	20	25	33	20	2	0

Table 4. Adjusted mean gingival index scores

Group	Baseline	3 months	6 months
Listerine antiseptic	2.234±0.022	1.328±0.064*	0.748±0.064
Peridex	2.281±0.031	1.032±0.064	0.810±0.065
control	2.221±0.023	1.409±0.064	1.166±0.063

* Standard error.

Table 5. % of gingival units which received a gingival index score of 0, 1, 2 and 3 at baseline and after 6 months of trial

MGI score	0	1	2	3
Listerine				
baseline	0	1	74	25
6 months	46	36	17	1
Peridex				
baseline	0	1	70	29
6 months	43	32	23	3
Control				
baseline	0	2	74	24
6 months	26	39	34	2

Gingivitis

Adjusted mean gingival index scores are presented in Table 4. Using baseline scores as covariate, gingivitis development was inhibited in the LA group by 5.8% at 3 months and by 35.9% ($p<0.001$) at 6 months, compared to the control. PX inhibited gingivitis development by 26.8% ($p<0.001$) at 3 months and by 30.5% ($p<0.001$) at 6 months, compared to the control. PX was significantly more effective than LA ($p<0.002$) at 3 months. There was no significant difference between the two mouthrinses at 6 months.

The distribution of gingival index scores at baseline and after 6 months are shown in Table 5. The % of clinical healthy gingival units (score of 0) at 6 months increased from 0% in all groups at baseline to 46% in the LA group, 43% in the PX group, and 26% in the control group. In total, 89% of the gingival scores in the LA group, 87% in the PX group and 77% in the control group improved between baseline and 6 months.

Extrinsic tooth stain

Extrinsic tooth stain was not observed in the gingival region in either the LA or control group (Table 6). The PX group showed significant increases ($p<0.001$) in stain at 3 and 6 months, compared to its baseline. In the PX group, the percent of subjects with scores of 1.0 or greater was 20.9% at 3 months and 58.5% at 6 months. There were no significant differences between LA and control at 3 or 6 months. Significantly greater ($p<0.01$) extrinsic stain was observed in the PX group when compared to LA and the control at both 3 and 6 months (Table 7).

Calculus

There was no increase in calculus formation in either the LA or control

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groups versus their respective baselines (Table 8). There was no significant difference in calculus formation between LA and control. The PX group, however, showed a significant increase ($p < 0.05$) in calculus formation versus its baseline and compared to LA or control at 6 months.

Bleeding index

There were significant ($p < 0.001$) reductions in the bleeding index for all three groups versus their respective baselines (Table 9). There were no significant differences among the 3 groups at 3 or 6 months.

Soft tissue

No abnormal soft tissue findings were noted in any group.

Discussion

This double-blind, controlled study conducted in accordance with CDT Guidelines compared the antiplaque/antigingivitis efficacies of LA and PX when used as adjuncts to daily oral hygiene and a professional prophylaxis. Results of this study confirmed those of 6-month or longer studies in which LA (Lamster et al. 1983, Gordon et al. 1985, DePaola et al. 1988) and PX (Lang et al. 1982, Grossman et al. 1986) were shown to have significant antiplaque/antigingivitis efficacy as supplements to mechanical plaque control methods.

In this study, LA and PX produced significant plaque reductions of 36.1% and 50.3%, respectively, compared to control at 6 months. Corresponding gingivitis reductions were 35.9% for LA and 30.5% for PX, both of which were also significant compared to control. Statistical comparison of LA and PX showed PX to be significantly more effective in plaque reduction, but both rinses were comparable in gingivitis reduction at 6 months. As has been suggested with chlorhexidine (De la Rosa et al. 1988), mouthrinses could exert more of an antigingivitis effect by altering the pathogenic aspects of plaque, as opposed to affecting plaque area. The statistically equivalent antigingivitis activities of LA and PX indicates that either agent could be used with comparable efficacy in a regimen for the control of gingivitis.

The finding of a statistically significant increase in both extrinsic tooth stain and calculus in the PX group compared to baseline is consistent with previous reports (Lang et al. 1982, Grossman et al. 1986). The LA and control groups, by comparison, did not form either stain or calculus. Although stain and supragingival calculus are generally recognized as aesthetic problems, they must be considered clinically and individually for each patient to the extent that they may interfere with patient compliance in long-term treatment regimens.

In summary, this 6-month controlled study demonstrated PX to be more effective in inhibiting plaque formation, and LA and PX to be comparable in inhibiting the development of gingivitis, when the rinses were used as adjuncts to routine oral hygiene following a professional prophylaxis.

Table 6. Extrinsic tooth stain index scores gingival region

Group	Baseline mean	3 months mean	6 months mean
Listerine	0.07±0.023*	0.08±0.028	0.13±0.037
Peridex	0.11±0.032	0.71±0.093	1.45±0.199
control	0.05±0.014	0.08±0.020	0.07±0.022

* Standard error.

Table 7. % of subjects having a minimum average extrinsic tooth stain score of 1.00 (gingival region)

Group	Baseline	3 months	6 months
Listerine	0%	0%	0%
Peridex	0	20.9	58.5
control	0	0	0

Table 8. Supragingival calculus index scores

Group	Baseline mean	3 months mean	6 months mean
Listerine	0.19±0.052*	0.10±0.026	0.14±0.034
Peridex	0.21±0.049	0.15±0.035	0.36±0.058
control	0.17±0.036	0.09±0.022	0.09±0.021

* Standard error.

Table 9. Bleeding index scores

Group	Baseline mean	3 months mean	6 months mean
Listerine	0.71±0.048*	0.40±0.056	0.29±0.042
Peridex	0.72±0.056	0.28±0.038	0.25±0.045
control	0.66±0.062	0.37±0.061	0.33±0.057

* Standard error.

Acknowledgement

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Zusammenfassung

Vergleich der Wirkung von zwei chemotherapeutischen Mundspülmitteln auf die Entwicklung von supragingivaler Plaque und Gingivitis
Eine kontrollierte klinische Doppelblindstudie von sechsmonatiger Dauer wurde an 124 erwachsenen Probanden durchgeführt, um die Wirksamkeit von zwei Mundspüllösungen, Listerine (LA) und Peridex (PX), zu bestimmen. Sie wurden als Ergänzung zu den regulären Mundhygienemaßnahmen zur Reduzierung von supragingivaler Plaque und Gingivitis benutzt. Nach einer Auswahluntersuchung zur Feststellung des bestehenden Gingivitis- und Plaqueniveaus wurden die Eingangswerte des Gingiva- und Plaqueflächenindex, der Zahnverfärbung, des supragingivalen Zahnsteins, der Blutung sowie des Weichgewebezustandes aufgezeichnet. Alle Probanden erhielten eine komplette professionelle Zahnreinigung zur Entfernung von Plaque, Zahnstein und Zahnverfärbung. Die Probanden wurden nach einem Randomisierungsplan einer der drei Gruppen zugeteilt und führten sechs Monate lang unter Überwachung, zweimal täglich für 30 Sekunden, zusätzlich zur normalen Mundhygiene eine Mundspülung durch. Alle Indizes wurden

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wiederum nach drei und sechs Monaten bewertet. Nach sechs Monaten hemmten LA und PX sowohl signifikant ($p<0.001$) die Entwicklung der Plaque um 36.9% bzw. 50.3% als auch die Entwicklung einer Gingivitis um 35.9% bzw. 30.5%, verglichen mit einer Hydroalkoholkontrolle. PX war in der Plaquehemmung wirkungsvoller und beide Mundspüllösungen schienen gleich effektiv bei der Hemmung der Gingivitis. Verglichen mit der Eingangsuntersuchung und der Kontrolle entwickelten LA-Patienten nach sechs Monaten keine signifikanten Mengen an Zahnverfärbungen und supragingivalem Zahnstein. Verglichen mit der Eingangsuntersuchung und der Kontrolle entwickelten PX-Patienten signifikante Mengen an Zahnverfärbungen und supragingivalem Zahnstein. Obwohl PX effektiver als LA bei der Plaquekontrolle war, zeigt diese Studie, daß sowohl LA als auch PX wirkungsvolle Mittel bei Maßnahmen zur Kontrolle von Plaque und Gingivitis sind.

Résumé

Effets comparés de 2 bains de bouche chimiothérapiques sur le développement de la plaque dentaire sus-gingivale et de la gingivite

Une étude clinique avec contrôle en double aveugle a été pratiquée chez 124 adultes en bonne santé pour déterminer l'efficacité de 2 bains de bouche. Listerine (LA) et Peridex (PX), utilisés comme complément des mesures habituelles d'hygiène bucco-dentaire pour réduire la plaque dentaire sus-gingivale et la gingivite. Après des examens pour situer les niveaux existant de gingivite et de plaque, on a enregistré les valeurs initiales de l'indice gingival, de l'indice de la surface de la plaque, les colorations dentaires externes, le tartre sus-gingival, le saignement et l'état des tissus mous. Tous les sujets ont reçu un nettoyage dentaire complet pour éliminer la plaque, le tartre et les colorations externes. Les sujets ont été répartis au hasard dans l'un des 3 groupes et ont pratiqué pendant 6 mois sous surveillance des rinçages de 30 secondes 2 fois par jour, en plus de leur soins habituels d'hygiène bucco-dentaire. Tous les indices ont de nouveau été enregistrés à 3 mois et à 6 mois. Après 6 mois, LA et PX donnaient une inhibation significative ($p<0.001$) du développement de la plaque, respectivement 36.1% et 50.3%, et du développement de la gingivite, respectivement 35.9% et 30.5%, par rapport a une solution hydroalcoolique témoin. PX était plus efficace pour l'inhibition de la plaque, mais les 2 bains de bouche semblaient être également efficaces pour l'inhibition de la gingivite. Les patients du groupe LA n'avaient pas, à 6 mois, développé de niveaux significatifs de coloration ou de tartre sus-gingival par rapport aux niveaux initiaux et par rapport au groupe témoin. Les patients du groupe PX développaient des niveaux significatifs de coloration externe et de tartre sus-gingival par rapport aux niveaux initiaux et au groupe témoin. Bien que PX ait été plus efficace que LA pour contrôler la plaque, cette étude indique que ces 2 produits sont des agents efficaces dans un programme établi pour contrôler la plaque et la gingivite.

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Effects of an Oral Rinse on Experimental Gingivitis, Plaque Formation, and Formed Plaque

Dental plaque is an important etiologic factor in the initiation and progression of dental caries and periodontal disease. Even though the precise mechanisms of action are not yet completely understood, sufficient data are available to implicate bacterial components of dental plaque in the development of most dental diseases.

Presently, the only safe and effective method available for daily removal of bacterial plaque is by mechanical means. The problems encountered in motivating patients to accomplish effective plaque control are well recognized. Also, not all patients have sufficient manual dexterity to accomplish the intricate mechanical procedures involved in tooth cleaning.

An effective, safe, and inexpensive chemotherapeutic agent is needed if a major impact is to be made on the burgeoning number of patients with dental disease. Many agents have been proposed and investigated, and the effectiveness of several agents has been reported.[1] However, many of the more promising drugs are still under investigation and their safety and effectiveness are questionable. Undoubtedly, an agent will be developed which can destroy formed plaque and prevent its formation. Until then, however, drugs or agents which are already available to the public, such as oral antiseptic mouthwashes, should not be overlooked. Their usefulness and effectiveness is generally criticized[2] but their public appeal is great,[3] and their actual value in plaque removal, or prevention of plaque formation, may be underestimated. As an example, one recent study[4] demonstrated a significant reduction in dental plaque formation when a commercial mouthwash was used as an adjunct to regular oral hygiene procedures.

The purpose of this study was to investigate the effects of one commercial mouthwash on experimental gingivitis, on formed plaque, and on plaque formation.

Materials and Methods

Thirteen periodontists, whose ages ranged from 30 to 45 years, and who had at least 26 teeth, were selected as subjects for the study. The examiner was a dental officer (S.S.L.) experienced in plaque

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TABLE 1
MEAN GINGIVAL SCORES FOR EACH EXAMINATION

	Water Rinse		Experimental rinse		
Examination	Initial examina-ation (mean, SD)	1 minute, 3 times a day (mean, SD)	1 minute, 3 times a day (mean, SD)	5 seconds, 3 times a day (mean, SD)	1 minute, once a day (mean, SD)
Gingival score	0.025 ± 0.006	0.56 ± 0.33* (96% greater than initial score)	0.12 ± 0.27†† (79% less than score of water rinse)	0.388 ± 0.47† (31% less than score of water rinse)	0.57 ± 0.40

*Significantly greater ($p < 0.01$) than initial score.
† Not significantly greater than initial score.
†† Significantly less than score of water rinse.

recognition and in the methodology of the Navy Periodontal Disease Index[5] and the Navy Plaque Index (Modified).[5] At no time during the study was the examiner aware of the purpose or of the materials and methods of the study.

Each subject was examined for gingivitis around all teeth on the first day and was scored by the gingival scoring system of the Navy Periodontal Disease Index. The mouth was flushed with an air-water spray to remove any loose debris around the teeth, and the subjects rinsed with a 1.3% erythrosine dye disclosing solution. The left maxillary and right mandibular quadrants, which were chosen to provide an accurate picture of plaque distribution,[6,7] were evaluated and scored using the Navy Plaque Index (Modified). All plaque on the right maxillary and left mandibular teeth was carefully removed with a curet, placed on preweighed aluminum disks, and weighed on an electric balance* within one minute from the time it was removed from the teeth.[8] Plaque on the facial surfaces was weighed separately from that on the lingual surfaces for each quadrant.

The subjects received an oral prophylaxis in order to establish a plaque score of zero as determined with a disclosing solution. They were advised to refrain from all plaque control procedures for 12 days and to rinse vigorously with tap water for 1 minute, three times daily: after breakfast, after lunch, and before retiring.

On the twelth day, the subjects were examined for both plaque and gingivitis, as before. Following the examination, plaque zero was established except for the mandibular right quadrant. This quadrant was not cleaned, in order to determine the effects of the test mouthwash on a 12-day plaque accumulation (formed plaque). The subjects were instructed to rinse vigorously, for the next 12 days, with the test mouthwash** for 1 minute, three times daily: after breakfast, after lunch, and before retiring; just as they had done with water. No other plaque control procedures were to be performed.

Twelve days later (day 24) the subjects were examined and scored for gingivitis and plaque, as previously described.

Once again, plaque zero was attained except for the mandibular right quadrant. The subjects were randomly divided into two groups of six subjects each (one subject dropped out) in order to study the effect of a change in duration and frequency of rinsing on plaque formation and on formed plaque. One group rinsed with the test mouthwash for 5 seconds after breakfast, after lunch, and before retiring. The other group was instructed to rinse with the test solution for 1 minute just before retiring. No other plaque control procedures were to be performed by either group.

Twelve days later (day 36) the subjects were examined and scored for gingivitis, for plaque formation, and for formed plaque, as previously described.

Results

The mean gingival scores (and standard deviations) are shown in Table 1. The increase in gingivitis was significant ($p < 0.01$)† after 12 days of rinsing with water and using no plaque control procedures, when compared to the initial gingival scores. There was significantly less gingivitis ($p < 0.01$) when the test mouthwash was used for 1 minute, three times per day, for 12 days, compared to the scores after the first 12 days of comparable rinsing with water. In addition, less gingivitis was present after using the test mouthwash for 5 seconds, three times daily,

*Ainsworth electric balance Type 10NT, Wm. Ainsworth & Sons, Inc., Denver, Colorado.

† Determined by the Student t test.

**An antiseptic rinse containing essential oils, thymol, and eucalyptol: *Listerine*, Warner-Lambert Pharmaceutical Company, Morris Plains, N.J.

when compared to the water rinse, but the difference was not significant. The mean gingival scores after 12 days of rinsing with the test rinse for 1 minute only before retiring were no better than when the subjects rinsed for 1 minute, three times daily, with water.

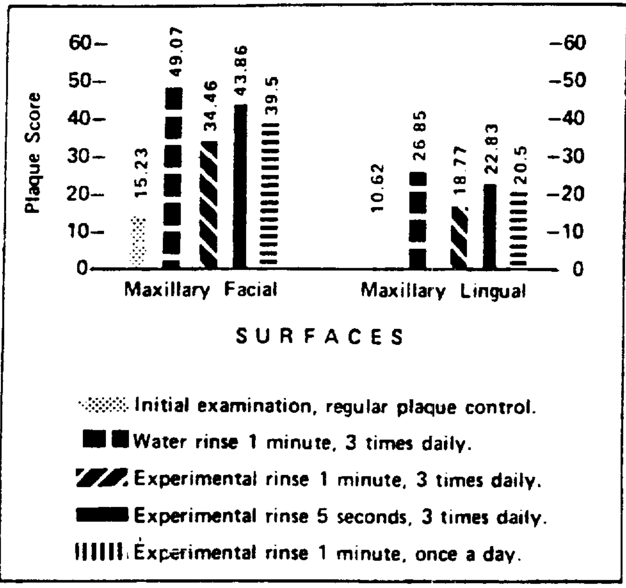

Figure 1 Mean plaque scores for plaque formation by quadrant at each examination.

The test mouthwash used for 1 minute, three times daily, for 12 days, produced a significant reduction in plaque formation scores ($p < 0.01$) when compared to 12 days of rinsing with water (Figure 1). There was also a significant reduction ($p < 0.01$) in plaque formation scores on the maxillary facial surfaces when the test solution was used only for 1 minute, once a day, when compared to the water rinse. The reductions of plaque formation scores on the maxillary lingual surfaces under the same regimen were significant at $p < 0.02$.

The mean plaque scores were significantly greater ($p < 0.01$) for the test rinse, regardless of how it was used, when compared to mean plaque scores after the subjects' own plaque control regimen.

A significant reduction in mean plaque weight ($p < 0.01$) on all tooth surfaces was seen when the test mouthwash was used for 1 minute, three times daily, compared to the water rinse (Figure 2). There was even less plaque by weight on the lingual surfaces than after the subjects' own plaque control methods. However, the difference was not significant.

More plaque by weight formed on the teeth when the mouthwash was used for less time (5 seconds) or less frequently (once a day) than when the mouthwash was used 1 minute, three times daily. However, the differences were not significant ($p < 0.1$). There was less plaque formation by weight under all experimental conditions compared to rinsing with water. This reduction was significant at the $p < 0.02$ level on the maxillary lingual and the mandibular facial surfaces when the test rinse was used for 5 seconds, three times daily, and on the mandibular facial surfaces when used 1 minute, once a day.

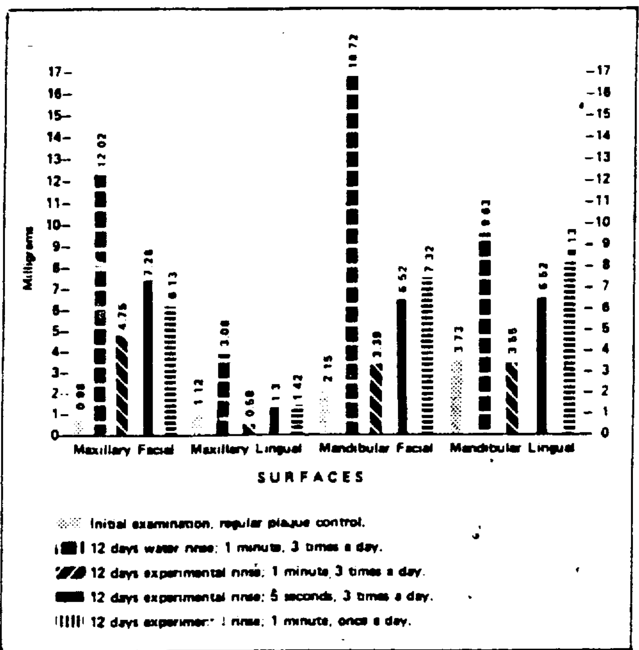

Figure 2 Mean plaque weight for new plaque formation by quadrant at each examination.

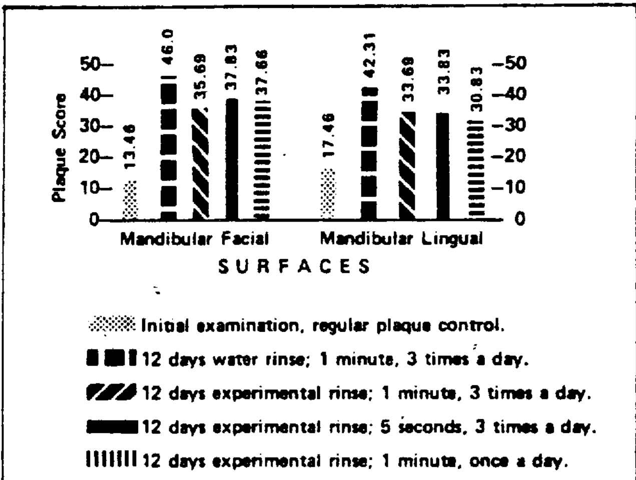

Figure 3 Mean plaque scores for formed plaque by quadrant at each examination.

The test mouthwash generally had less effect on formed plaque than on plaque formation. Figure 3 gives the mean plaque scores for formed plaque at each of five examination periods. All mean plaque scores for formed plaque were significantly higher ($p < 0.01$) after each test period use of the test mouthwash when compared to the subjects' own methods of cleansing. There was, however, a considerable reduction of formed plaque with each test period for the mouthwash compared to the plaque scores after the water rinse. Significant reductions

($p < 0.01$) in formed plaque on all tooth surfaces of the mandibular right quadrant occurred when the test mouthwash was used for 1 minute, three times daily. There was a significant reduction ($p < 0.01$) in formed plaque on the mandibular lingual surfaces after using the mouthwash for 1 minute, once a day. The reductions were significant at the $p < 0.02$ level on the mandibular facial surfaces when the test rinse was used for only 5 seconds, three times a day, or for 1 minute, once a day.

Discussion

The results of this study indicate that the test mouthwash was effective in reducing experimental gingivitis, plaque formation, and formed plaque when compared to a water rinse. It was most effective when used for 1 minute, three times daily, and somewhat less effective when used 1 minute, once a day, or for 5 seconds, three times daily. However, there was always less plaque and the reduction was significant on some surfaces even when the mouthwash was used for only 5 seconds, three times daily. The reduction in plaque was not due to the act of rinsing alone, since all subjects used a water rinse for 1 minute, three times daily during the first 12 days of the study and demonstrated an increase in plaque formation.

The test mouthwash was generally not as effective as the subjects' own methods of plaque control. There was less plaque formation (by wet weight) on the lingual surfaces when the test mouthwash was used for 1 minute, three times daily than was observed following the patients' own method of plaque control. However, this difference was not significant.

Plaque scores and plaque weight were used in this study to determine plaque formation. The results seem to indicate a greater reduction in plaque formation when determinations were made by wet weight. There was also a closer correlation betwen plaque formation and gingivitis scores when using the wet weight measurements. This finding supports the work of Loesch and Green,[8] who reported significant correlations between plaque wet weight and gingivitis scores and not between stained plaque scores and gingivitis.

During the study, several subjects complained of a dryness or burning sensation, or both, when the test mouthwash was used for 1 minute, three times a day. When the test rinse was used for a shorter period or less frequently, there were no complaints. However, the test rinse was not as effective when this was done.

The results of this study suggest the need for further investigation of the test rinse and raise such questions as: What is the active ingredient(s) of the test rinse? Will continued use of the test rinse for 1 minute, three times daily, alter the oral flora or result in soft tissue changes? Will the test rinse significantly reduce plaque formation when used as an adjunct to regular control methods, as reported in a pilot study by Gomer *et al*[4]? Are there other commercial mouthwashes which have the potential to retard plaque formation or alter formed plaque if used properly?

Hopefully, a chemotherapeutic agent will be developed for the prevention, treatment, and control of dental plaque disease. Current investigation suggests an eventual breakthrough, but the development and acceptance of such an agent will take time. Perhaps commercial products are already available and accepted by the Food and Drug Administration that could be used in conjunction with mechanical removal to control plaque until better agents are available.

Summary

The effects of a commercial mouthwash on experimental gingivitis, formed plaque, and plaque formation were studied clinically. The subjects first used a water rinse for 1 minute, three times daily, as their only method of oral hygiene and had signicant increases in plaque scores and plaque weights, accompanied by a significant increase in gingival inflammation. When a commercial rinse was used instead of water for 12 days, the plaque scores and plaque weights were significantly reduced. There was likewise an accompanying significant reduction in gingival inflammation and formed plaque. Reductions of bacterial plaque and gingivitis were also observed when the duration of rinsing was reduced to 5 seconds, three times daily, or when the frequency was reduced to one minute, once a day. ☐

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Efficacy of mouthrinses in inhibiting dental plaque and gingivitis in man

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Abstract. The aim of the present trial was to determine the effect of different mouthwash preparations used as supplements to regular oral hygiene measures on dental plaque and gingivitis in humans. 96 volunteers were recruited for the study. Following a baseline examination, each subject was given a careful prophylaxis, following which the mouthrinse regimens were initiated. During the 6 weeks of trial, the subjects continued to exercise their regular non-supervised, self-performed plaque control measures. The 96 volunteers were assigned either to 1 or 3 different treatment groups or to a control group according to a randomized code. The members of the control group and the listerine group rinsed with 20 ml of the mouthrinse for 30 s, twice daily, while the members of the chlorhexidine groups (using either a 0.2% or a 0.1% solution) rinsed with 10 ml of the antiseptic solution for 60 s twice daily. Examinations regarding extrinsic stain and plaque were performed at baseline and after 3 and 6 weeks, while the conditions of the gingiva were examined at baseline and after 6 weeks. Extrinsic stain was evaluated using the Lobene index, plaque was assessed by the Turesky modification of Quigley-Hein index and the gingival condition was examined using the gingival index system of Loe & Silness. The results of the trial demonstrated that the 3 active mouthwash preparations used as supplements to regular tooth cleaning measures markedly improved both the oral hygiene status and the gingival conditions of the participating human volunteers, compared to the control rinse. The findings thus agree with data previously reported on the effects of chlorhexidine digluconate and listerine antiseptic both in terms of plaque inhibition and resolution of gingivitis.

Key words: Plaque – gingivitis – extrinsic stain – clinical trial – chlorhexidine – listerine – mouthrinsing.

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Most forms of plaque associated periodontal disease start with inflammatory lesions of the gingivae which, if left untreated, with time may progress and eventually involve and compromise the entire periodontal attachment apparatus of the affected teeth. Even if the composition of the supragingival plaque is markedly different from the subgingival microbiota of periodontal disease sites, most if not all subgingival plaque develop subsequent to the formation of supragingival plaque (for review see Carlsson (1983)). This fact suggests that periodontal disease prevention should be based on measures which also prevent supragingival plaque formation. It has been demonstrated that gingivitis can be resolved and periodontitis prevented from developing or recurring in subjects who are enrolled in carefully supervised plaque control programs (for review see Kristoffersen & Meyer (1983), Frandsen (1985)) including both mechanical and/or chemical methods (for review see Loesche (1976), Kornman (1985)). The maintenance of proper standards of oral hygiene over prolonged periods of time by the use of mechanical tooth cleaning methods, even in a well-maintained patient population is, however, laborious (Lindhe et al. 1984). Consequently, efforts have been made to utilize chemical agents, often incorporated in mouthwashes or dentifrices, as adjuvants to traditional mechanical tooth cleaning procedures. Over the years, a number of enzyme preparations, antiseptics (e.g., bisbiguanides, quaternary ammonium compounds, phenolic compounds, alkaloids, fluorides) and surface-active agents have been developed and tested in various preparations in both short- and long-term clinical trials (for review see Kornman (1985)). The present study describes a clinical trial in which various mouthrinse preparations were used as adjuvants to mechanical tooth cleaning in an attempt to reduce the development of supragingival plaque and gingivitis.

The objective of the trial was to determine in some detail the clinical effect of 2 chlorhexidine digluconate solutions and listerine used in mouthwashes to supplement regular oral hygiene measures on dental plaque and gingivitis in humans.

Material and Methods

96 volunteers, 16–50 years of age, were recruited for the study. They all had signs of varying degrees of gingivitis in different parts of the dentition but were free of periodontal attachment and

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bone loss. Following a baseline examination, each subject was given a careful prophylaxis including scaling and professional tooth cleaning (Axelsson & Lindhe 1974). Immediately after the prophylaxis, the participants started on the mouthrinse regimens but continued to exercise their regular non-supervised, self-performed plaque control programs.

The 96 volunteers were assigned either to 1 of 3 different treatment groups or to a control group according to a randomized code by which double-blinding was maintained. The members of the control group and the listerine group rinsed vigorously with 20 ml of the mouthrinse preparation for 30 s twice daily (in the morning and in the afternoon), while the members of chlorhexidine groups (group hibitane 0.2% and group hibitane 0.1%) rinsed with 10 ml of the antiseptic solutions for 60 seconds twice daily.

Chlorhexidine is a cationic bisbiguanide the antiseptic properties of which are not unusual when tested in vitro (Gjermo et al. 1970). Unlike most other antiseptics, however, chlorhexidine is retained in the oral cavity following rinsing and is subsequently released over a period of 8–12 h. An antibacterial milieu of long standing can therefore be established in the mouth and bacterial colonization on tooth surfaces prevented or retarded. The ability of chlorhexidine, incorporated in a mouthwash to prevent plaque formation and in part resolve existing plaque is well documented (for review, see Kornman (1985)).

Listerine mouthrinse contains components such as ethanol, menthol, thymol, methyl salicylate and eucalyptol. The mouthwash preparation has been shown to be effective in reducing plaque both when used alone or when used as a supplement to regular toothbrushing (for review see Gordon et al. (1985)).

The rinsings were initiated the day of the first prophylaxis. During the weekdays (Monday through Friday), the rinsings were performed under supervision, while unsupervised rinsings were carried out over the weekends. During the course of 6 weeks of trial, the various participants continued their usual oral hygiene and dietary habits but were instructed to refrain from using commercial mouthrinses. They were each supplied with a Lactona® soft nylon toothbrush and Colgate® MFP toothpaste.

A complete intraoral soft tissue examination was performed at baseline and after 3 and 6 weeks of trial to evaluate the condition of the oral mucosa. The buccal, labial, and sublingual mucosa, the tongue, the hard and soft palate, the uvula and the oropharynx were examined for inflammation, ulcerations or other lesions. Aberations were recorded, their severity accessed and a judgement made as to whether they were attributable to the mouthrinse preparations and regimens utilized.

Extrinsic stain was scored at baseline, 3 and 6 weeks, by the use of a modification of the Lobene index (Lobene 1968). The examinations of stain were limited to the labial surfaces of the 12 anterior teeth. Each tooth was visually divided into 2 regions: the *gingival region* consisted of about a 3 mm wide area of the tooth next to the gingiva, and the *body region* which constituted the remaining part of the labial tooth surface. Only the *gingival region* was scored both for area and severity of staining according to the following criteria:

area
- 0 – no stain detected
- 1 – stain up to ⅓ of the gingival tooth region
- 2 – stain over ⅓ to ⅔ of the gingival tooth region
- 3 – stain over more than ⅔ of the gingival tooth region

Severity
- 0 – no stain
- 1 – light stain
- 2 – moderate stain
- 3 – heavy stain

The area and severity scores were multiplied with each other for a tooth score; tooth scores were added and a *stain index score* for the individual hereby calculated.

Gingivitis was scored at baseline and 6 weeks by the use of the gingival index system (Loe & Silness 1963). The buccal and lingual gingival units and the interdental papillae of all available teeth were examined using the following criteria:

- 0 – absence of inflammation
- 1 – mild inflammation (slight change in color, little change in texture, no bleeding on pressure)
- 2 – moderate inflammation (moderate redness, edema, and hypertrophy; bleeding on pressure)
- 3 – severe inflammation (marked redness and hypertrophy; tendency to spontaneous bleeding or ulceration)

Plaque area was scores at baseline, 3 and 6 weeks by the Turesky modification of the Quigley-Hein index (Turesky et al. 1970). This index emphasizes plaque present on the gingival third of the tooth surface. The buccal and lingual surfaces of all available teeth were examined for plaque. Before scoring, the plaque was disclosed with an erythrosine dye solution:

- 0 – no plaque
- 1 – separate flecks or discontinuous band of plaque at the gingival margin of the tooth surface
- 2 – thin (≤1 mm) continuous band of plaque at the gingival margin of the surface
- 3 – band of plaque wider than 1 mm but less than ⅓ of the tooth surface
- 4 – plaque covering ⅓ or more but less than ⅔ of the tooth surface
- 5 – plaque covering ⅔ or more of the tooth surface

The order of examinations were (a) soft tissue condition, (b) stain index, (c) gingival index, and (d) plaque index. All intraoral examinations were performed by a single dental examiner (P.A.).

Statistical analyses

The study was designed to provide a minimal power of 0.70 for detecting a clinically important difference to be statistically different at the 0.05 probability level. The final sample size was based on the maximum determined among the separate requirements of the plaque and gingival index scores.

Average index or score was determined for each subject. The averages were analyzed by analysis of variance or covariance.

The Fisher exact test was utilized for pairwise comparisons of treatment groups for degree of stain.

Results

Of the 96 volunteers who initially were recruited for the study, 8 failed to complete the entire 6-week period of trial. The reason for termination of the mouthrinse regimens was mainly the development of lesions in the oral mucosa (the floor of the mouth, the gingiva or bucca). Mucosal lesions occurred in 6 subjects in the 0.2% hibitane group; 1

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Table 1. Results from the baseline examination with respect to plaque (Quigley & Hein: plaque index), gingivitis (Löe & Silness: gingival index) and extrinsic stain (Lobene: stain index). N = number of subjects. Mean ($\bar{X}$) and Standard error (SE)

Group	N	Plaque index $\bar{X}$ SE	Gingival index $\bar{X}$ SE	Stain index $\bar{X}$ SE
control	22	1.3±0.1	1.21±0.08	0.02±0.02
listerine	24	1.2±0.1	1.19±0.07	0.13±0.10
hibitane 0.2%	18	1.4±0.1	1.18±0.08	0.01±0.01
hibitane 0.1%	24	1.2±0.1	1.26±0.07	0.12±0.09

subject in the control group complained of oral irritation which worsened during study, 1 control subject did not continue the rinsing regimen for personal reasons. The alterations that occurred between the baseline examination and 3 weeks and 6 weeks of trial with respect to plaque, gingivitis and extrinsic tooth stain are thus reported for 88 subjects: 22 in the control group, 24 in the listerine group, 18 in the 0.2% hibitane group and 24 in the 0.1% hibitane group.

The examinations performed at the baseline examination for the 88 subjects that completed the trial revealed that the 4 study groups had similar mean plaque index, gingival index and stain index scores (Table 1). After the first 3 weeks of non-supervised, self-performed plaque control combined with supervised rinsings, the mean plaque index score of the control group was found to have remained unchanged (1.3±0.1 versus 1.2±0.1), while in the 3 test groups, the plaque scores had become significantly ($p<0.001$) reduced (Table 2A and Fig. 1). The improvement was of similar magnitude (50–60%) in the 3 groups and occurred both in incisors (Table 2B) and molars (Table 2C) and on buccal (Table 3A) and lingual (Table 3B) surfaces (Fig. 2). The degree of improvement was more pronounced in the incisor than in the molar tooth regions. Thus, while the plaque scores of the incisors in the 3 test groups after 3 weeks of trial varied between 0.3–0.4, the corresponding scores in the molars were significantly ($p<0.001$) higher and amounted to between 0.8 and 1.1. The measurements performed at the 6-week examination revealed that the improved oral hygiene status noted in the test groups after the first 3 weeks remained unchanged or improved further (Tables 2 and 3). The individual mean plaque index score of the control group at 6 weeks was not significantly different from the value calculated at baseline. It should be observed, however, that the plaque scores representing the molar regions decreased in this group from 1.9 at baseline to 1.4 after 6 weeks ($p<0.01$). Table 4 reports the % of tooth surfaces which received a plaque index score of 0, 1, 2, 3, 4 or 5 at the baseline and at the 6-week examination for the control group as well as for the 3 test groups. A plaque index score of 0 represents a tooth surface that is entirely free of clinically detectable plaque. At baseline, between 41% and 31% of all surfaces examined were found to be plaque-free. At the 6 week examination, in comparison to the baseline data, there was in the 3 test groups, but not in the control group, a marked increase in the % of plaque-free tooth surfaces. Thus, in the listerine group, the % of surfaces with plaque index score 0 had increased from 40% to 66%, while in the 2 hibitane groups, the corresponding change was 31% to 76% (hibitane 0.2%) and 41% to 67% (hibitane 0.1%).

Table 5 presents the individual mean stain index scores for the 4 study groups at baseline, 3 weeks and 6 weeks. Significant extrinsic tooth discolorations did not develop during the 6 weeks of trial in any of the groups.

The gingival index alterations are re-

Table 2A. Plaque index (Quigley & Hein Index) calculated from measurements made at baseline, 3 weeks and 6 weeks; overall mean±SE

	Control $\bar{X}$ SE	Listerine $\bar{X}$ SE	Hibitane 0.2% $\bar{X}$ SE	Hibitane 0.1% $\bar{X}$ SE
baseline	1.3±0.1	1.2±0.1	1.4±0.1	1.2±0.1
*3-weeks	1.2±0.1	0.6±0.1	0.5±0.1	0.5±0.1
*6-weeks	1.2±0.1	0.6±0.1	0.3±0.1	0.5±0.1
*Adjusted means.				
% reduction from control				
at 3-weeks		50%	61%	57%
at 6-weeks		51%	77%	54%
		$p<0.001$	$p<0.001$	$p<0.001$

Table 2B. Plaque index scores ($\bar{X}$±SE) for incisor tooth regions

	Control $\bar{X}$ SE	Listerine $\bar{X}$ SE	Hibitane 0.2% $\bar{X}$ SE	Hibitane 0.1% $\bar{X}$ SE
baseline	1.1±0.1	0.9±0.1	1.1±0.1	0.8±0.1
*3-weeks	1.0±0.1	0.4±0.1	0.4±0.1	0.3±0.1
*6-weeks	1.1±0.1	0.3±0.1	0.2±0.1	0.3±0.1
*Adjusted means.				
% reduction from control				
at 3-weeks		62%	65%	75%
at 6-weeks		69%	82%	71%
		$p<0.001$	$p<0.001$	$p<0.001$

Table 2C. Plaque index scores ($\bar{X}$±SE) for molar tooth regions

	Control $\bar{X}$ SE	Listerine $\bar{X}$ SE	Hibitane 0.2% $\bar{X}$ SE	Hibitane 0.1% $\bar{X}$ SE
baseline	1.9±0.1	1.8±0.1	1.9±0.2	1.9±0.1
*3-weeks	1.7±0.1	1.1±0.1	0.8±0.1	0.9±0.1
*6-weeks	1.4±0.1	1.0±0.1	0.5±0.1	0.8±0.1
*Adjusted means.				
% reduction from control				
at 3-weeks		31%	53%	48%
at 6-weeks		29%	65%	45%
		$p<0.009$	$p<0.001$	$p<0.001$

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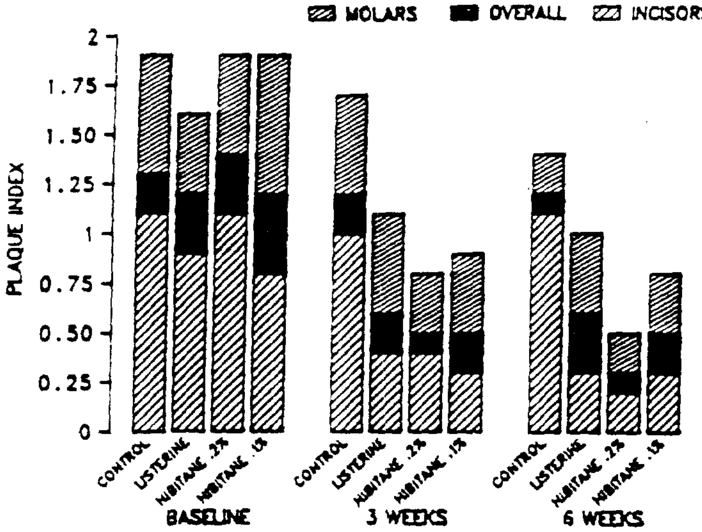

Fig. 1. Individual mean (overall) plaque scores, and scores representing *molars* and *incisors*, calculated for measurements made at *baseline* and after *3 weeks* and *6 weeks* of trial. In both the control and the 3 test groups, a reduction of the plaque scores occurred between the *baseline* and the 3- and 6-week examinations. The improvements were, however, more marked in the test groups than in the control group.

ported in Tables 6, 7 and in Figs. 3, 4. Between the baseline and the 6-week examinations, there were only minor improvements in the condition of the gingiva of the control group participants. In all 3 test groups, however, the mean GI scores were significantly ($p<0.001$) reduced during the 6 weeks of trial. Thus, in the listerine group, the individual mean GI score was reduced from 1.19 ± 0.07 to 0.48 ± 0.06 (51% compared to control) while in the hibitane groups the GI scores were brought down from 1.18 ± 0.08 to 0.65 ± 0.07 (0.2% hibitane group) and , from 1.26 ± 0.07 to 0.61 ± 0.06 (0.1% hibitane group). The improvement of the gingival conditions was more marked in incisor than in the molar regions (Tables 6B and 6C and Fig. 3) and was least pronounced at lingual surfaces (Tables 7A, B, C and Fig. 4). A further analysis of the changes that occurred during the 6 weeks of trial with respect to the gingival conditions is presented in Table 8. At the baseline examination, between 37% and 43% of all gingival units examined showed clinical signs of moderate to severe inflammation (gingival index scores 2 or 3). In all 4 study groups, the % of gingival units scored GI 2+3 was reduced between the baseline and the 6-week examination. At the end of trial, 25% of the gingival units in the control group received a GI score of 2 or 3, while the corresponding figures for the test groups were 7% (listerine group), 12% (hibitane 0.2% group) and 11% (hibitane 0.1% group). The improvement of the gingival conditions in the 4 study groups is also illustrated by the increased % of clinical healthy units. At baseline, between 18% and 22% of the gingival sites received a GI score of 0. After 6 weeks, the corresponding figures were 27% (control group) and 59, 46 and 50% in the listerine and the 2 hibitane groups respectively.

Discussion

The results of the present clinical trial demonstrated that each of the 3 active mouthwash preparations used as supplements to regular tooth cleaning measures markedly improved both the oral hygiene status and the gingival conditions of the participating human volunteers. Most of the improvement with respect to the oral hygiene, i.e., reduction of the plaque scores, occurred during the first 3 weeks of the trial, but was maintained or slightly further enhanced during the second 3-week period. Our findings thus agree with data previously reported on the effects of chlorhexidine digluconate and listerine antiseptic used as mouthrinses both in

Table 3A. Result of plaque index (Quigley & Hein index) measurements representing buccal surfaces

	Control $\bar{x}$ SE	Listerine $\bar{x}$ SE	Hibitane 0.2% $\bar{x}$ SE	Hibitane 0.1% $\bar{x}$ SE
baseline	1.2 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	1.1 ± 0.1
*3-weeks	1.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
*6-weeks	1.1 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
*Adjusted means.				
% reduction from control				
at 3-weeks		55%	59%	62%
at 6-weeks		52%	76%	61%
		$p<0.001$	$p<0.001$	$p<0.001$

Table 3B. Result of plaque index (Quigley & Hein index) measurements representing lingual surfaces

	Control $\bar{x}$ SE	Listerine $\bar{x}$ SE	Hibitane 0.2% $\bar{x}$ SE	Hibitane 0.1% $\bar{x}$ SR
baseline	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.2 ± 0.1
*3-weeks	1.3 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
*6-weeks	1.2 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
*Adjusted means.				
% reduction from control				
at 3-weeks		47%	62%	57%
at 6-weeks		51%	77%	50%
		$p<0.001$	$p<0.001$	$p<0.001$

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PLAQUE INDEX SCORES

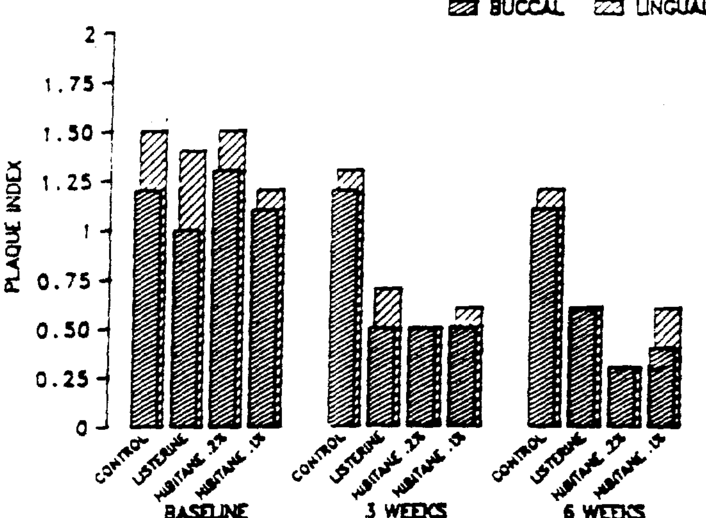

Fig. 2. Mean plaque scores representing *buccal* and *lingual* surfaces at the *baseline* examination and after *3* and *6 weeks* of trial.

terms of plaque inhibition and resolution of gingivitis (for review see Kornman (1985)).

The mere participation in a clinical trial which involves prophylaxis and repeated dental examinations may, even if no active attempts are made to change the quality of the self-performed plaque control, stimulate the subjects involved to improve their mechanical tooth cleaning measures. This so called "placebo effect" was examined in the present study in great detail. Both with respect to plaque reduction and gingivitis resolution, significant changes occurred during the 6 weeks of trial in the control group. The individual mean plaque index score of the control group did not change between the baseline and the 6-week examination (1.3±0.1 versus 1.2±0.1), but a significant reduction occurred in the molar tooth regions (1.9±0.1 versus 1.4±0.1) and at lingual surfaces (1.5±0.1 versus 1.2±0.1). Furthermore, with respect to the % of "plaque-free" tooth surfaces (plaque index scores 0+1), it was observed that such surfaces increased from 58% at baseline to 68% at 6 weeks. In addition, surfaces that harbored abundant amounts of plaque (i.e., plaque index scores 3+4+5) were reduced from 17% at baseline to 11% at 6 weeks. During the 6-week period of observation, the individual mean gingival index score of the control group was also reduced from 1.2 to 1.0. A further analysis of the individual score data revealed that the % of clinically healthy gingival units (GI score 0) increased from 20 to 27%, and markedly inflammed gingival units (GI score 2+3) were reduced from 40 to 25%. Because of the significant changes that occurred in the control group, the improvements registered in the test groups were expressed in terms of % reductions in comparison to those that occurred in the control.

All 3 active mouthwash preparations tested were effective in reducing plaque and signs of gingivitis in individuals who continued their regular tooth cleaning habits during the trial. This efficacy was reflected by improvements in the individual mean plaque and gingival index scores, but became even more obvious when the frequency distributions of the various scores were considered. Let us, for example, assume that the objective of using a mouthwash to supplement mechanical tooth cleaning is to reach tooth surfaces with abundant amounts of plaque left following regular toothbrushing. After 6 weeks of trial, only between 2% and 4% of such surfaces (scored plaque index 3+4+5) remained in the test groups: all were located in the molar tooth regions. It should be emphasized that this effect was obtained in individuals who already had a rather high standard of oral hygiene prior to the trial. This fact is illustrated by the observation that at baseline, between 58% and 67% of all tooth surfaces were "plaque-free" (had a plaque index score of 0 or 1). The dramatic improvement of the oral hygiene condition in the test groups is also demonstrated by the increased % of "plaq-

Table 4. % of tooth surfaces scored plaque index 0, 1, 2, 3, 4 and 5 at baseline and after 6 weeks of trial

	Plaque index (Quigley & Hein)					
Score	0	1	2	3	4	5
Control						
baseline	34%	24%	25%	11%	4%	1%
6-weeks	23%	45%	21%	9%	1%	1%
Listerine						
baseline	40%	24%	20%	11%	3%	1%
6-weeks	66%	20%	10%	3%	1%	0%
Hibitane 0.2%						
baseline	31%	27%	24%	11%	5%	2%
6-weeks	76%	17%	4%	2%	0%	0%
Hibitane 0.1%						
baseline	41%	26%	17%	10%	4%	2%
6-weeks	67%	20%	10%	2%	1%	0%

Table 5. Extrinsic stain index (a.m. Lobene (1968)) calculated from assessments made at baseline and after 3 and 6 weeks of trial: mean and standard error

	Control $\bar{x}$ SE	Listerine $\bar{x}$ SE	0.2% Hibitane $\bar{x}$ SE	0.1% Hibitane $\bar{x}$ SE
baseline	0.02±0.02	0.13±0.09	0.00±0.00	0.13±0.09
3-weeks	0'05±0.03	0.07±0.04	0.07±0.04	0.14±0.12
6-weeks	0±0	0.09±0.05	0.14±0.07	0.10±0.06

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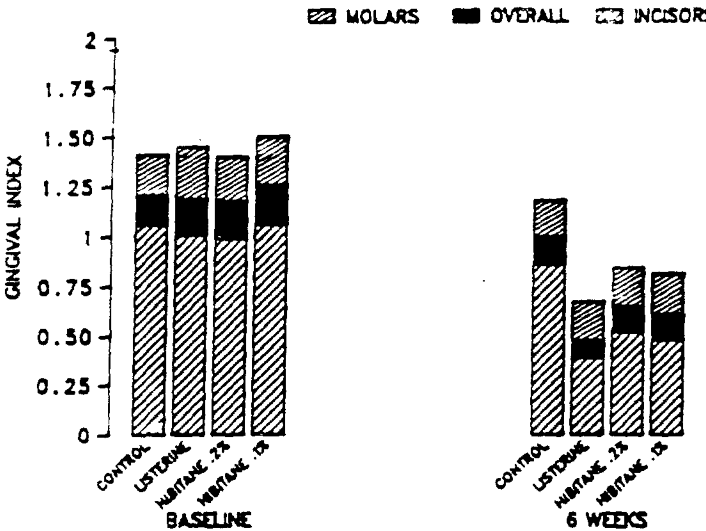

Fig. 3. Individual mean *(overall)* gingival index scores and scores from *molar* and *incisor* tooth regions calculated for measurements made at the *baseline* examination and after *6 weeks* of trial. The improvement of the gingival conditions was more pronounced in the test groups than in the placebo control group.

ue-free" tooth surfaces at the 6-week examination, i.e., 86% (listerine), 93% (hibitane 0.2%) and 87% (hibitane 0.1%), compared to 68% in the control group.

Furthermore, the present plaque data demonstrate that while the improvement in the control group occurred in molars and at lingual tooth surfaces, in the test groups, the most obvious plaque reductions were observed in incisors. This indicates that following prophylaxis on day 0, the members of the control group paid more attention than before to plaque removal in the posterior segments of the dentition. It also indicates that mouthrinsing as exercised in the present trial was less effective in bringing the active ingredients to the posterior than to the anterior teeth.

The active mouthrinse preparations were also effective in comparison to the control in terms of gingivitis resolution. Thus, out of all gingival units which at the baseline were considered inflammed (GI score 2 or 3), 9% had become healthy (GI score 0) in the control group, after 6 weeks, while the corresponding figures for the test groups were 39% (listerine), 30% (hibitane 0.2%) and 29% (hibitane 0.1%). In other words, the active mouthwashes were 3 to 4 times as effective as the placebo rinse in eliminating clinical signs of gingivitis.

In the listerine group, the individual mean plaque index scores, in comparison to the control data, were reduced by about 50% after 3 and 6 weeks of trial. At 6 weeks, the individual mean gingival index score was also reduced by 50%. This degree of retardation of new plaque formation and resolution of gingivitis obtained by listerine antiseptic, when used as a supplement to normal oral hygiene procedures, is compatible with or even greater than reductions previously reported from similar studies (e.g., Gomer et al. 1972, Lusk et al. 1974). The observations made in the present trial also corroborate results reported by Fornell et al. (1975). They assessed in a 2-week cross-over study the rate of plaque formation and gingivitis development when all efforts toward active mechanical oral hygiene were withdrawn. The 10 participants rinsed 3 times a day with 20 ml of listerine or a placebo solution. The authors reported that after the 2 weeks of trial, there was in the active group a 53% reduction of the mean plaque index score, a 93% reduction of plaque wet weight, and a 47% reduction of the mean gingival index score.

A comparison between the listerine group and the two hibitane groups in the present trial revealed that the chlorhexidine-containing mouthrinses were equally or more effective in reducing plaque than was listerine but not quite as effective in enhancing gingivitis resolution. This conclusion is based on comparisons made both between the mean plaque and gingival index scores

Table 6A. Individual mean gingival index scores ($\bar{X} \pm SE$) calculated from assessments made at baseline and after 6 weeks of trial

	Control $\bar{X}$ SE	Listerine $\bar{X}$ SE	Habitane 0.2% $\bar{X}$ SE	Habitane 0.1% $\bar{X}$ SE
baseline	1.21±0.08	1.19±0.07	1.18±0.08	1.26±0.07
6-weeks	1.00±0.06	0.48±0.06	0.65±0.07	0.61±0.06
% reduction from control		51% $p<0.001$	35% $p<0.001$	38% $p<0.001$

Table 6B. Gingival index scores for *incisor tooth regions*

	Control $\bar{X}$ SE	Listerine $\bar{X}$ SE	Hibitane 0.2% $\bar{X}$ SE	Hibitane 0.1% $\bar{X}$ SE
baseline	1.06±0.09	1.01±0.09	0.99±0.1	1.06±0.09
6-weeks	0.86±0.07	0.39±0.06	0.52±0.07	0.48±0.06
% reduction from control		55% $p<0.001$	39% $p<0.001$	44% $p<0.001$

Table 6C. Gingival index scores for *molar* tooth regions

	Control $\bar{X}$ SE	Listerine $\bar{X}$ SE	Hibitane 0.2% $\bar{X}$ SE	Hibitane 0.1% $\bar{X}$ SE
baseline	1.41±0.07	1.45±0.06	1.40±0.07	1.50±0.06
6-weeks	1.18±0.07	0.67±0.06	0.84±0.07	0.81±0.06
% reduction from control		43% $p<0.001$	29% $p<0.001$	31% $p<0.001$

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Table 7. Gingival index scores calculated from measurements made at baseline and after 6 weeks of trial

(A) *Buccal units* ($\bar{X}$ ± SE)

	Control $\bar{X}$ SE	Listerine $\bar{X}$ SE	Hibitane 0.2% $\bar{X}$ SE	Hibitane 0.1% $\bar{X}$ SE
baseline	0.78±0.08	0.76±0.08	0.81±0.09	0.81±0.08
6-weeks	0.64±0.04	0.28±0.04	0.46±0.05	0.39±0.04
% reduction from control		57% $p<0.001$	29% $p<0.001$	40% $p<0.001$

(B) *Lingual units* ($\bar{X}$ ± SE)

	Control $\bar{X}$ SE	Listerine $\bar{X}$ SE	Hibitane 0.2% $\bar{X}$ SE	Hibitane 0.1% $\bar{X}$ SE
baseline	0.81±0.08	0.93±0.08	0.90±0.09	0.96±0.08
6-weeks	0.71±0.05	0.43±0.05	0.48±0.06	0.52±0.05
% reduction from control		39% $p<0.001$	32% $p<0.004$	27% $p<0.007$

(C) *Interproximal units* ($\bar{X}$ ± SE)

	Control $\bar{X}$ SE	Listerine $\bar{X}$ SE	Hibitane 0.2% $\bar{X}$ SE	Hibitane 0.1% $\bar{X}$ SE
baseline	1.41±0.08	1.36±0.08	1.33±0.09	1.44±0.08
6-weeks	1.15±0.07	0.55±0.07	0.74±0.08	0.69±0.07
% reduction from control		52% $p<0.001$	36% $p<0.001$	40% $p<0.001$

and on alterations, between the baseline and the 6-week examinations, of the % distribution of the various score categories.

Zusammenfassung

Die Erfolge von Mundspülungen bei der Beseitigung dentaler Plaque und bei der Ausheilung der Gingivitis des Menschen

Die vorliegende Studie wurde konzipiert, um die Wirkung zusätzlich zu oralen Hygienemassnahmen eingesetzter Mundspülungspräparate auf die Plaque und die Gingivitis beim menschlichen Probanden zu bestimmen. An dieser Studie nahmen 96 freiwillige Probanden teil. Nach einer Ausgangsuntersuchung wurde jeder Proband eingehend propylaktisch behandelt. Daran anschliessend wurden Mundspülungsanweisungen ausgegeben. Während der 6-wöchigen Beobachtungsperiode setzten die Versuchspersonen ihre regelmässige, nicht überwachte häusliche Plaquekontrolle fort. Nach einem zufälligen Verteilungsschlüssel wurden die 96 freiwilligen Probanden entweder einer der 3 verschiedenen Behandlungsgruppen oder einer Kontrollgruppe zugeteilt. Die Mitglieder der Kontrollgruppe und die Listeringruppe spülten 30 Sekunden lang mit ihrer Mundspülflüssigkeit, während die Angehörigen der Chlorhexidingruppen (0.2%-ge oder 0.1%-ge Lösung) 2 mal täglich 60 Sekunden lang mit 10 ml. der entspr. antiseptischen Lösung spülten. Untersuchungen über externe Verfärbungen und das Vorkommen von Plaque wurden bei der Ausgangsuntersuchung und 6 Wochen danach beurteilt. Die externe Verfärbung wurde nach dem Lobene-Index bestimmt. Die Plaque wurde nach der Turesky'schen Modifikation des Quigley-Hein Index beurteilt und die gingivale Gesundheit nach dem System von *Löe & Silness*. Im Vergleich zu den Kontrollgruppen zeigen die Resultate dieses Versuches, dass sowohl der orale Hygienestatus als auch die Zahnfleischgesundheit freiwilliger menschlicher Probanden durch zusätzlich zu regelmässigen Zahnreinigungsmassnahmen vorgenommene Spülungen mit 3 aktiven Mundspüllösungen, deutlich verbessert wurde. Die Resultate stimmen mit kürzlich veröffentlichten Daten über die Effekte des Chlorhexidin-Glukonates und des antiseptischen Listerin überein, und zwar sowohl hinsichtlich der Plaquebeseitigung als auch der Ausheilung der Gingivitis.

Table 8. % of gingival units which received a gingival index score of 0, 1, 2 and 3 at the baseline and the 6-week examinations

Score	Gingival index (Löe & Silness 1963) 0	1	2	3
Control				
baseline	20%	40%	39%	1%
6-weeks	27%	48%	23%	2%
Listerine				
baseline	22%	39%	38%	1%
6-weeks	59%	34%	7%	0%
Hibitane 0.2%				
baseline	20%	43%	37%	0%
6-weeks	46%	41%	12%	0%
Hibitane 0.1%				
baseline	18%	40%	42%	1%
6-weeks	50%	38%	11%	0%

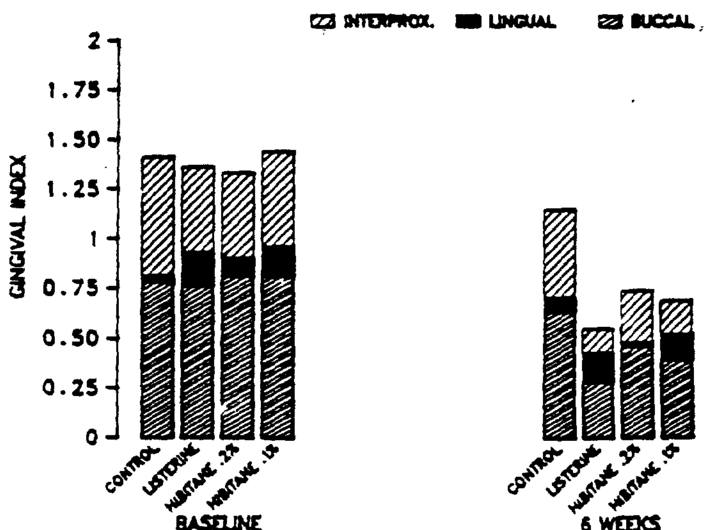

Fig. 4. Mean gingival index scores representing *interproximal*, *lingual* and *buccal* units calculated for measurements made at the *baseline* examination and after 6 weeks of trial.

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Résumé

Efficacité des bains de bouche pour l'inhibition de la plaque et de la gingivite chez l'homme

Le but du présent travail expérimental était de déterminer l'effet de différents produits pour bains de bouche, utilisés comme adjuvants des soins habituels d'hygiène bucco-dentaire, sur la plaque et sur la gingivite chez l'homme. 96 volontaires ont été recrutés pour l'étude. Après un examen initial, tous les sujets ont reçu un nettoyage dentaire minutieux, suivi d'un période de rinçages de bouche. Pendant les 6 semaines de l'expérience, les sujets continuaient à pratiquer les soins habituels personnels d'hygiène bucco-dentaire, sans surveillance. Les 96 sujets ont été répartis suivant une méthode aléatoire soit dans un des 3 groupes de traitement, soit dans un groupe témoin. Les membres du groupe témoin et ceux du groupe de la listérine faisaient 2 fois par jour des rinçages de bouche de 30 s avec 20 ml du bain de bouche; les membres des 2 groupes de la chlorhexidine (utilisant soit une solution d'hibitane à 0.2% soit une solution à 0.1%) faisaient 2 fois par jour des rinçages de bouche de 60 s avec 10 ml de la solution antiseptique. Des examens concernant les colorations externes et la plaque ont été pratiqués à l'origine et après 3 et 6 semaines; l'état de la gencive a été examiné à l'origine et après 6 semaines. Les colorations externes ont été évaluées par l'indice de Lobene, la plaque par la modification de Turesky de l'indice de Quigley-Hein et l'état de la gencive a été enregistré au moyen de l'indice gingival de Loe & Silness. Les résultats de cette expérience ont montré que les 3 produits actifs pour bains de bouche utilisés comme adjuvants des soins habituels d'hygiène bucco-dentaire donnaient une nette amélioration du degré de l'hygiène buccale ainsi que de l'état de la gencive des sujets volontaires par rapport au bain de bouche témoin. Ces résultats sont en accord avec les données présentées antérieurement sur les effets du digluconate de chlorhexidine et de l'antiseptique listerine, tant pour l'inhibition de la plaque que pour l'amélioration de la gingivite.

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(VI) SUMMARY OF DATA AND RATIONALE

This section sets forth the scientific basis for the conclusion that Listerine is safe and effective for the prevention and reduction of plaque and gingivitis. Summaries are provided of all referenced reports and articles.

Listerine safety is discussed in the first part of this section. As described there, FDA and a number of expert panels have determined that Listerine ingredients are safe for use in both food and drugs. Listerine safety is demonstrated through extensive clinical trials and through 110 years of consumer use.

The second part of this section summarizes the scientific data demonstrating the effectiveness of Listerine in preventing and reducing plaque and gingivitis. The antibacterial activity of Listerine is discussed as well. In addition, data is presented on the antibacterial properties of the individual active ingredients and the contribution of each to the overall antibacterial properties of the formulation. We also explain the etiology of plaque and gingivitis and the need for a chemical means to combat these conditions. Finally, we discuss the American Dental Association's (ADA's) review and acceptance of Listerine as safe and effective for the prevention and reduction of plaque and gingivitis and the support of the American Academy of Periodontology for the ADA decision.

We have included all major short and long term clinicals that clearly support the safety and efficacy of Listerine Antiseptic for the prevention and reduction of supragingival plaque and gingivitis. We have not included numerous other studies on Listerine for other claims, e.g., bad breath, etc., nor have we included studies on plaque or gingivitis where Listerine was included as a control or to make a comparison to another product. The omitted studies are not inconsistent with the studies that are included in this submission.

A. SAFETY

1. FOOD AND DRUG REGULATORY STATUS

The four oils in Listerine (thymol, eucalyptol, methyl salicylate and menthol) are all recognized and commonly used in food as flavoring agents. They are generally recognized as safe (GRAS) or approved as food additives by current FDA regulations and/or are deemed to be GRAS by the Expert Panel of the Flavor Extract Manufacturers Association (FEMA). The FEMA panel consists of a group of expert pharmacologists and toxicologists whose mission is to determine, on the basis of all available data, including experience based on common use in food, what substances are GRAS. FDA has regularly deferred to the expertise of FEMA in assessing the safety of flavoring agents. The pertinent regulatory citations are identified in the attached (Table I) and the full reports can be found in Section IV. A. - IV. D. In addition, the full Scientific Literature Review documents prepared by FEMA are attached, as appendices I through IV.

These ingredients are ubiquitous in foods, particularly confectionery products. The National Academy of Science survey, of industry on the use of food additives reported the use of these flavors in cough drops, hard candies, soft candies and chewing gums, among other products (Appendices I-IV). All of these confectionery products are used by the general population on an unlimited basis.

Additionally, these oils are found in a multiplicity of OTC drug product formulations and, as such, have been reviewed by different OTC drug expert advisory panels, for both internal and external indications. All of these panels have found these essential oils safe for their intended use. A summary of the respective panel findings are also appended (Table II). The complete panel reports can be found in Section IV. A. through IV. D.

2. ORAL MUCOSAL SAFETY

1) Introduction

Over the past several decades Listerine has been the subject of extensive clinical studies (reported herein) designed to assess efficacy against plaque and gingivitis. These studies have been of various durations, but more recently, longer term (6 and 9 month) studies have been conducted following American Dental Association (ADA) guidelines. Listerine has also been the subject of two *in vivo* microbiology studies in which the oral microflora was assessed following long-term use.

These studies, representing over 20 years of data derived from controlled supervised clinical settings encompassing over 1500 subjects, show conclusively that Listerine is safe for use in the oral cavity. In fact, not one case of oral soft or hard tissue aberration attributable to Listerine was reported in any of these studies. Additionally, long-term use of Listerine has not been found to adversely alter the oral microflora.

Various investigators have looked at the possible link between mouthwash use and oral mucosal damage and oral cancer. These studies, which do not show a causal relationship between mouthwashes and oral cancer, are also described herein.

The following sections summarize the above noted clinical studies from the standpoint of oral safety.

2) Oral Mucous Membrane Safety

a) Lamster, I. et al.: The effect of Listerine Antiseptic on reduction of existing plaque and gingivitis. Clin. Prev. Dent.; 5: 12-16, Nov.-Dec. 1983/Warner-Lambert Research Report # 931-0170, March 24, 1982. (See Section IV Safety Studies Ref. a.)

This was a double-blind placebo controlled six month study designed to assess the efficacy of Listerine against plaque and gingivitis. It included 145 subjects, 45 of whom were in the Listerine group. Subjects rinsed twice a day with Listerine under labeled directions for use. This study included specific evaluation of oral soft and hard tissue at baseline, 1, 3 and six months for any pathology. The oral tissues examined were buccal mucosa, labial mucosa, sublingual mucosa, tongue, hard/soft pallat and uvula/oropharynx. as part of the examinations, aberrations were to be recorded, their severity assessed and a judgment made as to whether or not they were attributable to test materials. There were no reports of any oral tissue abnormalities or aberrations.

b) Gordon, J. et al.: Efficacy of Listerine Antiseptic in inhibiting the development of plaque and gingivitis. J. Clin. Periodontol.; 12: 697-704, 1985/Warner-Lambert Research Report # 931-0491, March 5, 1984. (See Section IV Safety Studies Ref. b.)

This nine month, plaque/gingivitis, placebo-controlled study was conducted in 127 subjects, 38 of whom were in the Listerine treatment groups for six months with 27 remaining for 9 months. The protocol was similar to the study noted above. Oral soft tissue exams were performed at baseline, 1, 3, 6 and 9 months. No effects were observed in any group, in terms of soft or hard tissue abnormalities.

c) De Paola, L. et al.: Chemotherapeutic inhibition of supra-gingival dental plaque and gingivitis development. J. Clin. Periodontol.; 16: 311-315, May 1989/Warner-Lambert Research Report # 931-0647, December 17, 1985. (See Section IV Safety Studies Ref. c.)

One hundred and seven (107) subjects participated in this six-month plaque/gingivitis study, 54 of whom were in the Listerine treatment group. Following a protocol similar to the Lamster and Gordon

studies reported above, subjects rinsed with Listerine or placebo twice a day under label directions. Intraoral soft tissue examinations were performed at baseline, and after 1, 3 and six months. No soft tissue aberrations attributable to either rinse were noted.

d) Mankodi, S.: Efficacy of Listerine (W2194-92) and Listerine Plus Mint (W2194-194) in inhibiting the development of dental plaque and gingivitis. Warner-Lambert Research Report # 931-0780, July 18, 1989. (See Section IV Safety Studies Ref. d.)

This six-month controlled clinical study compared **the anti-plaque/** anti-gingivitis efficacy of Listerine to an **experimental mint** flavored Listerine containing identical levels of active ingredients. 124 subjects completed the study. All subjects rinsed twice a day with test material for 30 seconds. **Intraoral soft** tissue examinations were performed at baseline, **3 and 6 months.** Tissues were examined for inflammation, infection ulceration or lesions. No mention of adverse reactions appeared in the research report.

e) Overholser, D. et al.: Comparative effects of 2 chemotherapeutic mouthrinses on the development of supragingival dental plaque and gingivitis. J. Clin. Periodontol.; 17:575-579, September 1990/Warner-Lambert Research Report # 931-0730, February 2, 1988. (See Section IV Safety Studies Ref. e.)

This six-month controlled clinical study compared the anti-plaque/ anti-gingivitis efficacy of Listerine to that of chlorhexidine (Peridex, Procter & Gamble). A total of 124 subjects completed the study with 41 in the Listerine group. As in previous studies, oral soft tissue was examined at baseline, and after 3 and 6 months. There were no abnormal soft tisue findings reported.

f) Mankodi, S.: Effect of Listerine Antiseptic (W2194-92) and Peridex (W14948-6) compared to a hydroalcholic control (W2194-167P) in inhibiting the development of dental plaque and gingivitis. Warner-Lambert Research Report # 931-0792; March 26, 1990. (Published as an abstract J. Dent. Res.; 69: Abstr. 1099, 1990). (See Section IV Safety Studies Ref. f.)

One hundred and seven (107) subjects completed this six month controlled clinical study comparing the efficacy of Listerine to Peridex. Thirty four (34) subjects were in the Listerine treatment group. Oral mucosa tissue condition was recorded at baseline, 3 and 6 months. No adverse reactions were reported.

3) <u>Other Oral Safety Issues</u>

g) Kowitz, G., Lucatorto, F., Cherrick, H.: Effects of mouthwashes on the oral soft tissues. J. Oral Med. 31-47, 1976. (See Section IV Safety Studies Ref. g.)

h) Kowitz, G., Lucatorto, F., Bennett, W.: Effects of dentifrices on soft tissues of the oral cavity. J. Oral Med. 28:105, 1973. (See Section IV Safety Studies Ref. h.)

Kowitz reported that 2-week use of various unidentified commercial mouth washes resulted in inflammation, ulceration, epithelial peeling and geographic tongue - like lesions, but could not attribute these effects to any specific ingredients. The validity of this study is questionable. No oral examination was reported to have been made prior to the experimental use of the mouthwashes. Rigorous brushing could also have contributed to the findings of this study as previously reported by the same investigator.

i) Rothstein, A., Piccozi, A., E., Doyle, J., Cancro, L., Singer, E.: Soft Tissue responses to high frequency use of experimental and commercial mouthwashes. Pharmacol. and Therap. in Dent. 3:25:, 1978. (See Section IV Safety Studies Ref. i.)

A study by Rothstein using stringent test conditions was unsuccessful in confirming these findings. Several commercial mouthwashes, including Listerine, did not produce any soft tissue conditions beyond those which would be expected in the normal population.

j) Bernstein, M.: Oral mucosal white lesions associated with excessive use of Literine mouthwash. Report of two cases. Oral Surg. 46:781, 1978. (See Section IV Safety Studies Ref. j.)

Bernstein reported two cases of asymptomatic, diffuse, filmy white lesions in patients using Listerine. Both patients had prolonged surface contact through excessive use of the product. (One patient held the product in his mouth for 15 minutes per day while he shaved). Following discontinuation of the product, all symptoms completely disappeared in both patients.

k) Warner-Lambert Research Report #955-0831. Oral Irritation and sensitization potential of Listerine Antiseptic (W2194-92): A clinical study in humans. (See Section IV Safety Studies Ref. k.)

A well controlled study by Pallazollo, following exaggerated use of Listerine failed to show irritation or sensitization of the oral mucous membranes.

l) Weaver, A., Fleming, S., Smith, D.: Mouthwash and oral cancer: carcinogen or coincidence? J. Oral Surg. 37:250, 1979. (See Section IV Safety Studies Ref. 1.)

Weaver, et al; suggested that mouthwash use may be an etiological factor in the development of oral cancers. However, his study suffered from major limitations, a small number of cases (nine) who were non-smokers and non-drinkers and the lack of comparability between cases and controls.

m) Blot, W., Winn, D., Fraumeni, J.: Oral cancer and mouthwash. JNCI 70:251, 1983. (See Section IV Safety Studies Ref. m.)

n) Wynder, E., Kabat, G., Rosenberg, S., Levanstein, M.: Oral cancer and mouthwash use. JNCI 70:255, 1983. (See Section IV Safety Studies Ref. n.)

Two separate 1983 studies by Blot and Wynder on oral cancer, which considered mouthwash use, were unable to confirm the findings of Weaver. No dose response relationships were established and no causal significance was attributable to daily mouthwash use and oral cancer. In the study by Blot, the relative risk did not rise with increasing years of mouthwash use, nor were there consistent trends with frequency of mouthwash use, length of time the mouthwash was retained in the mouth, or concentration (full strength vs. diluted) of mouthwash.

In fact, where significant differences were observed they were frequently inversely related. For example, in Wynder's study, duration of use was highly significant but inversely associated

with disease as was the cumulative mouthwash index, which cumulatively measured daily use and duration of use. In terms of duration, the highest relative risk was seen in women who used mouthwash daily for 1-4 years. This effect, in short term users, and lack of increased risk in more frequent users is, according to the authors, most likely due to the use of mouthwash in response to symptoms of the disease rather than a cause of the disease. This study also did not establish a tumor-enhancing effect of mouthwash in smokers and alcohol consumers among men and women.

o) Kabat, G.C., Hebert, J.R., Wynder, E.L.: Risk factors for oral cancer in women. Cancer Research 49:2803, 1989. (See Section IV Safety Studies Ref. o.)

A follow-up study by Kabat, Hebert, and Wynder undertook a further evaluation of a potential relationship of mouthwash to oral cancer. They confirmed that mouthwash was not associated with increased oral cancer risk in terms of frequency, duration of use, dilution, or rinsing practices.

p) Mashberg, A., Barsa, P., Grossman, M.: A study of the relationship between mouthwash use and oral and pharyngeal cancer. J.Amer. Dent. Ass. 110:731, 1985. (See Section IV Safety Studies Ref. p.)

In a later study by Mashberg, et al. when the effects of age, smoking, and drinking habits were removed, while controlling for all other factors, oral and pharyngeal cancer did not appear related to mouthwash use. There was no evidence that mouthwash is a risk factor.

q) Warner-Lambert Research Report #931-0659. Listerine: Mutagenicity assay. II. Rat hepatocyte primary culture/DNA repair test. (See Section IV Safety Studies Ref. q.)

Recent studies have also confirmed that Listerine is not a mutagen. Listerine was demonstrated not to interact with DNA in the rat hepatocyte primary culture/DNA repair test. It was also negative in the micronucleus test and the Ames Salmonella/microsome plate test.

r) Warner-Lambert Research Report #931-0660. Listerine: Mutagenicity assay. III. Micronucleus test. (See Section IV Safety Studies Ref. r.)

s) Warner-Lambert Research Report #931-0662. Listerine: Mutagenicity assay. I. Ames Salmonella/Microsome plate test. (See Section IV Safety Studies Ref. s.)

We understand that Dr. Blot will publish a second paper in the very near future. Once available, we will submit a copy, together with our comments.

4) **Oral Microbial Safety**

In addition to oral tissue condition, two in vivo studies were conducted to assess the effect of Listerine on the oral microbial flora. These studies are described as follows:

t) Minah, G.E. et al.: Effects of 6 months use of an antiseptic mouthrinse on supragingival dental plaque microflora. J.Clin. Periodontol.; 16:347-352/ Warner-Lambert Research Report #931-0647, December 17, 1985. (See Section IV Safety Studies Ref. t.)

This study was conducted as an adjunct to the De Paola study described above (See Section V Definitive Studies Ref. 3.) the microbial flora of 83 subjects (42 in the Listerine group) was assessed at baseline, and after 3 and 6 months, based on plaque

samples. These plaque samples were analyzed via darkfield microscopy, culture on a series of selective and nonselective bacterial media and by recognition of microbial forms by recognition of distinct colony on nonselective media.

Results of the analysis showed no significant increases in presumptive oral pathogens, spirochetes, black-pigmented Bacteroides, Streptococcus mutans or Candida albicans. No detectable rise in either staphylococci or enteric bacteria, potential opportunistic pathogens, was observed.

u) Walker, W. et al.: Long-term effect of Listerine antiseptic (W2194-92) on dental plaque microbial composition. Warner-Lambert Research Report # 931-0654, December 18, 1985. (Published as an abstract J. Dent. Res.; 68: Abstr. 1845, 1989) (See Section IV Safety Studies Ref. u.)

This was an independent 6 month in vivo study. Eighty nine (89) subjects completed the study. Twenty (20) subjects rinsed with Listerine for three months and 25 subjects rinsed with Listerine for 6 months. The Listerine group rinsed twice a day for 30 seconds, per label directions. Microbial examination of plaque samples was conducted at baseline and either 3 or six months depending on the subject's length of time in the study. The microbial composition of the samples were characterized using three microbiological approaches: microscopic enumeration of cocci, motile and nonmotile rods, and spirochetes; recovery on selective and nonselective culture media; and enumeration by colony morphology on a nonselective medium. This study found no clinically significant shifts in in the composition of the microbial flora. There was no significant increase in presumptive oral pathogens or in opportunistic pathogens. Additionally, the ratio of facultative anaerobes to anaerobes remained the same in both groups relative to baseline.

3. CONSUMER EXPERIENCE

A major determinant of product safety is its history in the marketplace. Listerine has been marketed now for more than one hundred ten years making it one of the most widely used consumer products in the world. No significant untoward human adverse effects have been documented during this extensive period of human use and exposure. Reviews of our consumer correspondence files for the years 1984 through 1990 confirm the extremely low incidence of consumer complaints. During this period, we received, on the average, one report of alleged injury due to the use of the product for every 38,700,000 doses of product sold, an extremely low rate of occurrences. These reported reactions include, primarily, transient sensory phenomena, such as bite, burn, stinging as well as minor unconfirmed oral irritation reports.

4. SUMMARY

The totality of the data presented above, along with the long marketing history of Listerine, demonstrate very clearly that Listerine is safe for use in the oral cavity. Twenty years of rigorous controlled clinical testing documents that there are no significant adverse oral mucosal effects from long term use of Listerine. Recent microbiological studies show that there are no adverse effects on the oral microflora. For many years Listerine has been used twice a day by millions of people, thereby meeting the criteria of safe use for a material time and material extent. Based on the above clinical data, acceptance by the ADA Council on Dental Therapeutics and demonstrated safe marketing history Listerine is indeed a very safe product.

B. EFFICACY

1. Antibacterial Properties of Listerine

It has been well established and generally recognized and accepted by the dental scientific community that plaque is a bacterial milieu and gingivitis is a bacterially mediated disease. It is also acknowledged that the primary treatment for plaque and gingivitis is an antibacterial agent. In the OTC Oral Cavity report of May 25, 1982 (41 Fed. Reg. 22760-22930) the minority report recommended specific antibacterial testing criteria to evaluate products making antiplaque claims. (Attachment I) The testing criteria were not included in the final majority report, which did not address the issue of plaque and gingivitis. However, it is important to note that they had been previously established and adopted by the Oral Cavity Panel, and therefore represented a concensus of this group. The testing criteria provided that microorganisms, known to cause disease in the oral cavity, should be used as test cultures to assay the potency of oral products. For the purpose of the test, the panel selected Streptococcus mutans, a gram-positive cocci, Actinomyces viscosus, a gram-positive rod and Candida albicans, a yeast. These organisms, are all found in the oral cavity and plaque and have all been implicated as the cause of various dental disease conditions. Streptococcus mutans has been associated with the development of dental caries and dental plaque. Actinomyces viscosus has been associated with periodontal disease and is also found in dental plaque. Candida albicans is a recognized cause of oral yeast infections. These specific microorganisms were chosen as representative of their particular classes with the presumption that an antibacterial agent demonstrating activity against these organisms would be similarly effective against others in the respective classes.

The test guidelines specify that to be considered an effective antiseptic, the test material must kill the three microorganisms within five minutes. Listerine has consistently passed these test criteria. A study using the above methodology was was conducted to measure the antibacterial effectiveness of the individual active ingredients in Listerine as compared to the total formula. Results of this test showed that all of the Listerine active ingredients, when tested at the respective concentrations in Listerine, possessed antibacterial activity against the three oral microorganisms. Importantly, the test demonstrated that the total Listerine formulation was more effective than any of the individual oils, demonstrating that each of the active ingredients is necessary for the antiseptic activity of the complete formulation. (Attachment II)

Finally, it is important to note that Listerine antiseptic meets the definition of antiseptic, as defined in the Food, Drug and Cosmetic Act [21U.S.C. 201.(O)] According to this section "the representation of a drug in its labeling, as an antiseptic shall be considered to be a representation that it is a germicide". Listerine has consistently met this criterion, whether tested by the traditional phenol coefficient method of the U.S. Department of Agriculture or more modern microbiological methodologies.

2. Plaque and Gingivitis

1. Precursors of Periodontitis

Plaque and gingivitis have long been of concern to dental health experts. If these conditions are not controlled, they can develop into periodontitis, an advanced stage of periodontal disease which destroys the gums and supporting structures of the teeth. Periodontal disease is the foremost cause of adult tooth loss in the United States and throughout the world. Largely as a result of its

ravages, an estimated 23 million Americans were missing all of their teeth in 1971. A 1985 national survey by the National Institute of Dental Research ("NIDR") found that 41 percent of senior Americans were missing all of their teeth and only two percent had all of their teeth.

NIDR reports that chronic adult periodontitis and gingivitis, the inflammation of the gums which precedes periodontitis, are "endemic" in the United States, affecting 75 percent of all adults, 68 percent of youths, and 39 percent of children. Other estimates of the disease's prevalence among adults are as high as 90 percent. Because the disease tends to be both most prevalent and most severe among older people, a segment of the population that is rapidly growing, the disease has become increasingly more significant in recent years.

In addition to the human cost of periodontitis in terms of pain, distress, and loss of function, the financial cost of the disease is enormous. FDA reported in 1984 that periodontitis costs the American public an estimated $4 billion each year in dental fees alone. There are also substantial costs to employers in reduced productivity and to employees in lost wages.

2. The Progression From Plaque To Periodontitis

It is well-established that plaque accumulation is the most important factor in the development of chronic adult periodontitis. Plaque is a sticky, colorless film composed primarily of bacteria. Everyone has plaque. It begins to accumulate on the surface of teeth within hours after the teeth are cleaned. If plaque is not removed frequently, the bacteria contained within the plaque can proliferate and produce harmful toxins. These toxins irritate the gums, making them red, tender, swollen, and susceptible to bleeding. This condition is called gingivitis, literally, an inflammation of the gingiva or gums.

Gingivitis can become more severe if the unremoved plaque hardens into calculus (tartar), a rough, porous deposit that collects under the gumline. Calculus is not in itself pathogenic, but its rough texture offers the plaque an easier surface to which to adhere than a smooth and polished tooth and thus both fosters plaque build-up and makes plaque removal more difficult.

Gingivitis is completely reversible if the plaque is removed while its impact is confined to the inflammation of the gums. If the plaque is removed at this stage, the inflammation will disappear, and there will be no lasting damage.

If gingivitis is not arrested, however, the supragingival plaque will start to proliferate subgingivally, and the gingivitis can progress to periodontitis. Paradoxically, the human body's own defense mechanisms may assist in this process. When the bacteria-laden plaque is not removed, defending white blood cells and antibodies accumulate at the affected areas and may be subverted into attacking the body's own tissue. In the meantime, the toxic products from the plaque will have begun to destroy the fibers that bind the teeth to the gums. Eventually, as more and more of these gum-to-tooth connective fibers are destroyed, the gums start to separate from the teeth. The spaces that develop when the gums separate from the teeth are call "periodontal pockets." These pockets become veritable nests for bacteria and their pathogenic products to grow and flourish. As gingivitis progresses into periodontitis, the pockets between the teeth and the gums become steadily deeper, until finally the toxic products affect the bone in which the teeth are embedded. When this occurs, the teeth become looser because their foundation is eroding away. Ultimately, the teeth become too loose to function properly. The bone is resorbed by the body. Just as a post in the ground loses its stability once the ground supporting it is lost or washed away, even otherwise healthy teeth whose support is diseased frequently cannot be saved. This bone loss is irreversible.

Whether gingivitis progresses to periodontitis and the rate of that progression can vary considerably from one individual to another, depends upon individual susceptibility to bacterial infection. A number of investigators have suggested that the disease progression is episodic rather than linear. Acute episodes of tissue destruction may alternate with periods of relative quiescence. Moreover, the onset and progression of gingivitis and periodontitis usually varies from one tooth to another in the same mouth. Nevertheless, while not all gingivitis progresses to periodontitis, virtually all adult periodontitis is preceded by gingivitis. There is currently no way to determine which gingival inflamed sites will proceed to periodontitis and which will not.

Another characteristic of periodontitis is that it is largely asymptomatic until the condition becomes severe. Except for the mild discomfort of inflamed gums and occasional bleeding on brushing, there is little or no pain associated with the disease's progression. Lack of pain is one reason why there is widespread tooth loss as a result of periodontitis. By the time people discover they have the disease, it is sometimes too late to save their teeth.

3. Prevention of Plaque Accumulation and Gingivitis

Lost teeth need not be the inevitable consequence of aging. It is well-established that the control, reduction, or elimination of plaque accumulations is effective in preventing gingivitis and periodontitis. NIDR has declared that plaque control" is the cornerstone of all programs aimed at preventing gum disease."[1/]

Unfortunately, as NIDR reports, the conventional mechanical methods of eliminating plaque -- toothbrushing and flossing -- have

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1/ Challenges for the Eighties/National Institute of Dental Research Long-Range Research Plan FY 1985-89 at 49 (NIH 1983) [Hereinafter referred to as NIDR]

not been effective because they demand time, skill, and diligence which most Americans are unwilling or unable to provide.[2/] At their best, mechanical home procedures are cumbersome and inefficient, seldom reaching all surfaces vulnerable to infection.[3/] Dental investigators have concurred that it may be unrealistic to expect that plaque control, using the principle of regular mechanical plaque removal, will become effective in a significant part of the population.

The realization that toothbrushing and flossing are unlikely to provide adequate protection against plaque accumulation has led to widespread interest in less demanding plaque control methods. NIDR has taken the step of identifying the development of new chemical or alternative means to prevent or control periodontal disease as an important objective for the future. Dental experts have articulated similar goals. If the plaque diseases are to be dealt with on anything approaching a mass basis, chemical agents that supplement or even supplant the purely patient-dependent mechanical regimen are essential.[4/] There is no question that a commercially available mouthwash which is both safe and effective in preventing and reducing plaque and gingivitis would offer enormous public health benefits by reducing the incidence of periodontal disease.

3. Study Summaries

The following section includes summaries of the definitive and supporting controlled clinical studies relating directly to the efficacy of Listerine Antiseptic in terms of plaque and gingivitis reduction and prevention. Also included are microbiology phases of the six month clinical studies and

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2/ NIDR at 47,49

3/ NIDR at 49

4/ Mandel, I., "New Approaches to Plaque Prevention" Dental Clinics of North America, 662 (1972)

additional studies relating to professional indications for example, pre- and post- procedural rinses and stomatitis. The studies will be presented in the following order (with attendant microbiology portions):

Definitive Studies

6 month and longer

6 week

Supporting Studies

6 month

6 week

2 week

Professional Indication Studies

Definitive Studies

These are studies conducted according to ADA guidelines. (The ADA Guidelines are provided in Section V, Efficacy Data and are discussed in Part 4.1.c. below)

6 Month and longer

1) **Lamster et al: The effect of Listerine Antiseptic on reduction of existing plaque and gingivitis. Clin. Prev. Dent.; 5:12-16, Nov.-Dec. 1983/Warner-Lambert Research Report # 931-0170, March 24, 1981.** (See Section V Definitive Studies Reference 1)

The purpose of this study was to evaluate the effect of Listerine on existing plaque and gingivitis over a six-month period. The study found that Listerine was effective in reducing existing plaque and gingivitis by statistically significant levels.

a. Methodology

There were 145 subjects participating in the study ranging from 18 to 54 years. These subject met the following qualifications: (1) they had a minimum of 20 natural teeth which could be "scored" for gingivitis and plaque; they were not currently receiving medications that could affect the oral tissues such as systemic antibiotics or anti-inflammatory drugs; and (3) they had teeth which met pre-established minimum gingival and plaque scores of 2.0 and 1.8, respectively, but no clinically observable periodontitis.

Gingival scores for each subject were calculated on the basis of a "modified Loe Silness Index. "Scores" were assigned to the gum surrounding each tooth, based on the following criteria:

0 - normal (no inflammation)
1 - mild inflammation (e.g., slight changes in color) of any portion of the gum tissue surrounding the tooth)
2 - mild inflammation (but no edema) of all gum tissue surrounding the tooth
3 - moderate inflammation (moderate glazing, redness, edema and/or hypertrophy
4 - severe inflammation (marked redness and edema/ hypertrophy, spontaneous bleeding or ulceration). After each tooth was scored using this index, an average overall score for each subject was calculated.

Similarly, plaque scores for each subject were calculated using the Turesky modification of the Quigley-Hein Index. This index focuses on plaque on the third of the tooth surface closest to and in contact with the gums. After the subjects' teeth were stained with a disclosing dye, the teeth were scored as follows:

0 - no plaque

1 - separate flecks or a discontinuous band of plaque at the gingival margin

2 - thin (up to 1 mm) continuous band of plaque at the gingival margin

3 - band of plaque wider than 1 mm, but covering less than 1/3 of the gingival third of the tooth surface

4 - plaque covering more than 1/3, but less than 2/3 of the gingival third of the tooth surface

5 - plaque covering 2/3 or more of the gingival third of the tooth surface

After each tooth was scored, the average overall plaque score for each subject was calculated. In addition, a third index, a modified Lobene Stain Index, was used to score extrinsic tooth stain so that it would later be possible to determine the effect of the experiment on that factor.

The subjects were randomly assigned to one of three groups - Listerine, colored water control, or a true vehicle control (consisting of the total Listerine formulation except for the four active ingredients). All subjects rinsed with 20 ml. of their assigned product for 30 seconds twice each day for six months. The rinses were supervised on weekdays and unsupervised on weekends and holidays. The subjects were required to maintain a diary of their unsupervised rinses.

All the subjects were provided with the same brand toothbrush and toothpaste and instructed to follow their usual oral hygiene regimen, although they were asked not to use commercial mouthwashes or oral irrigators. The study was conducted on a double-blind basis so that neither the subjects nor the investigator who scored the teeth would be aware of the groups to which the subjects had been assigned. A single investigator conducted all examinations.

b. Results

Listerine plaque scores were statistically significantly lower than the scores of the control groups at the one month, three months, and six months measurement times ($p < 0.001$). At six months, plaque scores in the Listerine group had been reduced by 28.6 percent compared to baseline scores, 20.8 percent compared to the vehicle control scores, and 22.2 percent compared to the water control scores.

Listerine gingivitis scores were also statistically significantly lower than the scores of the control groups at the one month ($p \leq 0.002$), three months ($p < 0.001$), and six months measurement times ($p < 0.001$). Gingivitis scores for the Listerine group at six months were reduced by 55.7 percent compared to baseline scores, 27.7 percent compared to the vehicle control scores, and 28.2 percent compared to the water control scores. There was no evidence of soft tissue damage or extrinsic tooth stain in any group.

2) **Gordon, J., et al: Efficacy of Listerine Antiseptic in inhibiting the development of plaque and gingivitis. J. Clin. Periodontol.; 12:697-704, 1985/Warner-Lambert Research Report # 931-0491, March 5, 1984.** (See Section V Definitive Studies Reference 2)

This nine-month study evaluated the effectiveness of Listerine in inhibiting the development of supragingival plaque and gingivitis following extensive professional care, rather than its effectiveness in reducing existing plaque and gingivitis. At the conclusion of the study, the plaque and gingivitis scores for the group using Listerine were statistically significantly lower than the scores of the control groups. There was no statistically significant difference between control group scores.

a. <u>Methodology</u>

There were 144 subjects, ranging in age from 18 to 54 years. Criteria for participation included a minimum of 20 sound natural teeth, a minimuim gingivitis score of 2.0, using the modified Loe-Silness Index, a minimum plaque score of 1.8, using the Turesky modification of the Quigley-Hein Index and no clinically discernible periodontitis.

Prior to the initiation of the study, participating subjects were given three full scalings and prophylaxes (professional dental cleanings) and one prophylaxes, (polishing) at designated times over a period of four weeks to remove all plaque, calculus, and extrinsic tooth stain and to minimize existing gingivitis. Baseline examinations were made of subjects' oral soft tissue, and extrinsic tooth stain, as well as of plaque and gingivitis. Following four weekly professional prophylaxes to minimize gingivitis and eliminate all plaque, a second gingivitis baseline was scored.

Subjects were assigned randomly to one of three groups: Listerine, a sterile colored-water control, or a true vehicle control which was identical to Listerine, except that it lacked the four active ingredients in Listerine. The subjects began a regimen of rinsing twice each day for 30 seconds with with 20 ml. of the product assigned to their groups. For the first six months, the rinsing was supervised on weekdays. For weekends and holidays, the appropriate products were supplied to each group, and the subjects were required to maintain a record of their unsupervised rinsings.

During the study, subjects followed their usual oral hygiene habits, but were instructed to refrain from using other mouthrinses. All subjects were provided with a non-therapeutic, low-abrasive dentifrice and soft toothbrush.

All scoring was performed by one examiner. The study was conducted double-blind in that neither the examiner nor the subjects knew to which group the subjects has been assigned. Of the 144 subjects participating in the study, 127 subjects completed six months of the supervised study and 85 subjects continued in the study for an additional three months of unsupervised rinsings.

b. Results

For the 127 subjects who completed the first six months of the study, the plaque score differences between the Listerine and control groups were statistically significant at one, three, and six months. (All differences were statistically significant at the level of $p < 0.01$, with the exception of the difference between Listerine and vehicle control scores at one month, which was statistically significant at the $p < 0.05$ level.) The Listerine group showed a 12.1 percent, 18.3 ercent, and 18.0 percent reduction in plaque scores, as compared to the water control group at one, three, and six months, respectively. When compared with the vehicle control group, the Listerine group showed an 8.1 percent, 16.0 percent, and 14.9 percent reduction in plaque scores at the one, three, and six-month measurement points. No statistically significant differences were found between the water and vehicle control groups at any of these times, thus confirming that the alcohol in Listerine is not responsible for its effect on plaque.

For the 85 subjects that continued in the study for nine months, the differences in plaque scores between the Listerine group and the control groups were statistically significant at one, three, six and nine months. (All differences were statistically significant at the level of $p < 0.01$, with the exception of the difference between Listerine and vehicle control scores at one month, which were statistically significant at the $p < 0.05$ level.) The Listerine group showed a 15.5 percent, 20.9 percent, 23.7 percent, and 19.5 percent reduction, as compared to the water control group at one, three, six

and nine months, respectively. When compared to the vehicle control group, the Listerine group showed a 11.7 percent, 21.6 percent, 18.6 percent and 13.8 percent reduction in plaque scores at one, three, six and nine months. Compared to the baseline measurements, the Listerine group had a mean plaque score 3.0 percent less than the baseline; in the water control group there was a 16.9 percent increase in the plaque score, and in the vehicle control group, there was a 7.3 percent increase in the plaque score. No statistically significant differences were found between the water and vehicle control groups at any of these times.

As for the gingivitis scores, a statistically significant decrease in the gingivitis scores was seen only in the Listerine group that completed the full nine months of the study ($p < 0.05$). At nine months, Listerine had reduced gingivitis scores by 23.9 percent, compared to the water control, and by 22.1 percent, compared to the vehicle control. Compared to the baseline scores, gingivitis scores for the Listerine group were 18.7 percent lower at nine months. In contrast, the gingival scores for the water control group and vehicle control group showed an increase over baseline scores of 7.0 percent and 5.1 percent, respectively. No significant extrinsic staining or soft tissue effects were observed in any of the groups.

The researchers concluded that the reason there was no statistically significant difference in gingivitis scores between the groups in the first six months was due to the initial improvement in gingival health resulting from the four prophylaxes. The study demonstrated that Listerine can usefully supplement good professional care, as well as reduce plaque in the absence of professional care.

3) **De Paola, L. et al: Chemotherapeutic inhibition of supragingival dental plaque and gingivitis development. J. Clin. Periodontol.; 16:311-315, May 1989/Warner-Lambert Research Report # 931-0647, December 17, 1985..** (See Section V Definitive Studies Ref. 3)

The purpose of this six-month clinical and microbiological study was to determine the effect of Listerine on the inhibition of plaque and gingivitis and on plaque microbial composition. The study found that Listerine was effective in inhibiting the development of plaque and gingivitis. After six months of Listerine use, subjects' plaque and gingivitis scores were statistically significantly lower than control group scores. The results of the microbiological component of the study were that use of Listerine did not alter the microbial composition of plaque following prolonged use, nor foster an increase in potential opportunistic pathogens.

a. **Methodology**

There were 108 subjects, aged 18 to 60, with pre-existing plaque and gingivitis, but without periodontitis. The subjects were screened for a minimum of 20 sound, natural teeth, a minimum plaque mean score of 2.0, using the Turesky modification of the Quigley-Hein Index, and a minimum gingivitis mean score of 2.0, using the Modified Gingival Index.

Following a baseline examination, subjects were given a complete scaling and prophylaxis to remove all plaque, calculus, and extrinsic stain. Subjects were randomly assigned to either a Listerine group or to a 5 percent hydroalcohol control group. All subjects rinsed for 30 seconds with 20 ml. of their assigned product twice daily for six months.

All rinses were supervised on weekdays. For weekends and holidays, subjects were supplied with the appropriate rinses for their groups and instructed to maintain a diary of their rinsings. During the study, subjects followed their usual oral hygiene and dietary habits, but were instructed to rfrain from using commercial moutnrinses. The same brand toothpaste and toothbrush was provided to all subjects.

Supragingival plaque samples were collected from the subjects before treatment and following either three or six months use and were examined for microbial content. (Plaque was harvested from different subjects at the three-month and at the six-month stage to guard against the possibility that the three-month plaque harvest itself would alter the microbial flora composition.) Three distinct microbiological techniques were used to evaluate the microbial content of the plaque samples.

b. Results

At the conclusion of the study, the plaque scores of subjects in the Listerine group were statistically significantly lower, by 34.4 percent ($p < 0.001$), compared to the plaque scores of the control group. Gingivitis scores were statistically significantly lower by 34.0 percent ($p < 0.001$) in the Listerine group, compared to the control group.

The microbiological phase of the study showed that Listerine use did not lead to shifts in the composition of the plaque flora. Nor did Listerine use result n an increase in or emergence of potential opportunistic pathogens.

3a) **Minah, G.E., et at.: Effects of 6 months use of an antiseptic mouthrinse on supragingival dental plaque microflora. J. Clin. Periodontol.; 1989, 16:347-352/Warner-Lambert Research Report #931-0647, December 17, 1985.** (See Section V Definitive Studies Reference 3a)

This study was designed to determine if long-term use of Listerine caused a significant shift in the composition of dental plaque microbial flora during six-months use and three months following twice daily use. Emergence of opportunistic pathogens or presumptive oral pathogens were also determined. The study confirmed

that Listerine use did not cause a significant shift in the microbial composition of plaque or foster the development of opportunistic organisms or of specific microorganisms associated with periodontal diseases.

a. **Methodology**

Eighty-three subjects completed this double-blind controlled clinical study. All the subjects had pre-existing plaque and gingivitis, but not advanced periodontitis. At baseline, supragingival plaque was collected from specified teeth from each subject. Forty-two subjects were then randomly assigned to the Listerine group and 41 randomly assigned to the 5% hydroalcohol placebo control group.

Subjects were instructed to rinse twice daily for 30 seconds with 20 ml of their assigned rinse. After three months, plaque was harvested from the same sites on 40 subjects and at six months plaque was harvested from the remaining 43 using a random code for selection of sampling time. Plaque samples were maintained in an anaerobic atmosphere as sampling and during dilution and plating. A small alliquot was subjected to darkfield microscopy for enumeration of bacterial morphotypes and the remaining samples were diluted and plated on a battery of selective and non-selective culture media and subjected to appropriate cultural conditions. Results of darkfield microscopy were recorded as the number of organisms within each cell morphological type per 100 cells observed.

b. **Results**

The only significant differences between the treatment groups occurred at three-months, when significantly more cocci and fewer spirochetes were observed in the Listerine treatment group than in the control group. No significant intergroup treatment differences in counts on either selective or non-selective media were observed.

Examination of the data for treatment effects also failed to reveal any significant differences to pretreatment levels. No significant difference was detected between treatment groups or relative to pretreatment levels in ratios of facultative to anaerobic microorganisms. Differential counts determined by colonial morphology revealed a significant reduction in Bacteroides sp. and Fusobacterium sp. at six months but not at three months in the Listerine group but not in the controls. At three months, Veillonella counts were reduced in the Listerine group compared to the control group but, this difference was not maintained at six months. There was no detectable rise in either staphlococci, enteric bacteria or other potential opportunistic pathogens during the study. Twice daily use of Listerine resulted in a clinically detectable reduction in supragingival plaque and gingivitis as reported by DePaola et al without a significant disruption in the microbial ecologic balance of the oral microflora.

4) **Walker, W. et al.: Long term effect of Listerine antiseptic (W2194-92) on dental plaque microbial composition. Warner-Lambert Research Report # 931-0654. December 18, 1985. (Published as an abstract. J. Dent. Res.; 68: Abstr. 1845, 1989).**
(See Section V Definitive Studies Reference 4)

This six-month study, was designed to determine if long-term use of Listerine caused a significant shift in the composition of dental plaque microbial flora or led to the emergence of opportunistic or presumptive oral pathogens. The study confirmed that Listerine use did not cause a significant shift in the microbial composition of plaque or foster the emergence of resistant organisms or the development either of opportunistic organisms or of specific microorganisms associated with periodontitis.

a. Methodology

Eighty-nine subjects completed this double-blind study. The subjects were aged 20 to 58 and had pre-existing plaque and gingivitis, but not advanced periodontitis. The screening was similar to that in the prior studies. At a baseline point, supragingival plaque was collected from specified teeth from each individual. Subjects were then given a complete prophylaxis and subsequently began rinsing with their assigned rinse. Half of the subjects rinsed twice daily for 30 seconds with 20 ml. of either Listerine or a 5 percent hydroalcohol control for three months. The remaining subjects used one of these rinses for six months. Rinsing was conducted under supervision on weekdays. On weekends and holidays, subjects rinsed unsupervised.

Plaque was again collected at either three or six months for microbiological examination. The microbial composition of the plaque samples for each subject were analyzed using three distinct microbiological approaches.

b. Results

The results were that there were no significant shifts in the microbial composition of the plaque in either the Listerine group or the control group. Nor was there an emergence of resistant organisms or a significant increase in presumptive oral pathogens or opportunistic pathogens.

5) **Mankodi, S.: Efficacy of Listerine (W2194-92) and Listerine Plus Mint (W2194-194) in inhibiting the development of dental plaque and gingivitis. Warner-Lambert Research Report No. 931-0780. July 18, 1989.** (See Section V Definitive Studies Reference 5)

This research studied the effects of rinsing with Listerine and Listerine Plus Mint and a 5% hydroalcohol control on

dental plaque and gingivigis inhibition, and dental plaque microbial composition. Both Listerine and Listerine Plus Mint resulted in a significantly inhibited plaque formation and gingivitis development compared to control. All groups showed an increase in extrinsic tooth stain but there were no significant differences in calculus development among the treatment groups.

a. **Methodology**

One hundred twenty-four subjects, ranging in age from 18-56 years completed this study. They all had varying degrees of gingivitis, but did not show evidence of periodontal attachment or alveolar bone loss. Following baseline examination, each subject was given a professional prophylaxis and subjects continued their usual oral hygiene and began rinsing with 20 ml of their assigned rinse twice daily for 30 seconds, for six months. All clinical examinations were repeated at three and six months and microbial plaque was sampled at three, six and three months after completion of the study. The subjects were assigned to one of the two different treatment groups or to a control group, according to a randomized code by which double-blinding was maintained. During weekdays, the rinsings were performed under supervision, while unsupervised rinsings were carried out over weekends and holidays. All subjects were provided the same brand of toothbrush and toothpaste.

A complete intraoral soft tissue examination was performed at baseline, three and six months.

Extrinsic tooth stain was scored at each examination time by the Lobene Index on the labial surfaces of the 12 anterior teeth, according to the following criteria:

area:
0 - no stain detected
1 - stain up to 1/3 the region

2 - stain over 1/3 to 2/3 the region

3 - stain over more than 2/3 the region

<u>severity or intensity:</u>

0 - no stain

1 - light stain (can be seen with close examination)

2 - moderate stain (obvious but not aesthetically unacceptable)

3 - heavy stain (obvious and aesthetically unacceptable)

Supravingival calculus was measured at baseline, three and six months using a flat callibrated peridontal probe in three constant planes on the lingual surface of the six mandibular anterior teeth.

Gingivitis was scored at baseline, three and six months by the Gingival Index as follows:

0 - absence of inflamation

1 - mild inflammation (slight change in color, little change in texture; no bleeding on pressure)

2 - moderate inflammation (moderate glazing, redness, edema and hypertrophy; bleeding on pressure)

3 - severe inflammation (marked redness and hypertrophy; tendency to spontaneous bleeding or ulceration)

Plaque area was scored at the same examinations as above by the Turesky modification of the Quigley-Hein Index on the buccal and lingual surfaces of all scorable teeth.

b. Results

After six months, Listerine and Listerine Plus Mint mouthrinses, used as a supplement to usual oral hygiene, significantly inhibited ($p < .001$) development of gingivitis by 22.4% and 21.6% respectively compared to control. There were no significant differences between Listerine and Listerine Plus Mint groups.

Listerine and Listerine Plus Mint mouthrinses significantly inhibited ($p < .001$) plaque development by 25.3 and 25.7% respectively when compared to the control. There were no significant differences between the two Listerine groups.

Lastly, there were no significant differences in microbiological parameters between groups.

6) **Overholser, D., et al: Comparative effects of 2 chemotherapeutic mouthrinses on the development of supragingival dental plaque and gingivitis. J. Clin. Periodontol.; 17:575-579, September 1990/ Warner-Lambert Research Report #931-0730, February 10, 1988.**
(See Section V Definitive Studies Reference 6)

The purpose of this study was to compare the effect of Listerine, Peridex(R) (Procter & Gamble) and a hydroalcohol control rinse, in inhibiting the development of dental plaque and gingivitis over a six-month period when used as a supplement to normal oral hygiene. The results indicated that both Listerine and Peridex statistically significantly reduced supragingival plaque and gingivitis when compared to the control group, and that Listerine and Peridex are comparable in their ability to prevent and control gingivitis.

a. Methodology

There were 128 subjects participating in the study, and 124 completed the study. These subjects had supragingival plaque scores of at least 2.0, using the Turesky modification of the Quigley-Hein Index and gingivitis scores of at least 2.0, using the Modified Gingival Index.

Prior to the study, baseline examinations for soft tissue condition, extrinsic tooth stain, supragingival calculus, gingivitis, bleeding, and plaque were conducted. These examinations were repeated at the three-month and six-month points in the study. Following a complete dental prophylaxis, subjects were randomly assigned to a Listerine, Peridex, or control group. Subjects rinsed with either 15 or 20 ml. of their assigned rinse for 30 seconds, twice daily, for six months and continued their normal oral hygiene.

b. Results

Both Listerine and Peridex statistically significantly ($p < .001$) inhibited development of plaque by 36.0 percent and 50.6 percent respectively, as compared to the control group after six months. Peridex was statistically significantly ($p = .043$) more effective than Listerine in inhibiting plaque. Both Listerine and Peridex inhibited the development of gingivitis by statistically significant levels ($p < .001$), 35.9 percent and 30.8 percent, respectively, compared to the control. There was no statistically significant difference ($p > .20$) between Listerine and Peridex with respect to gingivitis inhibition. Bleeding indices were significantly reduced in all groups compared to baseline values, with no significant differences among the groups.

Neither the Listerine nor the control group exhibited a statistically significant increase in extrinsic tooth stain or supragingival calculus at six months, nor was there any statistically

significant difference between the two groups. Peridex, however, showed statistically significant increases (p < .001) in both extrinsic tooth stain and calculus at six months compared to its baseline values. Peridex also showed statistically significantly greater (p < .05) extrinsic tooth stain and calculus formation than either Listerine or the control.

7) **Mankodi, S.: Effect of Listerine Antiseptic (W2194-92) and Peridex (W14948-6) compared to a hydroalcoholic control (W2194-167P) in inhibiting the development of dental plaque and gingivitis. Warner-Lambert Research Report No. 931-0792; March 26, 1990. (Published as an abstract J. Dent. Res.; 69:Abstr. 1099, 1990)** (See Section V Definitive Studies Reference 7)

This research studied the effects of rinsing with Listerine or Peridex (0.12% chlorhexidine) compared to a 5% hydroalcohol control over a six month period. Both Listerine and Peridex significantly inhibited development of plaque and gingivitis when used to supplement usual and oral hygiene for six months. Peridex use resulted in significantly more calculus and extrinsic tooth stain than did the use of Listerine or control mouthrinses.

a. Methodology

One hundred and seven subjects ranging in age between 20 and 57 years participated in this study. They all had varying degrees of gingivitis but did not demonstrate any evidence of periodontal attachment or alveolar bone loss. Following baseline examination, each subject received a professional prophylaxis.

The subjects were assigned to one of two different experimental groups or to a control group, according to a randomized code by which double-blinding was maintained. Beginning on the same day as the prophylaxis, subjects began rinsing with either 15 or 20 ml of their assigned rinse for 30 seconds twice daily for six months

while continuing their usual oral hygiene, all subjects were provided with identical toothbrushes and toothpaste.

At baseline, three and six months a complete intraoral soft tissue exam was performed along with the following:

Plaque area was scored by the Turesky modification of the Quigley-Hein Index. Extrinsic tooth stain was scored by the Lobene Index on the labial surfaces of the 12 anterior teeth as follows:

Area:

0 - no stain detected
1 - stain up to 1/3 the region
2 - stain over 1/3 to 2/3 the region
3 - stain over more than 2/3 the region

Severity or intensity:

0 - no stain
1 - light stain (can be seen with close examination)
2 - moderate stain (obvious but not aesthetically unacceptable
3 - heavy stain (obvious and aesthetically unacceptable)

Supragingival calculus was measured using a flat calibrated periodontal probe in three constant planes on the lingual surface of the six mandibular anterior teeth using the Volpe-Manhold method.

Gingivitis was scored by the Loe and Silness Gingival Index on the buccal and lingual surfaces and interdental papillae of all scorable teeth as follows:

0 - absence of inflammation

1 - mild inflammation (slight change in color, little change in texture; no bleeding on pressure)

2 - moderate inflammation (glazing, redness, edema and hypertrophy; bleeding on pressure)

3 - severe inflammation (marked redness and hypertrophy; tendency to spontaneous bleeding or ulceration)

b. Results

Listerine and Peridex mouthrinses used as supplements to usual oral hygiene for six months significantly ($p < .001$) inhibited development of plaque by 18.8% and 21.6% respectively compared to rinsing with the hydroalcohol control. There was no significant difference ($p=.59$) between the two treatment groups. Listerine and Peridex mouthrinses significantly ($p < .001$) inhibited development of gingivitis by 14% and 18.2% respectively. There was no significant difference ($p=.21$) between the two treatment groups in gingivitis inhibition. After 6 months, the Peridex group showed significantly ($p<.001$) more supragingival calculus than either the Listerine or the control group. There was no significant difference between Listerine and control ($p=.92$) in supragingival calculus formation. The Peridex group also showed significantly ($p<.001$) more extrinsic tooth stain than both the Listerine and control groups while the Listerine group showed significantly ($p<.01$) more stain than the hydroalcohol control group.

SUPPORTING STUDIES

6 Months

1) **Menaker, L., et al. Efficacy of Listerine Antiseptic (W-2194-92) and control (-92P) for inhibiting the development of dental plaque and gingivitis. Warner-Lambert Research Report No.931-0135, June 1, 1981.** (See Section V Supporting Studies Ref. 1)

The purpose of this six-month study was to determine the effectiveness of Listerine in inhibiting the development of plaque and gingivitis following prophylaxes. Measurements of plaque area showed that Listerine retarded the accumulation of dental plaque by a statistically significant amount.

Listerine also reduced plaque accumulation when measured by plaque weight from four selected teeth, but the results were not statistically significant. Although there were no statistically significant differences in treatment groups, with respect to gingivitis scores at the end of the study, those scores did not differ significantly from the "near zero" baseline scores.

a. **Methodology**

Eighty-five subjects qualified for this study by having 20 sound natural teeth and plaque and gingivitis scores which met pre-determined minimum standards. Assessments were also made of any extrinsic stain on the subjects' teeth. Repeated complete prophylaxes were performed on the subjects until zero plaque scores and "near zero" gingivitis scores were attained. Subjects were then assigned randomly, double-blind, to one of two groups: Listerine or a vehicle control identical to Listerine, except for the four active ingredients.

The subjects all rinsed with 20 ml. of their assigned mouthrinse for 30 seconds twice daily for a period of six months. Rinsing was supervised on weekdays. On weekends, subjects were asked to maintain a diary of their rinsings. Subjects were instructed in oral hygiene, and that instruction was periodically reinforced during the course of the study.

b. Results

Listerine statistically significantly ($p < 0.013$) retarded the accumulation of dental plaque by approximately 27.7 percent, 27.2 percent, and 24.4 percent at one, three, and six months respectively, compared to the vehicle control. The average wet weight of the plaque harvested from the first molars of the Listerine group at six months was 9 percent less than the average wet weight of the plaque harvested from the first molars of the control group, but the difference was not statistically significant. There was no statistically significant difference between the groups in the reduction of gingivitis after six months; however, the six-month gingivitis scores in the two groups did not differ significantly from the "near zero" baseline. The researchers concluded that this was due to the multiple prophylaxes and an improvement in subjects' oral hygiene practices during the study.

There were no clinically or statistically significant differences between the groups with respect to extrinsic tooth stain or soft tissue effects during the course of the study.

2) **Fine, D. et al: The effect of rinsing with Listerine Antiseptic on the properties of developing dental plaque. J. Clin Periodontol.; 12:660-666, 1985/Warner-Lambert Research Report # 931-0485, January 12, 1984.** (See Section V Supporting Studies Reference 2)

This study was designed to determine whether Listerine reduces the development of plaque mass and pathogenic activity as compared with vehicle and water control rinses. The theory behind the study was that plaque indices that measure plaque area, as does the Turesky modification of the Quigley-Hein Index, may be less sensitive than measurements of plaque mass or of toxic potential in reflecting changes in plaque and its relationship to gingivitis over the course of a study.

The study found that Listerine effected a statistically significant reduction in the development of plaque mass and pathogenic activity, compared to the control groups. The reduction in plaque mass was appreciably greater than that found by using the plaque area index.

a. **Methodology**

The study compared the wet and dry weights of the supragingival plaque harvested at nine months from the Listerine rinse group in the previous study with the plaque harvested at the same time from the control groups in the study. The protein present and the limulus lysate assay in the plaque harvested from the three groups was also compared.

b. **Results**

The plaque wet and dry weights of the Listerine group were statistically significantly lower, by 52.6 percent (p = 0.04) and 59.0 percent (p = 0.01), respectively, than the comparative weights for the water control group. Compared to the vehicle control group, the Listerine plaque wet and dry weights were lower by 55.1 percent (p = 0.03) and 59.6 percent (p = 0.01). The study also found a statistically significant Listerine group of 59.7 percent (p = 0.01) and 59.2 percent (p = 0.02), compared to the protein content of the plaque from the water and vehicle control groups, respectively. Other investigators have shown that decreased protein is indicative of decreased viable microbial content per given weight of plaque. Finally, the study found that when endotoxin activity was evaluated on the basis of limulus lysate assay of total plaque, the Listerine group limulus activity was reduced by 75.8 percent, compared to the water control group, and by 77.9 percent, compared to the vehicle control group (p = 0.01). These results demonstrated significantly reduced plaque pathogenic potential for the Listerine group.

2 Months

3) **Moser, E.: The clinical efficacy of Listerine Antiseptic in reducing preformed dental plaque. Warner-Lambert Research Report No. 955-0241, June 1, 1974.** (See Section V Supporting Ref. 3)

This two-month study was designed to determine the effect of Listerine on reducing pre-existing plaque. The results showed that Listerine statistically significantly reduced plaque, compared to a saline control rinse.

a. **Methodology**

Thirty-one dental students participated in ths study. These subjects received a thorough dental prophylaxis and then continued their normal, mechanical, oral hygiene routines for the next fourteen days. The purpose of this period was to establish in each subject a baseline level of plaque, gingivitis, and calculus formation. On the fifteenth day, the accumulation of plaque and calculus and the severity of gingivitis were assessed.

The subjects were then randomly divided, double-blind, into two groups and assigned to one of two mouthwash regimens: Listerine or a 0.9 percent saline solution. For the next 60 days, the subjects rinsed (unsupervised) with 20 ml. of their assigned mouthwash for 30 seconds three times daily, in conjunction with their normal mechanical tooth cleansing procedures. At four scheduled times during that 60-day period, the subjects were reassessed for plaque, gingivitis and calculus.

At the conclusion of the 60-day period, the subjects each received a second thorough dental prophylaxis and were again instructed to continue their normal mechanical tooth-cleansing procedures for the next 14 days. Assessments for plaque, gingivitis, and calculus were again made at the conclusion of this period.

The subjects then crossed-over treatment regimens. The subjects who had previously rinsed with Listerine received the 0.9 percent saline solution, while those who were originally on the saline regimen were assigned to the Listerine group. As in Phase I, the subjects rinsed three times daily with the assigned mouthrinse for 60 days and were assessed during that time for plaque, calculus, and gingivitis. Data was used only from those subjects who had minimum plaque, gingivitis, and calculus baseline scores in both Phase I and Phase II.

b. Results

Of the 31 subjects, 16 formed enough plaque to qualify for data analysis. Those subjects experienced a statistically significant 35.7 percent reduction in plaque accumulation ($p<0.01$) when using Listerine, compared to the mean baseline score. When these same subjects used a 0.9 percent saline rinse under the same conditions, they experienced a 11.5 percent reduction in plaque, compared to the baseline score (not statistically significant). The differences between the Listerine and saline rinse groups were statistically significant ($p < 0.05$)

There were insufficient subjects with adequate minimum baseline calculus scores to make data analysis practical. None of the subjects demonstrated the baseline level of gingivitis necessary to qualify them for final data analysis.

6 Week

4) **Lusk, S. et al: Effects of an oral rinse on experimental gingivitis, plaque formation, and formed plaque. J. Amer. Soc. Prev. Dent.; 4: 31-37, July-Aug 1974 Warner-Lambert Research Report # 956-0072, August 15, 1974..** (See Section V Supporting Studies Reference 4)

The purpose of this study was to determine the effect of Listerine on dental plaque accumulation and gingivitis. The results showed that rinsing with Listerine retarded overall plaque accumulation and gingivitis. When the inhibition in plaque accumulation was measured by plaque weight, the inhibition was seen to be even greater than that shown by measurements of plaque area.

a. **Methodology**

The subjects in this study were 13 periodontists (ages 30-45). Following a thorough dental prophylaxis to remove all plaque, the subjects refrained from all oral hygiene procedures for 12 consecutive days except to rinse with water three times each day. At the end of this time, gingivitis severity and plaque accumulation were evaluated. The plaque was then removed from all teeth, except for certain specified ones, and weighed.

Subsequently, the subjects underwent a second prophylaxis to clean all teeth, except for those on which the plaque had been left untouched. For the next 12 days, the subjects rinsed with Listerine for one minute, three times daily, but abstained from all other oral hygiene measures. On the last rinse day, the subjects were examined and scored for gingivitis and plaque.

Twelve of the subjects were then provided with a third prophylaxis, leaving untouched the plaque that had now accumulated for 24 days on the specified teeth. This group was divided in half. For the next 12 days, half of the subjects rinsed with Listerine for five seconds three times each day. The other half of the subjects rinsed with Listerine for one minute once daily. Both groups abstained from all other oral hygiene measures. Following this period, gingivitis and plaque were scored as before.

b. Results

The primary results, which were statistically significant ($p < 0.01$), showed that rinsing with Listerine on the one-minute, three times daily regimen, retarded overall plaque accumulation 30 percent better than rinsing with water when measured by plaque area, and 70 percent better when measured by plaque weight. Gingivitis levels were 79 percent less on the Listerine one-minute, three-times daily regimen, compared to the water-rinse regimen.

Other statistically significant results ($p < 0.01$) were that Listerine reduced plaque surface area scores by 21 percent when used once daily, compared to the water rinse control. Compared to the water rinse, Listerine reduced plaque weight by 48 percent when rinsed for five seconds three times daily and by 44 percent when rinsed for one minute once daily. In one of the areas where plaque had been left to accumulate after the first prophylaxis, Listerine reduced that plaque from water rinse control levels by 21.4 percent ($p < 0.01$) when used for one minute three times daily, by 18.9 percent ($p < 0.02$) when used for five second three times daily and by 22.4 percent ($p < 0.01$) when used for one minute once daily.

5) **Axelson, P. et al: Efficacy of mouthrinses in inhibiting dental plaque and gingivitis in man. J. Clin. Periodontol.; 14:205-212; 1987/Warner-Lambert Research Report # 931-0666, March 11, 1986.**
(See Section V Supporting Studies Reference 5)

This research studied the effects of rinsing with Listerine or a 0.1 percent or 0.2 percent chlorhexidine rinse. The study found that all three mouthwash preparations were effective at statistically significant levels in inhibiting and reducing plaque and gingivitis.

a. Methodology

Ninety-six subjects, ranging betwen age 16 and 50, were used in the study. They all had varying degrees of gingivitis, but did not show loss of periodontal attachment or bone loss. Following a baseline examination, each subject was given a professional prophylaxis.

The subjects were assigned to one of three different treatment groups or to a control group, according to a randomized code by which double-blinding was maintained. Members of the control and Listerine groups rinsed for 30 seconds with 20 ml. of their assigned mouthwash preparations twice each day. The members of the clorhexidine groups rinsed with 10 ml. of their solutions for 60 seconds twice daily. During the weekdays, the rinsings were performed under supervision, while unsupervised rinsings were carried out over the weekends. For the course of the six-week trial, the subjects continued their usual oral hygiene and dietary habits, but were instructed to refrain from using commercial mouthrinses. They were each supplied with the same brand of toothbrush and toothpaste.

Gingivitis was scored at baseline and six weeks using the Loe-Silness Index (unmodified) as follows:

0 - absence of inflammation
1 - mild inflammation (slight change in color, little change in texture, no bleeding on pressure)
2 - moderate inflammation (moderate redness, edema, and hypertrophy; bleeding on pressure)
3 - severe inflammation (marked redness and hypertrophy; tendency to spontaneous bleeding or ulceration)

Plaque area was also scored at baseline and six weeks, using the Turesky modification of the Quigley-Hein Index that was

employed in the other studies. Extrinsic tooth stain was scored at baseline, three, and six weeks, and an examination of the intraoral soft tissue was done at those time periods as well. All examinations were performed by a single dental examiner.

b. Results

After three weeks, the mean plaque score of the control group was unchanged, while in the three test groups the plaque scores were statistically significantly reduced by 50-60 percent ($p < 0.001$). The plaque scores at the six-week examination revealed that the improved oral hygiene status noted in the test groups after the first three weeks remained unchanged or had further improved, while the mean plaque score of the control group was still not statistically significantly different from the baseline score. Other results were that at basline, between 41 percent and 31 percent of all surfaces examined were found to be free of clinically detectable plaque. At the six-week examination, there was a marked increase in the percentage of plaque-free tooth surfaces in the three test groups, although not in the control group. In the Listerine group, the percentage of surfaces with a plaque index score of 0 had increased from 40 percent to 66 percent, while in the two other test groups, the corresponding change was from 31 percent to 76 percent (0.2 percent chlorhexidine) and 41 percent to 67 percent (0.1 percent chlorhexidine) Significant extrinsic tooth discoloration did not develop during the six weeks of trial in any of the groups.

As for gingivitis scores, these were statistically significantly reduced in all three test groups ($p < 0.001$), but not in the control group, during the six weeks of trial. Listerine reduced the mean gingivitis score by 51 percent compared to the control group, while the other two active mouthwashes reduced gingivitis scores by 38 percent (0.1 percent chlorhexidine) and by 35 percent (0.2 percent chlorhexidine). Thus, while the mouthwashes were more or less equally effective in reducing plaque (with slightly greater reductions for the

chlorhexidine rinses), Listerine was the most effective in reducing gingivitis.

3 week

6) **Ross, N. et al: The effect of toothbrushing and rinsing with Listerine in providing a cleaner mouth, FDU study, Warner-Lambert Research Report # 955-0875, August 18. 1976.** (See Section V Supporting Studies Reference 6)

The purpose of this study was to determine the effect of rinsing with Listerine as a supplement to regular toothbrushing in regarding the accumulation of plaque. The results showed that when any of three Listerine formulations were used, they retarded the accumulation of dental plaque by a statistically significant amount.

a. Methodology

Following a dental prophylaxis to remove all plaque and calculus, the 88 subjects (ages 18 - 53) rinsed for 30 seconds twice each day for 21 consecutive days with 20 ml. of either regular Listerine, one of two different variations of Listerine, or a placebo. The rinses were supervised on weekdays

b. Results

Mean plaque scores were statistically significantly reduced for Listerine and its two variations by 50.6 percent, 52.1 percent, and 46.5 percent ($p < 0.001$), compared to those for the colored, flavored 8 percent hydroalcoholic control rinse. None of the mouthwashes affected the subjects' oral soft tissue.

7) **Yankell, S., et al: The effect of tooth brushing and rinsing with Listerine in providing a cleaner mouth: University of**

Pennsylvania Study. Warner-Lambert Research Report # 955-0893, November 12, 1976. (Published as an abstract J. Dent. Res. 56: Abstract. 769, 1977.) (See Section V Supporting Studies Reference 7)

The purpose of this study was to determine the effect of rinsing with Listerine as a supplement to regular toothbrushing in retarding the accumulation of dental plaque. The results showed that Listerine and a variation of the Listerine formula **statistically** significantly reduced plaque accumulation when compared **to the placebo** rinse.

a. Methodology

Following a dental prophylaxis, the 66 subjects (ages 18-36) rinsed for 30 seconds twice a day for 21 consecutive days with 20 ml. of either regular Listerine, a variation of Listerine, or a placebo.

b. Results

The mean plaque scores for Listerine and its variation were 24 percent ($p < 0.05$) and 43 percent ($p < 0.01$) less than those for the placebo rinse.

8) **Menaker, L. et al: The effects of Listerine Antiseptic on dental plaque. Ala. J. Med. Sci. 16:71-77. 1979/Warner-Lambert Research Report # 964-0222, September 15, 1978.** (See Section V Supporting Studies Reference 8)

The purpose of this study was to determine the effect of rinsing with Listerine in retarding the accumulation of dental plaque. The results showed that Listerine retarded the accumulation of plaque by a statistically significant amount.

a. Methodology

Seventy-nine subjects completed this double-blind trial. After a dental prophylaxis, the subjects followed their normal oral hygiene procedures for four weeks to provide information regarding normal plaque growth. Subsequently, the subjects received another After a dental prophylaxis and were assigned to either a Listerine or a placebo group. The subjects rinsed for 30 seconds twice each day for 21 consecutive days with 20ml of their assigned product. The subjects followed their ordinary oral hygiene procedures during this time, but were instructed to refrain from using dental floss or commercial mouthwashes. Rinses were supervised on weekdays.

b. Results

Listerine significantly ($p < 0.001$) retarded the accumulation of dental plaque by 42.9 percent as compared to tooth-brushing and rinsing with a placebo mouthwash. The 38.3 percent reduction between baseline mean plaque scores and after-treatment mean plaque scores in the Listerine group was also statistically significant ($p < 0.001$).

There were no soft tissue aberrations in either group.

9) **Kennedy, P. and Kravets, T. Plaque removal and the use of an antibacterial mouthwash. Warner-Lambert Research Report # 956-0073, August 15, 1974. (Published as an abstract. U. S. Navy Med. Newsletter; 55: 39-40., Feb. 1970.)** (See Section V Supporting Studies Reference 9)

The purpose of this study, conducted by investigators at the United States Navy Dental School, was to determine the effect of Listerine on the accumulation of dental plaque. The study found that rinsing with Listerine reduced the accumulation of plaque by a statistically significant amount.

a. Methodology

Twenty subjects were provided with a thorough prophylaxis to remove all plaque. The subjects followed their normal oral hygiene routine for the next seven days. At that point, plaque was stained, harvested from six specified teeth, air-dried, and weighed for a baseline measurement. The 15 subjects who had formed 0.6 mg or more of plaque then underwent a second prophylaxis. These subjects were subsequently assigned to rinse (unsupervised) for 30 seconds three times a day with Listerine or a 0.9 percent saline solution, as a supplement to their normal oral hygiene practices. After seven days, plaque was stained and harvested from the same six teeth. The plaque was air-dried and weighed, and the resulting data was compared to the baseline data obtained prior to the initiation of the rinse regimen.

b. Results

Dry plaque weight averaged 0.5 mg in the Listerine group (a 50 percent reduction from baseline), compared to an average weight of 0.9 mg in the saline solution control group (a 10 percent reduction from baseline). The reduction from baseline for the Listerine group was statistically significant ($p < 0.01$).

10) **Gomer, R. et al: The effects of oral rinses on the accumulation of dental plaque. J. Amer. Soc. Preven. Dent.; 2:6-9, Mar-Apr., 1972/Warner-Lambert Research Report # 973-0007, February 24, 1970.** (See Section V Supporting Studies Reference 10)

The purpose of this study was to determine the effect of four commercial mouthwashes on dental plaque accumulation when used three times daily as part of an individual's oral hygiene program. The results showed that of the mouthwashes tested, a consistent statistically significant decrease in plaque accumulation was seen only with groups using Listerine.

a. Methodology

All the subjects accepted for participation exhibited moderate to heavy plaque accumulation, but were free from periodontal disease except for some areas of chronic gingivitis. The study was conducted in three phases. In Phase I, 60 naval enlisted personnel (ages 19-26) underwent a thorough prophylaxis to remove all plaque and then followed their normal oral hygiene routines for seven days. After this period, a baseline plaque measurement was made on six specified teeth, to determine the normal amount of one-week plaque accumulation. Following a second prophylaxis to remove all plaque, the subjects were randomly assigned, double-blind, to one of five treatment groups: 0.9 percent saline solution, Listerine, Sterisol, Colgate 100, and Lavoris. The subjects rinsed (unsupervised) for 30 seconds with 20 ml. of their assigned rinse, three times each day, in addition to following their normal oral hygiene routine. Plaque was scored on the seventh day. Fifty-four subjects completed Phase I.

Phase II was similar to Phase I except that only three rinses were studied. In this phase, 35 dental students (ages 22-31), following the same protocol as in Phase I, were assigned double-blind to one of three groups: 0.9 percent saline solution, Listerine, and Colgate 100. Thirty-two subjects completed Phase II.

In Phase III, 14 subjects from Phase II were evaluated for plaque seven days after cessation of the rinse regimen to determine if there were any residual effects.

b. Results

In Phase I, there was a diminution of plaque formation in all five groups, but only Listerine demonstrated a statistically significant reduction in plaque formation compared to the mean baseline plaque score ($p < 0.01$). Listerine inhibited plaque develop-

ment by 39 percent, compared to a 12 percent inhibition by the saline control.

In Phase II, there was again a reduction in dental plaque formation in all the rinse groups. Listerine, however, was most most effective. Listerine inhibited plaque development by 55 percent ($p < 0.01$) and Colgate 100 by 26 percent ($p < 0.05$), compared to a 10% reduction for the saline control.

In Phase III, no group showed a statistically significant residual effect at the end of the seven-day period following the cessation of the rinse regimes. The plaque scores of all groups approximated their baseline plaque scores.

2 week

11) **Fornell, J. et al: Effect of Listerine on dental plaque and gingivitis. Scand. J. Dent. Res.; 83:18-25, 1975/Warner-Lambert Research Report # 956-0074, August 15, 1974** (See Section V Supporting Studies Reference 11)

This cross-over study was designed to evaluate the effect of Listerine on the rate of plaque formation and gingivitis development during a two-week period when used as the sole oral hygiene measure. The results showed that Listerine allowed less plaque to accumulate and also inhibited the development of gingivitis, by statistically significant levels, compared to the water control. The plaque inhibition was greater when measured by plaque weight rather than plaque area.

a. **Methodology**

Ten subjects (ages 19-30) participated in the trial. The study was carried out during four consecutive two-week periods. At an initial examination, plaque and gingivitis were assessed.

Subsequently, the participants underwent a two-week preparatory period during which time they were subjected to repeated professional tooth-cleansings and instruction in toothbrushing and flossing. At the end of this preparatory period, plaque and gingivitis were again assessed for each subject.

For the following two weeks, half of the subjects rinsed with Listerine three times each day. The other half of the subjects rinsed with a placebo solution. All subjects abstained from all mechanical tooth cleaning. Plaque and gingivitis were assessed at four designated times during this two-week period. On the seventh and fourteenth day of the trial, the plaque formed on different specified teeth was sampled and weighed.

For the next two-week period, the subjects were again allowed to clean their teeth and underwent professional prophylaxis as in the initial preparatory period.

After this second preparatory period, there was a second test period during which the participants rinsed with Listerine mouthwash or with the placebo solution and refrained from other oral hygiene measures. Participants were assigned to the Listerine group if they had been in the placebo group in the first trial period and to the placebo group if they had previously been assigned to the Listerine group. Plaque and gingivitis were assessed as in the previous trial period.

b. **Results**

The results showed that compared to water control rinses, rinsing with Listerine allowed 53.3 percent less plaque to accumulate when measured by surface area ($p < 0.001$) and 93 percent less when plaque weights were compared ($p < 0.001$). Gingivitis severity when rinsing with Listerine was 47 percent less ($p < .05$) than when the subjects used the water rinse.

12) **Carter, H. and Barnes, G: Effects of three mouthwashes on existing dental plaque accumulation. J. Prev. Dent.; 2:6-11, May-June 1975.** (See Section V Supporting Studies Ref. 12)

This study was designed to determine the effects of three commercial mouthwashes on existing plaque accumulations. The study found that Listerine reduced plaque accumulation, but the result was not statistically significant.

a. Methodology

The study has two parts. In Part I, there were 65 male subjects (ages 18-43). Each subject was examined for plaque. Subsequently, the subjects were randomly divided, double-blind, into five groups. One group received no mouthwash, a second group received colored, flavored water, a third group received Colgate 100, a fourth group received Listerine, and a fifth group received Cepacol. The subjects rinsed with 30 ml. of their assigned mouthwash for 60 seconds twice daily for 14 days, while continuing their normal home oral hygiene procedures. Plaque was assessed at the conclusion of the treatment period.

In Part II, there were 96 female subjects (ages 18-40). After a plaque examination, the subjects were randomly divided into three groups and assigned either Colgate 100, Cepacol, or colored, flavored water to use as a mouthwash under the same procedures as in Part I. Listerine was not used in the second part of the study. After 21 days of rinsing with their assigned mouthrinses, the subjects were again examined for plaque.

b. Results

The Listerine group in Part I experienced a plaque reduction of 8.3 percent compared to the baseline mean plaque score, but that reduction was not statistically significant. The 19.9 percent

difference between Listerine and the water rinse control group was also not statistically significant. The only mouthwash that effected a statistically significant reduction in plaque accumulation in this study was Cepacol.

13) **Mankodi, S. et al: Clinical efficacy of Listerine in inhibiting and reducing plaque and experimental gingivitis. J. Clin. Perio.; 14: 285-288, 1987/Warner-Lambert Research Report# 931-0528, August 22, 1984.** (See Section V Supporting Studies Reference 13)

This two-week double-blind controlled clinical trial studied the effects of rinsing with Listerine or a vehicle control mouthrinse as the sole means of oral hygiene. The study found that rinsing with Listerine either twice or four times daily resulted in a significant inhibition of both plaque and gingivitis when compared to twice daily rinsing with the vehicle control.

a. Methodology

One hundred and three subjects ranging in age between 18-49 completed a two-week, double-blind, controlled, clinical trial. They all had varying degrees of gingivitis but did not show evidence of periodontal attachment or alveolar bone loss.

Following a screening examination, subjects were given a randomly assigned, half-mouth, dental prophylaxis and instructed to continue their usual oral hygiene and to return in four to five days. All subjects then received baseline examinations and were randomly assigned to a Listerine twice daily rinse group, Listerine four times daily rinse or a vehicle control twice daily rinse group. Subjects rinsed with their assigned products under supervision on weekdays and maintained a diary of unsupervised rinses on weekends. Subjects were instructed to avoid all other forms of oral hygiene during the course of the study.

At screening baseline and again at two-weeks, subjects received a complete intraoral soft tissue examination and both supragingival plaque and gingivitis were scored.

Plaque area was scored by the Turesky modification of the Quigley-Hein Index on the buccal and lingual surfaces of all scorable teeth.

Gingivitis was scored by the Lobene modification (noninvasive) of the Loe-Silness Index on the buccal and lingual surfaces and interdental papillae of all scorable teeth as follows:

0 - normal (absence of inflammation)
1 - mild inflammation (slight change in color, little change in texture, of <u>any portion</u> of the gingival unit)
2 - mild inflammation of the <u>entire</u> gingival unit
3 - moderate inflammation (modest glazing, redness, edema and/or hypertrophy) of the entire gingival unit
4 - severe inflammation (marked redness and edema/hypertrophy, spontaneous bleeding or ulceration) of the entire gingival unit

b. <u>Results</u>

The results showed that Listerine Antiseptic, used twice and four times daily, retarded the accumulation of dental plaque area scores by 46.0% (p=.001) and 56.6% (p=.001), respectively, compared to its vehicle control. Listerine four times daily retarded the accumulation of dental plaque area scores by 19.6% (p=.033) compared to Listerine twice daily. Listerine Antiseptic, used twice and four times daily, reduced existing plaque area scores by 39.1% (p=.001) and 49.6% (p=.001), respectively, compared to the vehicle control. Listerine four times daily reduced existing plaque area scores by 17.3% (p=.023) compared to Listerine twice daily.

The results of this test also showed that Listerine Antiseptic, used twice and four times daily, inhibited the development of gingivitis scores by 57.8% (p=.001) and 59.3% (p=.001), respectively, compared to its vehicle control. Listerine, used twice and four times daily, reduced existing gingivitis scores by 54.9% (p=.001) and 58.2% (p=.001), respectively, compared to its vehicle control. There were no significant differences (p>.20) between use of Listerine four times daily or twice daily in inhibiting the development of and/or reducing existing gingivitis scores. There were no adverse soft tissue effects in any group.

14) **Mankodi, S.: Efficacy of Listerine Antiseptic (W2194-92) compared to control (-167P) in inhibiting the development of and in reducing existing dental plaques and gingivitis. Duration of use study. Warner-Lambert Research Report No. 931-0703, May 15, 1987. (Published as an abstract. J. Dent. Res.; 68: Abstr. 1469, March 1989.)** (See Section V Supporting Studies Reference 14)

This study is published as an abstract entitle "Effect of Rinsing Time on Antiplaque-Antigingivitis Efficacy of Listerine" J. Dent. Res. 68:365 (abstract 1469) 1989.

This research studied the effects of rinsing times of either 30 seconds or 60 seconds with Listerine vs. 30 seconds with a 5% hydroalcohol control on reducing and inhibiting dental plaque development, gingivitis and gingival bleeding. Regardles of rinse time, twice daily use of Listerine resulted in improvement of all parameters evaluated.

a. <u>Methodology</u>

Ninety-four subjects ranging in age from 18-52 years participated in the study. They all had varying degrees of gingivitis, but did not show signs of periodontal attachment or bone

loss. Following baseline examination, each subject was given a randomly assigned, half-mouth supragingival prophylaxis.

The subjects were assigned to one of two different treatment groups or to a control group, according to a randomized code by which double blinding was maintained. All subjects rinsed twice daily with 20 ml of their assigned product for 30 seconds in the control troup and one Listerine group and for 60 seconds in the other Listerine group. During weekdays, the rinsings were performed under supervision while rinsings on weekends were unsupervised. For the duration of the two-week study, subjects refrained from all other forms of oral hygiène and were instructed to follow their usual dietary habits.

Intraoral soft tissue examinations were performed at baseline and two-weeks. Gingivitis was scored at baseline and two weeks using the modified Gingival Index as follows:

0 - normal (absence of inflammation)
1 - mild inflamation (slight change in color, little change in texture) of <u>any portion</u> of the gingival unit
2 - mild inflammation of the <u>entire</u> gingival unit
3 - moderate inflammation (moderate glazing, redness, edema and/or hypertrophy)
4 - severe inflammation (marked redness and edema/hypertrophy, spontaneous bleeding or ulceration)

Bleeding was scored at baseline and at two weeks by the Eastman Interdental Bleeding Index. A wooden interdental cleaner was inserted and removed midline between the teeth from the facial aspect four times and bleeding within 15 seconds was recorded.

Plaque area was also scored at baseline and two weeks using the Turesky modification of the Quigley-Hein Index that was employed in other studies. All examinations were performed by a single dental examiner throughout the course of the study.

b. Results

Rinsing with Listerine for 30 seconds twice daily resulted in a 16.67% and 16.75% reduction in gingivitis scores on the prophy and non-prophy sides , respectively, when compared to control Rinsing for 60 seconds resulted in similar reductions of 20% and 19.9% respectively.

Plaque scores in the control group increased from baseline to two weeks by 27.7% on the prophy side and 30.2% on and non-prophy side. Plaque inhibition of 19.8% and 29.9% was seen on the prophy sides in the Listerine 30 second and Listerine 60 second rinse group, respectively. Plaque reductions on the non-prophy sides were 20% and 29% for the Listerine 30 second and Listerine 60 second groups, respectively. Rinsing with Listerine for 60 seconds resulted in 12% greater plaque reduction than rising for 30 seconds but, these findings were not clinically significant as both groups were equally effective in reducing gingivitis scores and interdental bleeding when compared to the control group on both prophy and non-prophy sides.

PROFESSIONAL INDICATIONS STUDIES

1) **Minah, G.: Effect of Listerine antiseptic in reducing salivary microbial load. Warner-Lambert Research Report # 952-0144, August 20, 1990. Published as an abstract. J. Dent. Res.; 70: Abstr. 1995, April 1991** (See Section V Professional Indications Studies Reference 1)

This research studied the effects of rinsing for 30 seconds with either Listerine or a 5% hydroalcohol control upon the level of viable bacteria recoverable in the oral cavity. Rinsing with Listerine significantly decreased the level of recoverable viable bacteria at two minutes with a slight rise toward baseline during one hour when compared to control.

A. Methodology

Twenty subjects were used in this double blind, crossover design study, with random assignment of the first rinse used, and a wash out period of at least seven days between testing the first and second mouthrinses. Subjects reported to the clinical site having refrained from oral hygiene, eating, drinking or smoking that morning. A one ml sample of unstimulated saliva was collected in a sterile glass vial at baseline. Subjects were then instructed to rinse with 20 ml of their assigned product for 30 seconds and to avoid talking and swallowing during the collection period. Saliva samples were collected as above at 2, 15, 30 and 60 minutes and subsequently plated on culture media selected to enumerate anaerobes, facultative anaerobes, glucan-forming streptococci and Veillonella sp. Each saliva sample was assayed for protein and final results were reported as total colony forming units/mg protein.

B. Results

The Listerine and placebo groups exhibited statistically similar mean values for all four microbial groups at baseline. After rinsing with the placebo, total anaerobic and facultative anaerobic counts decreased by approximately 10 - 20% while rinsing with Listerine resulted in a 60 - 65% decrease in all four microbial groups at two minutes with a slight rise toward baseline thereafter. At 60 minutes after rinsing, the Listerine group exhibited bacterial levels that were 44%, 47%, 28% and 46% lower than baseline for total anaerobes, facultative anaerobes, streptococcal and Veillonella counts respectively. Rinsing for 30 seconds with Listerine resulted in a statistically significant ($p < .05$) reduction in total anaerobes, facultative anaerobes and streptococcal microoganisms thru 60 minutes when compared to the placebo rinse and at 15 minutes for Veillonella sp.

2) **Fine, D.: Reduction of aerosolized oral microbes by Listerine antiseptic. Warner-Lambert Research Report # 952-0148, September 27, 1990. (Published as an abstract J. Dent. Res.; 70: Abstr. 1165, April 1991)** (See Sec. V Professional Indications Studies Ref. 2)

This research studied the efficacy of rinsing with Listerine on reducing viable oral microbes aerosolized during ultrasonic cleaning. The results indicate that Listerine used as a pre-procedural rinse can significantly reduce the microbial content of aerosols generated during dental procedures.

A. Methodology

Eighteen subjects participated in this double blind, crossover design study, with random assignment of the first rinse used and a wash out period of seven days between testing the first and second mouthrinses. Subjects were instructed to abstain from all oral hygiene procedures for 24 hours prior to testing. Subjects received an ultrasonic scaling of one-half of their mouth (randomly assigned) for ten minutes. Subjects were then randomly assigned to either a Listerine or to a hydroalcohol control group and rinsed with 20 ml of their assigned rinse for 30 seconds under supervision. The other half of their mouth was then scaled as described above. During each ten minute scaling period, a modified air sampling device was used to collected the aerosol. The air sampling device was modified by the insertion of a 0.45 micron filter between the unit and the collection end of the intake tube. The viable oral microbes were collected on the filter which was overlaid on culture media for subsequent enumeration.

B. Results

Use of a Listerine rinse for 30 seconds resulted in a 1.23 log reduction in the recoverable colony forming units (CFU) in comparison to baseline levels. Use of the 5% hydroalcohol control rinse resulted in less than a 0.2 log reduction in recoverable CFU's. These results translated into a 94.1% reduction in aerosolized viable bacteria recoverable following rinsing with 20 ml of Listerine for 30 seconds compared to a 33.9% reduction following rinsing with the 5% hydroalcohol control mouthrinse.

3) **Ciancio, S. and Zambon, J.: Effect of Listerine antiseptic delivered by an oral irrigation device on plaque, gingivitis and subgingival microflora. J. Periodontol.; 60: 310-315, 1989/ Warner-Lambert Research Report # 931-0674, July 22, 1986.** (See Sec. V Professional Indications Studies Reference 3)

The purpose of this 6 week clinical and microbiological study was to determine the efficacy of Listerine delivered by an oral irrigation device, as a supplement to normal oral hygiene, in inhibiting and reducing supragingival dental plaque and gingival inflammation, and for its effects on subgingival microflora. The study found Listerine delivered by an oral irrigating device was effective at statistically significant levels in reducing and inhibiting supragingval plaque, moderately effective in controlling the subgingival microbial flora of pocket depths of < 5 mm, and appeared to suppress populations of recognized periodontal pathogens.

a. **Methodology**

Sixty-one subjects, ranging between age 21 and 60, completed the study. They all have preexisting plaque and varying degrees of gingivitis, but with no pocket depths greater than 5 mm. Following a screening examination, each subject was given a half mouth propylaxis. Baseline examinations were determined at seven days following the prophylaxis.

The subjects were assigned to either Listerine Antiseptic or the 5% hydroalcohol control according to a randomized code by which double-blinding was maintained. Starting the same day as baseline exams, all subjects used their assigned irrigating solution once daily under supervision, Monday through Friday, and unsupervised on weekends, for 6 weeks. For the course of the six week trial, the subjects continued their usual oral hygiene and dietary habits, but were instructed to refrain from using commercial mouthrinses. They were each supplied with the same brand of toothbrush and toothpaste.

Gingivitis was scored at baseline, 3 and 6 weeks by the visual criteria of the Loe-Silness Index, quantification of gingival crevicular fluid volume, and bleeding on probing using the modified Papillary bleeding index. Plaque area was scored at screening, baseline, 3 and 6 weeks by the Turesky Modification of the

Quigley-Hein Plaque Index. Subgingival plaque samples were collected from each subject's four deepest crevices at baseline and at 6 weeks. Darkfield microscopy and immunofluorescence were used to detect and quantitate the subgingival microflora. Pocket depth and attachment level were measured to the nearest millimeter at screening, baseline and 6 weeks. An intraoral soft tissue exam was also conducted at baseline and 6 weeks.

b. Results

At 3 and 6 weeks, both Listerine and control when used as oral irrigation solutions showed significant reductions in supragingival plaque compared to their screening scores. At 6 weeks, mean plaque scores were also significantly reduced from baseline in both groups. Listerine significantly reduced plaque compared to control by 12% at 3 weeks and 16% at 6 weeks, similarly on both the prophy and no prophy sides. Both Listerine and control significantly reduced gingival inflammation, gingival crevicular fluid volume, probeable pocket depth and attachment loss from pretreatment values. At 3 and 6 weeks, Listerine significantly reduced gingival bleeding compared to baseline on the prophy side; at 3 weeks, Listerine group gingival bleeding scores were significantly (41.8%) lower than control.

A moderate decrease or prevention of increase in the total bacterial counts detected by darkfield microscopy on both the prophy and non prophy sides was seen with the use of Listerine. No specific group of organisms within those enumerated accounted for the decreases, indicating that Listerine was not selective in its antimicrobial action. No meaningful shifts in the microbial populations were observed, indicating no emergence of strains resistant to Listerine. Using immunofluorscence, while differences between Listerine and control were not statistically significant, a strong trend in flavor of Listerine was evident in the reduction of three specific periodontal pathogens at week 6.

4) **Zambon, J. et al: The effect of an antimicrobial mouthrinse on early healing of gingival flap surgery wounds. J. Periodontol.; 60: 31-34, 1989/Warner-Lambert Research Report # 931-0707, March 31, 1987.** (See Section V Professional Indications Studies Reference 4)

The purpose of this clinical study was to determine the effect of Listerine as compared to a saline control on plaque formation, gingival inflammation, gingival bleeding, wound healing and patient comfort following gingival flap surgery. The study found that Listerine was significantly more effective than saline at 7 days in reducing plaque. Listerine was also significantly more effective for improving wound healing as measured by tissue color and edema at day 7.

a. Methodology

Twenty-five subjects, ranging between age 29 and 67 completed the study. They all required bilateral posterior segment periodontal flap surgery involving a minimum of three adjacent molars and premolars. Each surgerized segment was selected by a predetermined randomized code as Listerine or physiological saline. At least 6 weeks elapsed between surgical visits providing a two week interim between use of postoperative products. Twenty ml. of the assigned rinses were rinsed for 30 seconds, 3 times a day for 28 days starting the day following surgery.

Plaque area using the Tursky modification of the Quigley-Hein Index, soft tissue appearance by color and edema, and patient assessment of pain/discomfort were recorded at 7, 14, and 28 days postsurgically. Plaque was also scored presurgically. Gingivitis scored by the Loe-Silness Gingival Index, gingival bleeding using the Modified Papillary Bleeding Index and pocket depth were recorded presurgically and at day 28.

b. Results

Listerine was significantly 28.9% more effective than saline in inhibiting plaque development at day 7. There were no significant difference in plaque between treatments at either day 14 or 28. Pocket depth was equally significantly reduced from presurgery baseline in both treatment groups. While use of Listerine resulted in a 22% decrease in gingival bleeding at day 28 compared to baseline and the saline a 7% increase, these differences were not statistically significant. No differences in visual gingival inflammation were seen. On day 7 Listerine demonstrated significantly (p=.0517) better tissue color with 20% of the tissue evaluated being red (inflamed) as opposed to 40% for the saline treatment. Twelve percent demonstrated normal (pink) tissue in the Listerine group, but none in the saline group. Additionally at day 7 edema was significantly less severe in the Listerine group. Less than 17% of the evaluations showed moderate to severe edema in the Listerine group as opposed to 44% in the saline treatment. There were no significant difference between groups at day 14 or 28. No significant difference in patient evaluations for pain/discomfort were found.

5) **DePaola, L.G., et al: The effect of antiseptic mouthrinses on oral microbial flora and denture stomatitis. Clinical Preventive Dentistry 1986; 8:3-8, 1986/Warner-Lambert Research Report # 931-0515, April 24, 1984.** (See Section V Professional Indication Studies Reference 5)

The purpose of this 4 week clinical and microbial cultural study was to determine the effect of Listerine antiseptic mouthrinse and denture soaks in reducing inflammation of the denture-bearing tissues and pathological denture microbial flora. The study found that Listerine rinses and/or soaks significantly reduced both area and intensity of denture-bearing tissue inflammation. These clinical reductions in inflammation were accompanied by reductions in microbial components including yeast.

a. <u>Methodology</u>

Forty-seven subjects ranging between age 30 and 80 completed the study. They all were in good systemic health, currently wearing a maxillary complete denture or a tissue supported removable partial denture and demonstrating mild to moderate inflammation of the palatal mucosa.

Each subject was entered by random code into one of the five following treatment regimens: (1) Listerine rinse, Listerine soak, (2) Listerine rinse, Nystatin soak, (3) Nystatin rinse, Listerine soak, (4) Nystatin rinse, Nystatin soak (5) sterile colored water rinse, sterile colored water soak. Subjects rinsed their mouths three times daily for 30 seconds with 20 ml. of Listerine or sterile water, or for 2 minutes with 10 ml. of Nystatin (100,000 U/ml.) solution. Subjects also soaked their dentures for 6 hours each night in 150 ml. of the assigned solution. Soak solution were changed daily in the water control group, every 4 days in the Listerine group and weekly in the Nystatin group. Subjects refrained from all mechanical oral hygiene during the study.

Clinical assessments and specimen collection for microbiological culture were conducted at baseline and following 4 weeks of product use. Palatal inflammation was assessed using an inflammation index that quantified the area and intensity of inflammation. Denture stability was evaluated by a modification of the Kapur Index. Two examiners performed the clinical evaluations independently an their scores for each index were averaged. Specimens for microbial evaluations consisted of (1) a saliva sample, (2) swabbing the most inflamed region of the palate and (3) denture plaque from the tissue bearing surface of one half of the denture. Plaque on the other half of the denture was quantified using the Abelson Tissue Surface Plaque Score.

b. Results

Both Listerine Antiseptic and Nystatin Oral suspension rinses and/or soaks significantly reduced both area and intensity of denture-bearing tissue inflammation compared to the sterile water control. Listerine Antiseptic and Nystatin appeared equally effective.

Clinical scores for denture plaque and denture stability were not changed significantly by the treatment or control regimens. Total yeast populations in denture plaque were significantly reduced over the study in only the Listerine rinse and soak group and the Nystatin rinse and soak group. Yeast from the palatal specimens were lower posttreatment, but not at statistically significant levels, for all groups except Listerine rinse and Nystatin soak and control group. Yeast levels in saliva did not change significantly in any of the five groups.

4. Pertinent Marketing Information

1. ADA SEAL OF ACCEPTANCE

On June 22, 1987 Listerine was awarded the Seal of Acceptance from the Council on Dental Therapeutics (the "Council") of the American Dental Association ("ADA"). (See Attachment III.) The Seal of Acceptance reflects the ADA Council's determination that Listerine is safe and effective to help prevent and reduce supra-gingival plaque accumulation and gingivitis when used in a conscientiously applied program of oral hygiene and regular professional care. Since we obtained the Seal, all Listerine labels, labeling and advertising have been approved in writing by the ADA.

Known for its independence and rigorous scientific standards, the ADA Council's determinations are respected by the scientific community both in the United States and abroad. As noted recently by the National Advertising Division of the Council of Better Business Bureaus, in reviewing complaints relating to the effectiveness of a product which bore the ADA Seal, "There can be no doubt that the ADA is recognized throughout the world as an authoritative, professional body."[5/] FDA itself frequently relied on ADA Council findings and recommendations in its review of the report of the OTC Dentifrice and Dental Care Drug Products Panel.[6/]

In response to FDA's letters of June and July 1988, asking dental product manufacturers to prove their plaque and gingivitis claims, the ADA issued the following press statement on October 11, 1988, defending the significance of its Seal of Acceptance:

"The Association, through its Council on Dental Therapeutics, has for many years insisted on proof, through rigorous research, of all claims made for products that apply for, and carry, the ADA's Seal of Acceptance. That seal, which signifies the ADA's acknowledgement of the safety and efficacy of the product, is not awarded lightly. Many products are denied approval, and some products that currently carry the Seal were required to extend their research to satisfy the scientific standards of the Association before the Seal was awarded. In addition, the ADA continually reviews all advertising claims for products that carry the Seal."

5/ 18 NAD Case Report 44, (January 16, 1989)

6/ See e.g., 50FED.Reg.39584, 39857 (September 30, 1985); see also 53Fed.Reg.22430, 22439 (June 15, 1988)

The ADA encouraged manufacturers that had been awarded the ADA Seal to supply FDA with the data on which the plaque and gingivitis claims were based so that FDA can share the same level of confidence the ADA has in products that bear the ADA seal.

a. The Council on Dental Therapeutics

The Council on Dental Therapeutics was established in 1930 in response to ADA membership concerns about unproved claims for dental product safety and effectiveness. Pursuant to ADA Bylaws, the Council is directed to study, evaluate and disseminate information with regard to the proper use of dental therapeutic agents, their adjuncts and dental cosmetic agents which are offered to the public or to the profession. The Council fulfills this mission primarily through its Acceptance Program, under which it evaluates data voluntarily submitted by product manufacturers or distributors. Under the aegis of the Program, the Council evaluates the safety and efficacy of submitted agents; reviews and approves product labeling, package inserts, advertising, and other promotional material, and informs the profession and public about safe and effective dental therapeutic products.

The Council views the Acceptance Program as a procedure by which an independent body conducts a dispassionate analysis of the scientific accuracy of data in order to determine whether a product is safe and effective. In order to maintain its independence, the Council has never accepted a fee for its evaluation services. The Council classifies the products it reviews into one of three categories: "accepted," "provisionally accepted," or "unaccepted." These three classifications are defined:

Accepted products include those for which there is adequate evidence of safety and efficacy.

<u>Provisionally accepted</u> products include those for which there is reasonable evidence of safety and dental efficacy, but which lack sufficient evidence of dental effectiveness to justify being accepted. These products meet other qualifications and standards established by the Council. The Council may authorize the use of a suitable statement to specifically define the effectiveness of a provisionally accepted product.

<u>Unaccepted</u> products include those for which the Council has determined that there is no substantial evidence of efficacy or that a question of safety exists.

The decision whether to award a Seal of Acceptance is the responsibility of the Council's members. The Council members, in turn, may rely on consultants nominated to assist in the evaluation process. Both Council members and consultants have traditionally been leading experts in the dental academic community. For example, the Chairman of the Council in 1987 was Dr. Alvin Solomon, an assistant clinical professor at the Columbia University School of Dental and Oral Surgery. Dr. Solomon has published a number of articles relating to the use of drugs in dentistry. Similarly, the 1987 Vice-Chairman of the Council, Dr. Samuel Holroyd, was a professor and Department Chairman at the Washington University School of Dental Medicine. Dr. Holroyd is also the author of a number of scholarly articles, mostly relating to pharmacology. The other five members of the Council in 1987 were also well-respected academicians and authors of a number of published articles on dental subjects. These members were: Dr. Tommy W. Gage, a professor and Department Chairman at the Baylor College of Dentistry; Dr. Peter L. Jacobsen, an assistant professor at the University of the Pacific School of Dentistry; Dr. Edwin D. Joy, Jr. a professor and Department Chairman at the School of Dentistry within in the Medical College of Georgia; Dr. Robert A. J. Olson, an associate professor and clinical director at the University of Iowa School of Dentistry; and Dr. Donald E. Van Scotter, a professor at the Marquette University School of Dentistry.

b. Council Evaluation of Listerine

The Council has established guidelines for evaluating the different types of products it considers for the Seal. Listerine was required to meet the Council's "Guidelines for Acceptance of Chemotherapeutic Products for the Control of Supragingival Dental Plaque Plaque and Gingivitis." (See section V, Efficacy Data.) These guidelines require that clinical studies of safety and effectiveness submitted to the Council have the following characteristics:

(1) Characteristics of the study population should represent typical product users;

(2) Active product should be used in normal regimen and compared with a placebo control or, where applicable, an active control;

(3) Crossover or parallel designed studies are acceptable;

(4) Studies should be a minimum of 6 months in duration;

(5) Two studies conducted by independent investigators will be required;

(6) Microbiological sampling should estimate plaque qualitatively to complement indices that measure plaque quantitatively;

(7) Plaque and gingivitis scoring and microbiological sampling should be conducted at baseline, 6 months, and at an intermediate period;

(8) Microbial profile should demonstrate that pathogenic or opportunistic microorganisms do not develop over the course of the study; and

(9) The toxicological profile of products should include carcinogenicity and mutagenicity assays in addition to generally recognized tests for drug safety

The Council reported in the Journal of the American Dental Association (Attachment IV) that the Data on clinical effectiveness, microbiology, and safety submitted by Warner-Lambert satisfied the Council's guidelines. The Council explained that its determination of the effectiveness of Listerine was primarily based on three published clinical studies of six months or more which showed, among other results, that Listerine significantly reduced plaque and gingivitis and that Listerine also achieved a significant inhibition of plaque and gingivitis. With respect to the safety of Listerine, the Council indicated that the clinical studies had found no mucosal aberrations or development of extrinsic tooth stain. The Council also reported that three established tests found the mutagenicity potential of Listerine to be negative. Finally, two independent six-month microbiological studies one of which was published as a companion paper to a clinical study (See Section V Definitive Studies Reference 3a) found that Listerine use did not cause a significant increase in presumptive oral pathogens or opportunistic pathogens. In addition, the positive results of these studies were corroborated by the other studies supplied to the Council.

c. Council Approval of Listerine Labeling

As a condition for receiving the Seal of Acceptance for Listerine, Warner-Lambert was required to submit to the Council, for prior approval, all labeling, package inserts, and professional and consumer advertising copy, whether or not the material carried the Association's name. Moreover, as a condition for retaining its Seal, Warner-Lambert is required to submit any proposed changes in these materials to the Council for prior approval. The purpose of this

requirement, and of the ADA's decision to allow its name to be used in commercial advertising, is so that the ADA can provide authoritative guidance to the public on matters pertaining to dental health.

The Council explains in its guidelines:

> "The acceptance program for therapeutic agents is specifically designed to provide accurate information on the safety and effectiveness of products and to insure that: 1) all advertising claims made for such products are scientifically accurate supporting laboratory and clinical test results, 2) such products are clearly positioned as only one part of the total dental health program, and 3) the profession is not portrayed as a promoter of any specific commercial products."

Pursuant to this policy, all current labeling and promotional material for Listerine has been specifically approved by the Council. For attribution to the Council, the following statement is approved:

> "Listerine Antiseptic has been shown to help prevent and reduce supragingival plaque accumulation and gingivitis when used in a conscientiously applied program of oral hygiene and regular professional care. Its effect on periodontitis has not been demonstrated. Council on Dental Therapeutics - American Dental Association."

2. American Academy of Periodontology

A recent report from the American Academy of Periodontology on "Chemical Agents for the Control of Plaque" further recognizes the efficacy of Listerine in the treatment of plaque and gingivitis (Attachment IV). This report which was distributed to all of the Academy's members is intended to inform periodontists of the available data on chemical agents claiming plaque-reducing abilities. With respect to Listerine, the Academy reported that "Short-term studies

have shown plaque and gingivitis reduction averaging 35%, and long-term studies have shown plaque reduction averaging 25% and gingivitis reduction averaging 29%." The only adverse effects reported by the Academy were a burning sensation and bitter taste. The report noted that Listerine has been accepted by the American Dental Association (ADA) for the control of plaque and gingivitis, the only agent reported, with the exception of chlorhexidine, that had been so accepted. The report emphasized the significance of ADA approval, quoting the statement of the Chairman of the ADA's Council on Dental Therapeutics ("CDT"):

> The dentist should realize that a product claiming a therapeutic effect which has not been approved by the CDT-ADA as evidenced by the seal of approval has either not been submitted for approval or has been found lacking in research evidence of therapeutic effectiveness, safety, or both.

The American Academy of Periodontology, founded in 1914, is a well-established, highly respected body composed of dentists who are trained as specialists in the treatment of gum diseases. This recognition by the Academy of the data showing the safety and effectiveness of Listerine, and its reminder to its members of the significance of the ADA approval of Listerine, adds to the body of evidence that Listerine is generally recognized among experts as safe and effective for the prevention and reduction of plaque and gingivitis.

XYLITOL

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June 17, 1991

William E. Gilbertson, Pharm. D.
Director
Division of OTC Drug Evaluation (HFD-210)
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

Re: Docket No. 81N-0033; OTC Dental and Oral Health Care Products for Antiplaque Use

Dear Dr. Gilbertson:

The scientific data and information herein are being submitted to the Food and Drug Administration (FDA) by Leaf, Inc. (Leaf) in response to the agency's call-for-data relating to products bearing antiplaque-related claims. See 55 Fed. Reg. 38560 (Sept. 19, 1990); 56 Fed. Reg. 9915 (Mar. 8, 1991).

Leaf firmly believes that its XyliFresh chewing gum products (sugarfree chewing gums sweetened with significant levels of xylitol) are foods legally marketed with antiplaque-related dental health messages on their labeling. As we shall demonstrate, Leaf's XyliFresh gums have long been marketed as foods, are intended for use as foods, and comply with both the legal definition of a food in the Federal Food, Drug, and Cosmetic Act (the Act) and the FDA's policies concerning health messages on food labeling. To the extent that the XyliFresh chewing gums might nonetheless be considered drug products, Leaf submits that the data and information provided herein fully support the inclusion of sugarless chewing gums which contain

significant levels of xylitol in the over-the-counter (OTC) monograph for antiplaque drug products.

Xylitol Chewing Gums

Xylitol-containing sugarfree chewing gums have long been marketed as noncariogenic food products in the United States and Europe. Significant clinical and nonclinical data concerning the dental health benefits of xylitol chewing gums have been developed over the last ten years. Leading dental researchers agree that, when chewed after meals and snacks, sugarless gums sweetened with significant levels of xylitol provide a cariostatic effect -- xylitol gums help prevent dental caries and reduce the incidence of new caries. See Dental Dialogue, Caries Prevention with Xylitol, A Symposium at the University of Michigan, Ann Arbor, 3 (1988) (Appendix A). Published clinical and nonclinical data confirm that xylitol sugarless chewing gums also reduce plaque accumulation and help prevent plaque acids when added to a dietary program that limits sugary snacks. See generally, comments regarding reproposed rule on health messages on food labeling [55 Fed. Reg. 5176 (Feb. 13, 1990)] submitted by Leaf, Inc. (Apr. 13, 1990) and American Xyrofin, Inc. (May 15, 1990) (Appendix A).

XyliFresh Chewing Gum Products

The only sugarfree chewing gums marketed in the United States that contain a significant level of xylitol are Leaf products. The original Leaf, Inc. candy company was founded in Chicago in 1921. For many years, the Company ranked as the largest manufacturer of ball gums in the world. In 1983, the Company was purchased by Huhtamaki Oy, a Finnish company and one of the leading confectioners on the European continent. Huhtamaki Oy has produced and distributed xylitol gums and candies bearing antiplaque and cariostatic labeling claims in Scandinavia and Europe for more than 15 years. The Company's Xylitol Jenkki gum is now the leading confectionery product in Finland. With the addition of other candy businesses, e.g., the Beatrice Corp. Candy Division, L.S. Heath and Company, and Hollywood Candy Company, the legal entity now identified as Leaf, Inc. was formed. Today, Leaf is one of the major suppliers of candy and chewing gum products in the United States and Europe.

Leaf currently markets in the United States two different sugarfree chewing gums labeled with the "XyliFresh" mark. One is the "Original Xylitol Gum," a sugarfree gum product that has been

available in this country at health food stores since at least 1981. Since that year, more than units gum pellets) have been sold. A summary of the sales data is provided in Appendix B. Original XyliFresh Gum is sweetened with xylitol and sorbitol. See Section II. Active Ingredient for xylitol content of Original XyliFresh (enclosed).

In June 1990, Leaf introduced another xylitol gum product, XyliFresh Sugarfree Gum (XyliFresh). XyliFresh is manufactured by Leaf and distributed by Leaf Specialty Products, a division of Leaf located in Bannockburn, Illinois. XyliFresh is now distributed in 13 midwestern states: Michigan, Ohio, Indiana, Iowa, Kentucky, Illinois, Wisconsin, Nebraska, Minnesota, North Dakota, South Dakota, Missouri and Kansas. Since its introduction, more than units (gum pellets) have been sold. A summary of the sales data is provided in Appendix B. The XyliFresh product contains a higher level of xylitol and is more suited to American taste preferences than the Original XyliFresh product. The XyliFresh product is sweetened with xylitol and acesulfame K. See Section II. Active Ingredient for xylitol content of XyliFresh (enclosed).

The aforementioned sales data and information clearly demonstrate that Leaf's XyliFresh Sugarfree Gum and XyliFresh Original Gum, the only sugarfree gum products sweetened with xylitol, have been marketed for a material time and to a material extent.

In Europe and Scandinavia, caries prevention and antiplaque-related labeling claims have appeared on labels and labeling for Leaf's xylitol gums and confectioneries since the mid-1970's. In the United States, the dental health message "Fights Plaque" first appeared on the Original XyliFresh labeling in 1988. Since its introduction, the XyliFresh labeling has included the following dental health message:

XyliFresh Gum, with Xylitol, will help you ...

* Prevent plaque acids that can cause cavities.
* Reduce plaque build-up that can lead to ugly tartar.
* Prevent new cavities.

Copies of XyliFresh and Original XyliFresh labeling materials are provided in the enclosed Section I. Labels and Labeling. Leaf firmly believes that its XyliFresh and Original XyliFresh chewing gums are traditional food products, and the dental health labeling messages do not make these products drugs.

Regulation of Chewing Gums

Chewing gum was widely-recognized as a traditional food product for many years prior to enactment of the Federal Food, Drug, and Cosmetic Act (the Act). The status of chewing gum as a food is expressly confirmed in the Act's definition of food: "(1) articles used for food or drink for man or other animals, (2) chewing gum, and (3) articles used for components of any other such article." Section 201(f) of the Act, 21 U.S.C. §321(f) (emphasis added). This definition of food has not been amended by Congress since enactment of the Act in 1938. See Pub. L. No. 717, Chap. 675, 75th Cong., 3d Sess., p.1, approved June 25, 1938. The courts have consistently interpreted the statutory food definition to include (1) products consumed by people for the ordinary reasons -- taste, aroma or nutritive value; (2) chewing gum (which is not intended to be swallowed); and (3) food additives. See, e.g., Nutrilab, Inc. v Schweiker, 547 F. Supp. 880 (N.D.Ill. 1982), aff'd, 713 F.2d 335, 337 (7th Cir. 1983); United States v General Nutrition Inc., 638 F. Supp. 556, 558 n.1 (W.D.N.Y. 1986); American Health Products Co., Inc. v Hayes, 574 F. Supp. 1498, 1504 (S.D.N.Y. 1983), aff'd, 744 F.2d 912 (2d Cir. 1984).

In addition to being a traditional food, a chewing gum product can also fit within the Act's definitions of a medical device and therefore be regulated as such. Section 201(h) of the Act, 21 U.S.C. §321(h), defines a medical device as:

> an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part, or accessory, which is --
>
> > (1) recognized in the official National Formulary, or the United States Pharmacopeia, or any supplement to them,
> > (2) intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or
> > (3) intended to affect the structure or any function of the body of man or other animals, and

> which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its principal intended purposes.

Section 201(h) of the Act, 21 U.S.C. §321 (h). Chewing gums that contain abrasives and are used solely to clean and polish the teeth are considered medical devices, i.e., Premarket Notification #K810037 for Check-Up Dental Chewing Gum (April 8, 1981).

The definition of a "cosmetic" in the Act can also encompass chewing gum products. Section 201(i) of the Act states:

> The term "cosmetic" means (1) articles 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, and (2) articles intended for use as a component of any such articles; except that such term shall not include soap.

Section 201(i) of the Act, 21 U.S.C. 321(i). FDA regulations confirm the cosmetic status of products intended to control or mask offensive mouth odors. 21 C.F.R. §720.4(c)(9)(ii) (includes breath fresheners in oral hygiene cosmetic product category). In addition, the report of the Advisory Review Panel on OTC Oral Cavity Drug Products states that "[t]he Panel considers products intended for elimination or suppression of mouth odor of local origin in healthy persons with healthy mouths to be cosmetics unless they contain antimicrobial or other drug ingredients." 47 Fed. Reg. 22760, 22844 (May 25, 1982). Thus, chewing gums that are marketed for purposes of freshening the breath (e.g., Clorets chewing gum) are regulated as cosmetic products.

Chewing gum products can also be subject to regulation as drug products. Section 201(g) of the Act, 21 U.S.C. §321(g), defines a drug as:

> (A) articles recognized in the official United States Pharmacopeia, official Homeopathic Pharmacopeia of the United States, or official National Formulary, or any supplement to any of them; and (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (C) articles (other than food) intended to affect the

> structure or any function of the body of man or other animals; and (D) articles intended for use as a component of any articles specified in clause (A), (B), or (C); but does not include devices or their components, parts or accessories.

Thus, chewing gum products that contain active drug ingredients and act solely as a drug delivery system are regulated as drugs. See 40 Fed. Reg. 12902, 12903 (Mar. 21, 1975) (Feen-a-Mint Chewing Gum included in OTC review of laxative drug products); 42 Fed. Reg. 35346, 35348 (July 8, 1977) (Aspergum included in OTC review of internal analgesic drug products); 47 Fed Reg. 22760, 22780 (May 25, 1982) (chewing gums containing aspirin included in OTC review of oral analgesic drug products).

XyliFresh Chewing Gums Are Foods

It is well-established that the intended use of a chewing gum product determines whether the product is subject to regulation as a food, a drug, a cosmetic, or a medical device. As stated in the legislative history of the Act:

> The use to which the product is to be put will determine the category into which it will fall. If it is to be used only as a food it will come within the definition of food and none other. If it contains nutritive ingredients but is sold for drug use only, as clearly shown by the labeling and advertising, it will come within the definition of drug, but not that of food. If it is sold to be used both as a food and for the prevention or treatment of disease it would satisfy both definitions and be subject to the substantive requirements for both. The manufacturer of the article, through his representations in connection with its sale, can determine the use to which the article is to be put. For example, the manufacturer of a laxative which is a medicated candy or chewing gum can bring his product within the definition of drug and escape that of food by representing the article fairly and unequivocally as a drug product.

S. Rep. No. 361, 74th Cong., 1st Sess. (1935), quoted in C.W. Dunn, Federal Food, Drug and Cosmetic Act, 240 (1938).

A sugarless gum product that is intended primarily to provide the traditional taste and organoleptic effects of a chewing gum should be deemed a food. XyliFresh Sugarfree Gum is just such a product. The fundamental characteristics of

XyliFresh are its sweet flavor and the soft chewable consistency of its gum base. The endothermic properties of xylitol provide a cool, refreshing sensation in the mouth. Both the sweet taste and the chewing motion act to stimulate salivation, a traditional function of chewing gums. XyliFresh looks, smells, chews and tastes sweet -- just like a sugarless chewing gum product should.

When added to the daily diet, XyliFresh chewing gum also happens to help prevent dental caries, and reduce plaque build-up and the production of plaque acids. XyliFresh exerts these effects when consumed over time because it is sweetened with xylitol, and xylitol cannot be fermented by Streptococcus mutans into plaque acids. The xylitol in XyliFresh does not support the Streptococcus mutans population in plaque. As noted above, a discussion of the dental health benefits of chewing XyliFresh after meals and snacks, as part of a total dietary practice that includes reduced consumption of sugary snacks, is provided to consumers on the product labeling. See Section I. Labels and Labeling for XyliFresh labeling materials.

Based on the parenthetical exclusion of foods from part C of the drug definition, labeling claims concerning the effect of a food on the body need not make the food a drug if the claims relate to how the food "affects the structure or any function of the body." Section 201(g)(1)C) of the Act, 21 U.S.C. §321(g)(1)(C). In American Health Products Co., Inc., the court commented in dicta on the extent to which the parenthetical exclusion in part C prohibits foods that are represented as affecting the structure or functions of the body from being regulated as drugs:

> [I]f an article affects bodily structure or function by way of its consumption as a food, the parenthetical precludes its regulation as a drug notwithstanding a manufacturer's representations as to physiological effect. The Act evidences throughout an objective to guarantee accurate information to consumers of foods, drugs, and cosmetics. [Citation omitted.] The presence of the parenthetical in part (C) suggests that Congress did not want to inhibit the dissemination of useful information concerning a food's physiological properties by subjecting foods to drug regulation on the basis of representations in this regard.

American Health Products Co., Inc. v Hayes, 574 F. Supp. at 1507. Therefore, a food labeling claim concerning the effects on the structure or functions of the body due to the food's nutritional value when consumed over time would generally not be a drug claim. See 55 Fed. Reg. 5176, 5177 (Feb. 13, 1990) (Food Labeling; Health Messages and Label Statements; Reproposed Rule).

Under part B of the Act's drug definition, however, labeling claims regarding the role of a food in the "diagnosis, cure, mitigation, treatment, or prevention" of a disease may evidence an intent to offer the food product as a drug. Section 201(g)(1)(B) of the Act, 21 U.S.C. §321(g)(1)(B). Historically, FDA has interpreted disease-related claims on food labeling as evidence that the product is intended to be used as a drug. Thus, labeling claims that refer to a specific disease or health condition have made food products subject to regulation as drugs pursuant to part B of the drug definition. See, e.g., Kordel v United States, 335 U.S. 345 (1948); United States v Hohensee, 243 F.2d 367 (3rd Cir.), cert. denied, 353 U.S. 976 (1957); United States v 250 Jars of ... Honey, 218 F. Supp. 208 (E.D. Mich. 1963), aff'd, 344 F.2d 288 (6th Cir. 1965).

In 1987 the agency decided that consumers would benefit from certain public health messages on food labeling. Recognizing that past legal precedent had generally discouraged health-related claims on food labeling, FDA issued a notice of proposed rulemaking on food labeling which stated:

> In light of advances in current knowledge ... the agency now believes that health-related messages, when appropriately formulated for use on food labels and consistent with existing law and regulations, may provide valuable information to health-conscious consumers.

52 Fed. Reg. 28843, 28845 (Aug. 4, 1987) (Food Labeling; Public Health Messages on Food Labels and Labeling). In the 1990 reproposed rule on food labeling, FDA reaffirmed its acceptance of health messages on food labeling:

> [I]t may be appropriate to allow expanded health information on products that are consumed primarily as foods. Such information, if based on sound scientific data and if properly presented, can be useful to consumers who desire to adopt a healthier dietary pattern.

55 Fed. Reg. at 5178.

On November 8, 1990, Congress subsequently endorsed the presence of health messages on food labeling when it enacted the Nutrition Labeling and Education Act of 1990 (the NLEA). The NLEA provides a statutory basis for food labeling statements that relate to specific disease and health-conditions, and directs FDA to promulgate final regulations permitting such claims. Nutrition Labeling and Education Act of 1990, Pub. L. No. 101-535, 104 Stat. 2353 (1990).

Accordingly, under the NLEA and FDA's policies concerning health messages on food labeling, XyliFresh chewing gum is a food product that may legally display labeling claims which properly describe the relationship of xylitol to plaque and dental caries. XyliFresh is not converted into a drug product solely by virtue of its dental health labeling claims.

XyliFresh Antiplaque and Cariostatic Labeling Claims Are Appropriate Health Messages

Based on growing scientific evidence of the relationship between specific dietary practices and specific disease conditions, and the findings of the 1988 Surgeon General's Report on Nutrition and Health (the "Surgeon General's Report") and the 1989 report, Diet and Health: Implications for Reducing Chronic Disease Risk, by the National Research Council's Food and Nutrition Board (the "NAS Report"), FDA now proposes to allow food labeling to bear health messages about the association of diet and chronic diseases. 55 Fed. Reg. at 5179.

The FDA views appropriate health messages for food as "descriptions of the nutritional value of the food." Id. The concept of nutritional value is therefore basic to the distinction between a drug and a food product. In addition to the ability of a food to supply nourishment needed to sustain life, "[n]utritional value may also include the usefulness of a food component, consumed as part of the total diet, in reducing the risk, or forestalling the premature onset of, a chronic disease condition." Id. at 5178. Assuming sufficient scientific evidence to support the claimed effect on a person's risk of developing a chronic disease, a discussion of that effect on labeling would not necessarily make a food a drug. Id.

FDA recognizes that the Surgeon General's Report and the NAS Report "represent the most generally agreed upon scientific basis for health messages" with respect to several chronic diseases. Id. Based on these reports, FDA has tentatively identified six topic areas: calcium and osteoporosis, dietary fiber and cancer,

lipids and cardiovascular disease, lipids and cancer, sodium and hypertension, and dietary fiber and cardiovascular disease, as appropriate subjects for initial consideration in developing standardized health messages in the form of scientific summaries, consumer health message summaries, and model label statements. Id. at 5184. The agency also acknowledges that health messages outside these areas may be warranted. Id.

Xylitol gum and dental caries (which includes the formation of dental plaque and resulting production of plaque acids, a necessary predisposing cause of caries) is clearly a topic worthy of a food labeling health message to consumers. Both the Surgeon General's Report and the NAS Report evaluated the effects of diet on dental caries as a chronic disease. Surgeon General's Report, Chapter 8, Dental Diseases, pp. 345-380; NAS Report, Chapter 26, Dental Caries, pp. 637-647. The NAS Report considers a number of clinical studies on the protective effect of xylitol-containing foods, including chewing gum, and concludes that:

> The addition of certain foods and nonnutritive sweeteners, such as cheddar cheese, cocoa, and xylitol, to the diet appears to reduce the cariogenic potential of a sucrose-containing meal.

NAS Report at 644 (emphasis added). See also id. at 639. The Surgeon General's Report recommends that the general public reduce sugar consumption levels and that food service programs promote noncariogenic foods. Surgeon General's Report at 368-369. The health message on XyliFresh labeling, which discusses the plaque reduction and cariostatic effects of chewing XyliFresh gum after meals and snacks as part of a total dietary practice that includes reduced consumption of sugary snacks, is fully consistent with the recommendations of the Surgeon General's Report and the NAS Report. By also reminding consumers to "brush and floss teeth, limit sugary snacks and visit your dentist regularly," the XyliFresh health message clearly promotes good dental health care practices.

The dental health message on the XyliFresh labeling is truthful and not misleading. Meaningful information concerning the role of XyliFresh in reducing plaque build-up and plaque acids, and preventing cavities is included on the labeling. XyliFresh gum clearly contains sufficient levels of xylitol to exert the plaque reduction and cariostatic effects when chewed daily after meals and snacks as recommended in the labeling. XyliFresh does not possess any other attributes or exert any adverse effects on health that would make the dental health message misleading.

Most importantly, the XyliFresh dental health message is fully supported by numerous studies on xylitol-containing chewing gums, including clinical trials using the actual XyliFresh gum products, which were conducted in accordance with generally recognized dental research procedures and principles. The scientific evidence is provided in the enclosed Sections II - VI.

Conclusion

Based on the data and information herein, Leaf submits that antiplaque and cariostatic labeling claims for xylitol chewing gums are appropriate health messages for a chewing gum food. Leaf strongly urges the Dental Products Panel and the FDA to confirm that such dental health labeling claims do not alter the food status of xylitol chewing gum products.

If the Panel and FDA nonetheless conclude that xylitol chewing gum products bearing antiplaque and cariostatic labeling claims are drug products, Leaf would urge the Panel and FDA to find that xylitol, at significant levels in sugarfree chewing gums, is generally recognized as safe and effective for use in the reduction of plaque build-up, the prevention of plaque acids and the prevention of dental caries, and should be included in the OTC monograph for antiplaque drug products.

Respectfully submitted,

LEAF, INC.

Diane B. McColl

By: Diane B. McColl
Counsel for Leaf, Inc.

DBM/jh-TS
Enclosure

DENTAL DIALOGUE

Caries Prevention With

XYLITOL

A Symposium
at the
University of Michigan
Ann Arbor

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DENTAL DIALOGUE is a synopsis of the proceedings of a symposium held at the University of Michigan, January 27, 1988, sponsored by a grant from Xyrofin, Ltd. Editorial content reflects the opinions of the speakers whose presentations are summarized here, and not necessarily those of Xyrofin, Ltd. © 1988 Xyrofin, Ltd. All rights reserved. Produced by MEDECommunications™, a division of Medical Economics Company Inc., 680 Kinderkamack Rd, Oradell, NJ 07649.

The symposium "Caries Prevention With Xylitol," which brought together a group of recognized experts in caries research, was initiated with three objectives in mind: to review and evaluate published scientific data on xylitol* in numerous animal studies and human field trials that have been conducted around the world; to examine data relating to the various mechanisms of action that can explain the effects of xylitol on caries; and to explore the kinds of claims and package labeling that would be appropriate for xylitol-containing products.

From these discussions, we concluded *unquestionably* and *unanimously* that xylitol is a noncariogenic substance; in other words, xylitol does not contribute to caries formation.

We also reached a general consensus that scientific evidence supports a cariostatic claim for xylitol-containing gum—that is, such products aid in the prevention of caries or reduce the incidence of new caries.

Clearly, xylitol exhibits more dental benefits than any other sweetener. Appropriately stated and scientifically supported oral health claims for foods containing xylitol can now be made under the proposed regulations for public health messages on food labels and labeling that have been developed by the Center for Food Safety and Applied Nutrition at the Food and Drug Administration (FDA).

Further-reaching claims would have to be based on additional studies that examine the effects of such things as dose and frequency of use, as well as mechanisms of action—especially the impact on *Streptococcus mutans*. Any claim that states or implies that xylitol is an anticariogenic agent would likely be viewed by the FDA as a drug claim.

The following pages provide you with a synopsis of our detailed discussions during the symposium.

Irwin Mandel, DDS
January 27, 1988

*Xylitol is a five-carbon sugar alcohol. Xylitol has the same sweetness as sugar and provides the same number of calories: 4 kilocalories per gram. It is found in small amounts in fruits and vegetables, and is produced in the human body during normal metabolism. Xylitol is produced commercially from plant material such as birch trees. Xylitol is approved in the United States as a direct food additive for special dietary purposes.

"Clearly, xylitol exhibits more dental benefits than any other sweetener."

Dr Mandel

DR MANDEL: Many magazines and newspapers are proclaiming the end of tooth decay. So why hold a symposium on the subject of dental caries prevention with xylitol, if tooth decay is on the verge of extinction? The fact of the matter is that rumors about the death of dental caries have been greatly exaggerated. The cariologist, at least for the moment, is not an endangered species.

A recent national study demonstrated that children between the ages of 5 and 17 are experiencing a substantial reduction in the incidence of caries. But the study noted that at the age of 17 there is still an average of 11 DMFS (decayed, missing, or filled surfaces) per child. Cavities, very definitely, exist—and in numbers that should cause concern.

Furthermore, young people are not the only ones who need to worry about cavities. The number of DMFS is rising among people over the age of 35. And root caries is becoming more of a problem as well, in an aging population whose members are retaining their teeth longer. Put simply, decay does not stop as we get older.

We need to make it clear that, despite impressive reductions—especially in young children—tooth decay as a disease is not obsolete in the United States, and that it certainly is not so in developing countries, where there has been a dramatic increase in the incidence of caries in recent years.

Several years ago the National Caries Program developed a preventive approach to caries. It focused on controlling the cariogenic microflora, modifying diet, and increasing host resistance. It is interesting that, when we read the dental literature, we find that xylitol appears to possess properties that place it in each of those categories.

First, there are data to indicate that xylitol combats the mi-

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"The results [of all these trials] have been similar: dramatic reductions in the incidence of dental caries."

Dr Mäkinen

croflora. Second, it certainly is a dietary modification that doesn't promote dental decay. Third, it may indeed increase host resistance. So I think it is highly appropriate that we discuss xylitol in detail and see how we, as cariologists, can use this information to fight the remnants of dental caries.

Let's begin by discussing the findings of clinical trials of xylitol by Drs Mäkinen and Kandelman.

DR MÄKINEN: Of the roughly 20 animal model caries studies conducted with xylitol in the last two decades, 18 demonstrated a reduction in the incidence of caries and showed some therapeutic effect.

In human trials, about ten studies have been conducted. In the Turku, Finland, studies [a total sucrose substitution study conducted in 1972—Editor], in which I participated, caries reduction with xylitol exceeded 82%. A study carried out in the Soviet Union in the late 1970s showed a reduction in caries incidence of about 73% with a dose of about 30 grams per day of xylitol. In studies sponsored by the World Health Organization [trials in Thailand, French Polynesia, and Hungary, in which children consumed up to 20 grams of xylitol in gums and candies daily—Editor], the researchers reported a protective effect of 37% to 45%. And a recent two-phase study in northern Finland found an overall reduction of 30% to 57% in children and of 59% to 84% among those considered at high risk of caries. [Daily dose of 7 to 10 grams per child—Editor] Despite the fact that all these trials were carried out by independent research teams in different countries, using different study designs and different xylitol products, the results have been similar: dramatic reductions in the incidence of dental caries.

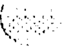
DR KANDELMAN: The objective of our two-year trial in Montreal was to measure the effect of chewing gum containing two levels of xylitol on the incidence and progression of dental caries. The children in the study were participating in an ongoing preventive dental program [in schools] performed by the Department of Community Health of the Montreal General Hospital, which included oral hygiene instruction and fluoride rinsing. Study participants were randomly assigned by school, not by child. It's almost impossible to do a randomization by child and follow them longitudinally for two years.

We found an [average] reduction of 55% in net progression of decay after one year in the two experimental groups of school children who chewed [under supervision] three sticks of 15% and 65% xylitol gum, compared to controls who received none.

Children who chewed the xylitol gums had a significantly smaller DMFS increment after the first year than controls did—[an average of] 1.58 surfaces versus 3.28. The results of the study clearly demonstrate the protective effect of xylitol chewing gum in school prevention programs. I believe chewing gum is an efficient way of using the caries-inhibiting properties of this sugar substitute. Our second-year results, although not finally tabulated, so far indicate a similar reduction in decay. [Second-year data are now available and indicate a 65% reduction in caries in the xylitol gum groups.—Editor]

DR BURT: These studies cannot collectively be called clinical trials, and, indeed, they're correctly referred to as field trials. . . . It is well described in the discussions of all the papers that there are inherent problems. The approach that one cannot strictly randomize children is reasonable, given that kids in a classroom are going to trade their gums. . . . So we see that, in a number of the studies, there are caries imbalances at baseline. . . . On the other hand, all results point in the same direction. . . . In summary, from my perspective, it's fair to say that although it's not hard to find flaws in the studies, it is very hard to argue against the efficacy of xylitol.

Now I draw an analogy with the initial water fluoridation studies of the mid-1940s, where I think we find a similar situa-



tion. There again, they couldn't randomize because obviously a community fluoridates or it doesn't. There's no in-between, so you can't randomize. No two communities are exactly the same, so there are always going to be differences between study and control communities. In summary, I would be satisfied that the material [xylitol] does work. But just as the argument for water fluoridation—whether it reduces caries 50%, 60%, 70%, or any other figure that people choose—I think they're empty arguments. . . . We want to be very, very careful about getting carried away with actual percentage reductions.

DR MANDEL: In xylitol studies of the 1970s and 1980s, a whole series of preventive services were built into routine care of the children. Fluoride mouth rinses, and in some cases fluoride in the drinking water, are examples. So we are now talking about xylitol not as a sole preventive agent but as an agent used in conjunction with available preventive services. This puts xylitol in an exciting category because, in a sense, it's a plus. It makes it more difficult in terms of conducting the study—because the caries rate is lower—but it shows an effect in a population already receiving preventive care.

DR KANDELMAN: Our policies were really to see if we could have an additional benefit in an already integrated preventive program. If the answer was "yes," then we could easily integrate such a preventive measure in an already existing preventive program. That is what I find so exciting.

DR MANDEL: Dr Schachtele, can you give us an overview of how xylitol might be exerting its effect?

DR SCHACHTELE: If you review the literature on xylitol, you will find the sweetener may influence each of the major factors involved in development of decay. It appears to have effects on the utilization of dietary substrates, the microorganisms that cause decay, the anticariogenic factors in saliva produced by humans, and the enamel portion of the tooth. I've summarized these potential mechanisms [see Table].

Possible Cariostatic Mechanisms of Xylitol

- Does not contribute to growth of plaque bacteria
- Reduces oral levels of *Streptococcus mutans*
- Is not metabolized to harmful plaque acids
- Reduces plaque accumulation
- Stimulates flow of protective saliva
- Favorably alters composition of saliva
- Retards demineralization
- Enhances remineralization

DR MANDEL: Let's explore xylitol's effect on the oral microflora in more detail.

DR LOESCHE: Xylitol's effect on cariogenic flora has been studied quite extensively in the United States and abroad. Most dental decay in humans is caused by *Streptococcus mutans*, which is one of the most acidogenic plaque bacteria.

S mutans uses sucrose in diverse ways—but at a price: it doesn't do well when sucrose is not around. That weakness is what xylitol and other polyols exploit.

"Xylitol may influence each of the major factors involved in development of decay."
Dr Schachtele

DR SCHACHTELE: An important feature of xylitol is that it isn't fermented to harmful acids by *S mutans*. Actually, little if any acid is produced when most plaque bacteria are supplied with xylitol. They simply do not metabolize it. Xylitol does not contribute to acid and caries formation, and is clearly noncariogenic.

DR LOESCHE: In contrast to xylitol, ingestion of sucrose induces a drop in the pH of the mouth, creating a suitable environment for the selection of *S mutans*. As Dr Mäkinen noted in his Turku studies, you did not see these nutrient pulses when xylitol was in the diet, and, therefore, there was no selection for *S mutans*.

"We all agree that xylitol is noncariogenic. . . . What we need is an explained mechanism for cariostasis."
Dr Newbrun

DR BOWEN: Let's say we accept Dr Loesche's very plausible hypothesis that the decrease in *S mutans* is simply loss of fermentable sucrose [substrates]. You then must ask if this is simply a generic effect attributable to *any* nonspecific, nonfermentable sweetener.

DR LOESCHE: . . . There may be some direct effect of xylitol on *S mutans*.

DR SCHACHTELE: Actually, the effect of xylitol appears to be much greater in comparison to that of other sweeteners. One theory is that xylitol is phosphorylated by *S mutans* while it is being transported into the cell, where it accumulates. What may happen is that xylitol-5-phosphate disrupts normal cell metabolism. For example, it could alter cell wall formation. *S mutans* survives in the mouth because it can tolerate varying environmental conditions. Xylitol may make this bacterium more sensitive to its environment and less likely to become dominant in plaque at caries-prone sites.

DR MANDEL: When it comes to xylitol's impact on *S mutans*, we have quantitative data that show a reduction in levels of the microorganism. But on the question of *why* that comes about, we must speculate.

DR NEWBRUN: We all agree that xylitol is noncariogenic and that it is not fermented. What we need is an explained mechanism for cariostasis.

DR TANZER: What Dr Loesche is saying is that it is a selective enrichment against *S mutans*, because by not providing *S mutans* its ecological advantage, you develop with repeated use [of xylitol] a multiplier effect such that, progressively, *S mutans* submerges in the ecology. That's a very good ecological argument.

DR BOWEN: You can supplement that argument by pointing out that each time you take xylitol, saliva pH increases by one or more units.

DR MANDEL: So what you're talking about is creating an environment that ecologically selects against *S mutans*.

DR KANDELMAN: We have observed reduced plaque formation in a few studies. Can we have some explanation of this plaque reduction from the microbiological point of view?

DR MANDEL: This is what we are trying to come to grips with. We have a clinical observation of reduced levels of *S mutans*, and we have some clinical observations of a reduced rate of plaque accumulation. What we are striving for is a specific mechanism by which this happens. We are suggesting that an alteration in the environment, a deprivation, seems to be generated by xylitol. This in turn ecologically alters the proportion of *S mutans* in the oral flora.

DR BÄR: Clearly, we would be more comfortable if we had mechanistic explanations for xylitol's action. On the other hand, I think the clinical end point is decisive. If something is proposed as a remedy for a headache, the end point—the fact that it relieves pain—is important. *How* it does so is less important. If we accept that clinical studies show a decrease in the incidence of caries, then we have shown xylitol is effective.

DR MANDEL: Let's move to another area where xylitol may function: the demineralization and remineralization equilibrium of tooth enamel. Dr Schachtele, what did you find in your review of the literature?

DR SCHACHTELE: With regard to remineralization, interesting studies have been performed in rodents. Xylitol appears to enhance remineralization of lesions where only minimal damage has occurred. In addition, however, there have been some studies in which enamel chips were placed in human subjects and enhanced remineralization was observed.

The models for studying remineralization are getting better; eventually we will be able to determine the extent of xylitol's effects beyond simple stimulation of saliva flow.

DR LOESCHE: Before sucrose is introduced into the mouth, remineralization and demineralization are in equilibrium. When you get a nutrient pulse of sucrose, the pH drops below the critical level. The tooth then acts essentially as a buffer, and calcium comes out of the tooth. On the other hand, when you introduce xylitol into the mouth, the pH does not fall. Consequently, you do not see demineralization occur.

DR MÄKINEN: One possible mechanism is that polyols, including xylitol, stabilize calcium phosphate solutions. The question is whether this will have any bearing on the clinical situation. In our laboratory, the introduction of polyols into a solution of saliva, microorganisms, and calcium does not induce a precipitate.

DR KOULOURIDES: It is possible that we are dealing with weak calcium-xylitol complexes that, under pH variations, may release calcium. In other words, you have more calcium in the fluid, and when the pH falls, this complex dissociates and deposits ionic calcium.

We have observed that xylitol added to mineralizing solutions prevents calcium deposition on enamel surfaces, in contrast to the action of sucrose. Another possibility is that by keeping the enamel surface clean and the pathways open, xylitol may enhance remineralization. I say that because we have other evidence that when remineralization occurs very quickly, you block the pathways and there is no influx of ions into the lesion. These two possible events may enhance remineralization.

DR STAMM: Some evidence from clinical trials discussed suggests a remineralization effect, but we still need more mechanistic, molecular biology–oriented studies to answer questions about calcium phosphate precipitation, remineralization, and demineralization.

DR MANDEL: That's a good point—and a good summary for this part of the discussion. In different protocols of clinical studies of xylitol, different concentrations and frequencies were used. They yielded positive, although diverse, results.

Let's move our discussion to the issue of dosage and frequency. Does increased frequency mean an increase in the total amount of xylitol available in the mouth? We have established that 15% xylitol three times a day has a significant effect. Would you speculate that the more often xylitol is consumed, the more it would make a difference?

DR KANDELMAN: In the Montreal study, we did not notice a relationship between dose and effect in the two groups given xylitol. But I think that if you increase the frequency—say to six times a day—you would get a better effect.

DR MÄKINEN: Frequency becomes important. You cannot explain the results of the Montreal study by dosage. Our studies in Finland indicate that a frequency of less than 1.4 sticks of gum a day produces a questionable effect or no effect at all. The number of intakes is crucial and decisive.

DR STAMM: I would prefer to say that it is possible that it is a question of both frequency as well as quantity.

"If we accept that clinical studies show a decrease in the incidence of caries, then we have shown xylitol is effective."
Dr Bär

DR MANDEL: Do the animal studies help us with regard to dosage and frequency?

DR BOWEN: Any animal study of sugar alcohols is fraught with problems. They induce major cathartic effects, which make interpretation of data difficult. Some researchers have attempted to circumvent the stumbling blocks of these studies by increasing the dose of the test substance gradually. But if you do that, the animals grow older and their susceptibility to caries diminishes, particularly on the smooth surfaces.

So animal studies are not easy to conduct, but they can be done if you're patient, use older animals, and are prepared to accept lower-than-normal baseline levels of caries.

DR SCHACHTELE: I must follow

DENTAL DIALOGUE

"Although it's not hard to find flaws in the studies, it is very hard to argue against the efficacy of xylitol."

Dr Burt

that comment with a plea for studies with human volunteers. There are excellent sampling systems and procedures that can now be used to do thorough investigations on the effect of xylitol dose and frequency on *S mutans* levels.

DR MANDEL: One of the most important issues surrounding xylitol is that of the specific claims for xylitol. How far can we go when we talk about its actions, especially when xylitol is used in various commercial products? We have invited Diane McColl, an associate in the Washington, DC, office of the law firm of Morgan, Lewis & Bockius, who have expertise in food and drug law, to answer these questions and help us lay down some guidelines for claims about xylitol.

MS McCOLL: Two federal agencies would regulate any health-related promotional claims concerning xylitol: the Federal Trade Commission (FTC) and the Food and Drug Administration (FDA). The FDA exercises primary responsibility over food labeling, and the FTC assumes primary responsibility for advertising.

The FTC encourages appropriately substantiated health claims in the belief that they serve a valuable function in the marketplace by giving consumers useful information about health benefits associated with food products. FTC officials are concerned that the public will be denied those benefits if unnecessarily high obstacles prevent dissemination of useful product information.

DR MANDEL: Several years ago, the Kellogg Company made a claim that a diet high in fiber could prevent cancer. Did that establish a precedent for health claims advertising?

MS McCOLL: The advertisement was not challenged by the FTC, so some caution is required in using the Kellogg claim as an affirmative FTC precedent for similar health claims.

Nonetheless, the Kellogg advertisement provides useful insight for an advertiser seeking to reduce the risk of an FTC challenge. The Kellogg anticancer claim was presented as a general health message concerning good nutrition and health. The claim was based on a National Cancer Institute study, and important qualifications to that study were included in the text of the advertisement.

The general rule is that the advertiser must possess and rely upon competent scientific evidence that substantiates the claim at the time the claim is made.

DR MANDEL: What about the FDA's role?

MS McCOLL: In the past, the FDA has taken the position that health- or disease-related claims for a food product bring the product under the statutory definition of a drug product. Any such products would essentially be illegal for at least two reasons: First, the absence of adequate and well-controlled studies would make the food an unapproved new drug; second, the food would also probably be misbranded for failure to include adequate directions for intended use.

On August 4, 1987, the FDA proposed regulations that would permit health-related claims and information on food labeling. The agency now believes that "health-related messages, when appropriately formulated for use in food labels and consistent with existing law and regulations, may provide valuable information to health-conscious consumers." [See 52 *Fed Reg* 28843, 28845 (August 4, 1987) (FDA's proposed regulations for public health messages on food labels and labeling).—Editor]

DR MANDEL: How does this affect what advertisers can say about xylitol?

MS McCOLL: The proposed food health claims regulations do not affect claims relating to the noncariogenicity of xylitol. Noncariogenic claims, such as "does not promote tooth decay," can continue to be included in labeling of food products that contain xylitol, without the risk of FDA challenge [as long as the food as a whole is noncariogenic.—Edi-

tor] Nor do the proposed health claims regulations alter the likelihood that an unqualified therapeutic claim, such as "xylitol prevents cavities," would be viewed by FDA as a drug claim.

However, the health claims proposal does permit health-related claims, such as "clinical studies show xylitol gum helps reduce the incidence of new cavities" in food labeling, provided such claims are properly formulated and scientifically supported.

On the other hand, a stated or implied claim that xylitol is adequate or effective in the prevention, cure, mitigation, or treatment of caries is likely to be viewed by the FDA as a drug claim. In summary, it is fair to conclude that, under current FDA regulatory policy, scientifically well-supported oral health care claims for xylitol-containing foods that do not promote xylitol as a therapeutic agent sufficient in and of itself to cure or prevent a disease condition are permissible.

DR BURT: As I understand it, the FDA's [Center for Drugs] has disallowed some foreign studies when evaluating evidence of claims. Because many studies of xylitol are foreign, will the agency's [Center for Foods] accept them?

MS McCOLL: Scientific evidence supporting claims relating to drug products are reviewed by the FDA's Center for Drug Evaluation and Research, whereas scientific support for food health claims will be monitored by the Agency's Center for Food Safety and Applied Nutrition. The legal standards and scientific requirements applicable to drug products are more stringent than those applicable to food products. Thus, a determination by the Center for Drugs that particular clinical studies are insufficient to support a therapeutic drug claim does not necessarily mean that the data are also inadequate to support a food health message under the Center for Food's new health claims proposal. The type and quality of required supporting evidence is likely to differ.

Whether a xylitol-containing product is viewed as a drug or a food by regulatory agencies is an issue that must be dealt with by the product's manufacturer.

DR MANDEL: Let's move on to the differences between the terms. We need to define the terms "noncariogenic," "cariostatic," and "anticariogenic" as they might be used in product claims for xylitol.

First, xylitol is unquestionably noncariogenic. It is not fermented by oral bacteria. Second, is it accepted that cariostasis means a slowing down or an arrest of caries progression, which is reflected in a reduced increment of caries in controlled human trials? Third, should anticariogenic be reserved for those compounds or products that reverse the caries process?

DR BURT: You used the word "progression." To me that implies a lesion that has already formed, even subclinically. It suggests that *something* has happened, as opposed to nothing beginning in the first place. Preventing progression implies reversal of a lesion through its stages.

DR LOESCHE: "Slowing down" implies that caries will eventually develop—and that's not the case. It is not a good descriptive phrase. If you want to say "reduces caries increment," however, that stands by itself.

"Under current FDA regulatory policy . . . oral health care claims for xylitol-containing foods . . . are permissible."
Ms McColl

DR BOWEN: Cariostatic means "stops caries"; if an agent does that, it can be distinguished quite clearly from a noncariogenic substance.

DR MANDEL: What I see happening in the literature—and in this discussion—is that we have used cariostatic and anticariogenic to mean almost the same. Is it worthwhile to make the distinction? Should we just let conventional usage continue?

MS McCOLL: Clinically, it may not be important to distinguish the two terms. From a legal perspective, however, it is important to clearly define the difference between an "anticariogenic" effect and a

"cariostatic" effect. For example, if "cariostatic" defines a reduction in the incidence of new caries, and "anticariogenic" describes a curative remineralization effect on existing lesions, then the "cariostatic" effect of xylitol could be characterized as a preventive effect appropriate for inclusion in a food health claim. However, the "anticariogenic" remineralization properties of xylitol would likely be considered a therapeutic effect, which would constitute a drug claim and subject the product to "new drug" requirements, if included in food labeling.

DR MANDEL: It is becoming clear that nobody is comfortable yet with the word "anticariogenic." We've already begun to differentiate noncariogenic as "passive, does not promote." We need to sharpen what we mean by cariostasis.

DR KOULOURIDES: One complication is that we have a natural cariostasis that can go to work when you remove the challenge. In such a scenario, you have something that is cariogenic, you remove the challenge, and natural remineralization by saliva occurs—arresting the caries and inducing cariostasis.

There may be another definition that implies stimulation of remineralization. Fluoride is an example. You stop the lesion and you have also a residual effect. In other words, you end up with a surface that is more resistant than it was to begin with.

DR STAMM: From a labeling perspective, wouldn't you agree that we are likely to see language such as "helps prevent formation of caries" or "helps fight cavities"? I think that such specific wordings will be submitted to regulatory agencies.

DR MANDEL: Cariostatic defined as "aids in the prevention of caries" sounds like a comfortable claim. I think that's an excellent definition of cariostatic and is one that appropriately characterizes the findings in the xylitol chewing gum studies. As far as an anticariogenic claim is concerned, it would appear that we need more clinical studies and clearer definitions of the progressive steps. We need to demonstrate reversals of lesions with defined criteria. I do not see anything on the horizon that will allow us to classify xylitol as anticariogenic without continuing the kinds of studies that Dr Kandelman is performing.

DR BÄR: When it comes to differentiating between a gum-chewing effect and the xylitol effect, would this group agree that xylitol-containing gum has a cariostatic effect? Should we limit the claim of cariostatic action to a xylitol-based gum?

DR BOWEN: I would feel a lot more comfortable [making such a claim] for the product [gum containing xylitol], as opposed to xylitol [itself].

DR BÄR: A [specific product] claim can be made only for the kind of product that has been subjected to clinical studies.

DR MANDEL: Ms McColl, if the scientific community generally concludes that the available clinical data support a generic claim that "xylitol-containing gums reduce the incidence of new caries," would manufacturers of all xylitol gum products be able to make this claim?

MS McCOLL: Yes. However, each manufacturer must have or develop data demonstrating that, under intended conditions and frequency of use, its particular xylitol gum formulation would produce results comparable to those demonstrated for xylitol gums in the clinical studies published in scientific literature.

DR MANDEL: What other xylitol-containing products can we anticipate?

DR KANDELMAN: One possibility would be to combine fluoride and xylitol in chewing gum. Such a combination might promote remineralization *and* effectively prevent decay.

DR BOWEN: I am wary about putting fluoride in chewing gum. There is a growing concern in the community about the amount of fluoride in society.

DR MÄKINEN: Another potential area for use is in elderly subjects. Xylitol is suitable for elderly subjects because their mucous membranes do not tolerate hard products. Soft products would stimulate the flow of saliva, which is very important in the elderly.

DR MANDEL: We have several levels of studies that can be anticipated. We have, first, additional laboratory-related or animal model studies to define more precisely aspects of xylitol's mechanism of action and dose and frequency effects; second, short-term studies of xylitol's impact on *S mutans*; and third, clinical studies, for which we recommend an additional group that uses a non-xylitol-sweetened or -flavored gum to give us some measure of salivary effects.

With xylitol-containing chew-

ing gum, we are more comfortable with a cariostatic claim at this point. We have also identified the kinds of studies necessary, and the difficulties they pose, if the manufacturer wants to move towards an anticariogenic claim.

DR BOWEN: I think there's an important point to add about getting to the stage where we can make a claim for xylitol in generic terms, as we do with fluoride. Personally, one of the biggest tragedies that has befallen dental research is the length of time it took to get to the stage to make a claim with fluoride because people simply took the clinical end result; namely, a reduction in holes as the only thing they were interested in. Nobody ever measured fluoride levels in the mouth or what the optimum therapeutic concentrations were. That's why it's so critical to do all the biochemical and microbiological and mechanistic studies that are possible with xylitol.

DR MANDEL: In conclusion, even though there are differences in views and different degrees of enthusiasm, I think there is basic agreement that xylitol is a worthwhile agent that people are excited about again. Once again the US research community should be investigating all these properties and all the potential of xylitol.

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Summary of Key Xylitol Studies

Scheinen A, Bánóczy J, Szöke J, et al: Collaborative WHO xylitol field studies in Hungary. I. Three-year caries activity in institutionalized children. *Acta Odontol Scand* 1985;43:327-347.

Hungarian school children, aged 6-11, divided into three groups: control (fluoridated dentifrice/normal diet); systemic fluoride (0.75 mg/day in milk or from water having natural 1.2-ppm fluoride level, plus dentifrice/normal diet); xylitol (fluoridated dentifrice, 14-20 g xylitol/day in between-meal candies and gums plus normal diet). The three year Δ DMFS results were 7.7 (control), 6.5 (systemic fluoride), and 4.2 (xylitol).

Kandelman D, Bar A, Hefti A: Collaborative WHO xylitol field study in French Polynesia. I. Baseline prevalence and 32-month caries increment. *Caries Res* 1988;22(1):55-62.

French Polynesian school children aged 6-12 divided into two groups: control (fluoridated dentifrice normal diet) and xylitol (fluoridated dentifrice normal diet plus no more than 20 g xylitol/day in candies and gums). The 32-month Δ DMFS results were 7.1 (control) and 4.5 (xylitol).

Kandelman D, Gagnon G: Clinical results after 12 months from a study of the incidence and progression of dental caries in relation to consumption of chewing-gum containing xylitol in school preventive programs. *J Dent Res* 1987;66(8):1407-1411.

Montreal school children, aged 8 and 9, divided into three groups: control (oral hygiene instruction, periodic brushing control, weekly 0.2% NaF mouthrinse, screening, and restorative treatment); 15% xylitol chewing-gum (0.75 g xylitol/day, 3 sticks of gum chewed 5 minutes each throughout school day) plus control oral hygiene activity; 65% xylitol chewing-gum (3.4 g xylitol/day, 3 sticks of gum as above) plus control oral hygiene activity. The 12-month Δ DMFS results were 3.35 (control), 1.76 (15% xylitol), and 1.35 (65% xylitol). Each group followed their normal daily diet.

Isokangas P, Alanen P, Tiekso J, Makinen KK: Xylitol chewing gum in caries prevention. A field study in children at caries-active ages. *J Am Dent Assoc* 1988, to be published.

The two-year study of Finnish school children aged 11 and 12 involved four groups: a control receiving no chewing-gum and three xylitol gum groups in which 5, 7, or 10 g xylitol was consumed daily in the form of 1.5, 2, or 3 pieces of gum, respectively. Both control and xylitol groups followed their normal diets and same caries prevention program, which included fluoridated dentifrice, fluoride tablets, topical fluoride, monthly fluoride rinse (0.2% NaF), and first molar fissure sealants prior to the trial. The measured Δ DMFS results were 2.4 (control), 2.4 (1.5 gums), 1.6 (2 gums), and 1.0 (3 gums).

Isokangas P, Alanen P, Tiekso J, Makinen KK: Xylitol chewing gum in caries prevention. A field study in children at caries-active ages. *J Am Dent Assoc* 1988, to be published.

A three-year study parallel to the Finnish two-year trial in which children with high caries susceptibility were divided into control, 1.5-, 2-, or 3-gum groups. The high-risk groups were formed based on their total caries experience at baseline examination (11-year-olds with DMFT $\geq$5; 12-year-olds with DMFT $\geq$7). Each group followed their normal diet and the basic caries prevention program noted above. The measured Δ DMFS results were 7.0 (control), 7.5 (1.5 gums), 3.5 (2 gums), and 1.5 (3 gums).

XYROFIN

A CULTOR COMPANY

May 15, 1990

Dockets Management Branch (HFA-305)
Food and Drug Administration
Room 4-62
5600 Fishers Lane
Rockville, MD 20857

Dear Sir or Madam:

American Xyrofin Inc. ("Xyrofin") submits the following comments pursuant to a notice of a reproposed rule on health messages and label statements published in the Federal Register, 55 Fed. Reg. 5176 (Feb. 13, 1990). In addition to the comments set forth below, Xyrofin also incorporates by reference, and has attached, its earlier comments on the appropriate regulatory framework for health messages submitted in response to the Food and Drug Administration (FDA) Advanced Notice of Proposed Rulemaking on Health Messages published in 1989, 54 Fed. Reg. 32610 (Aug. 8, 1989), and presented orally by an American Xyrofin representative at FDA's public hearing on health messages in Seattle.

Xyrofin is the U.S. distributor of Xylitol, a natural nutritive sweetener which provides oral health benefits when used in food applications. Xylitol, a naturally occurring five-carbon sugar alcohol, is a constituent of many fruits and vegetables. The human body produces five to fifteen grams of Xylitol per day during normal metabolism. Xylitol was first

85N-0061 C 682

AMERICAN XYROFIN Inc., 1400 North Meacham Road, Schaumburg, Illinois 60173-4808, USA. Telephone (708) 843 3200. Telefax (708) 843 3368. Telex 6871134

produced commercially in the early 1970's. Xylitol is approved by FDA as a direct food additive for use in foods for special dietary uses. 21 C.F.R. §172.395. Xylitol has several recognized health advantages compared to fermentable sweeteners including, most importantly, its ability to resist fermentation by oral bacteria, thus making it non-cariogenic and cariostatic. There is also significant evidence that consumption of Xylitol-containing foods causes a decrease in the formation of dental plaque. Based on the extensive scientific data, which will be described below, Xyrofin believes there is a substantial scientific basis to support an appropriately-qualified health claim concerning the effects of consumption of Xylitol-containing foods and, therefore, is interested in the framework established for the use of such claims.

Xyrofin encourages and supports FDA's efforts to establish a regulatory framework to permit manufacturers to use on food labeling scientifically-supported health messages that are truthful and non-misleading. Because Xyrofin believes that U.S. consumers will benefit substantially through improved health from the use of health messages in food labeling,[1/]

1/ See FTC Bureau of Economics, Health Claims in Advertising and Labeling: A Study of the Cereal Market (August 1989). The cereal study found that consumer dietary patterns can (continued...)

A CULTOR COMPANY

Xyrofin requests that FDA designate oral health as an appropriate additional topic for health claims should the Agency determine to proceed in the manner outlined in the reproposal. In addition, because oral health is a recognized, significant public health problem, Xyrofin believes that FDA should adopt a regulatory framework for health claims which permits the use of appropriately substantiated health claims in the interim period pending any development of final claims through the Agency's proposed committee review process.

I. ORAL HEALTH SHOULD BE INCLUDED AS AN APPROPRIATE TOPIC FOR DEVELOPMENT OF HEALTH CLAIMS IN FOOD LABELING

FDA has indicated in its reproposal that, based on the Surgeon General's Report, it has tentatively identified six topic areas as appropriate for its initial consideration for health claims. 55 Fed. Reg. at 5184. As recognized in the Surgeon General's Report, and several other recent public health documents, however, diet and oral health is also a significant public health concern and an appropriate topic for health claims. Consequently, use of the sugar alcohol Xylitol in confectionery and other food products to assist in the

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1/ (...continued)
be substantially and positively modified through the use of health messages in food labeling.

AMERICAN XYROFIN Inc., 1400 North Meacham Road, Schaumburg, Illinois 60173-4808, USA. Telephone (708) 843 3200. Telefax (708) 843 3368. Telex 6871134

reduction of dental caries should be included as an appropriate topic.

The exclusion of diet and dental health as an area for initial review is inconsistent with FDA's statement in the reproposal that it chose areas that "relate to problems of major health significance" and that "have been subject of sufficient scientific study to establish a scientific base for review by FDA." Id. at 5184. It is also inconsistent with the identification of dental health as a major health problem by every major public health policy review in the last five years. For example, the Surgeon General's Report states:

> Dental caries and periodontal disease are important and widespread public health problems in the United States. They are rarely life threatening but can cause substantial expense, pain, restriction of activity and work loss (Corbin, Kleinman, and Lane, 1985). Although dental caries among children, as well as some forms of adult periodontal disease, appear to be declining, the overall prevalence of these conditions imposes a substantial burden on Americans. Of the 13 leading health problems in the United States, dental disorders rank second in direct cost (Carter Center Health Policy Task Force 1984). Dental care costs $23.3 billion in 1985 (U.S. Department of Commerce, 1986).[2/]

2/ Surgeon General's Report on Nutrition and Health 347 (1988).

The inclusion of dental health as one of the priority areas of the Public Health Service's 1990 Health Objectives for the Nation and the Year 2000 Health Objectives, to be published in July 1990, as the second "Surgeon General's Report on Disease Prevention and Health Promotion," indicates the widespread recognition of the critical role that dental health plays in the comprehensive pursuit of disease prevention and health promotion. The 1990 Health Objectives emphasize that consumers do not have adequate information about the role of dietary factors in dental disease prevention.[3/] In addition, the report discusses the lack of sugar substitutes as an impediment to achieving improved dental health.[4/] Id. Similarly, the 1986 Report of FDA's Sugar Task Force concluded that "present evidence supports the contention that current average 90th percentile consumption levels of sugar contribute to caries incidence."[5/] The report further observes that "the consumption of sucrose and fermentable carbohydrates

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3/ Public Health Service, The 1990 Health Objectives for the Nation: A Mid-Course Review 147, 152 (Nov. 1986).

4/ Id.

5/ Glinsmann, M., Irausquin, H., and Clark, Y., Evaluation of Health Aspects of Sugars Contained in Carbohydrate Sweeteners J. of Nutrition: 116 (115) (Nov. 1986 Supp.).

facilitates the development of plaque, dental caries, and periodontal disease."[6/]

The FDA itself, in the 1987 proposed rule on health claims, acknowledged that tooth decay is one of the health problems that is linked with dietary practices.[7/] The Agency noted that "consumers are taking a greater interest in healthful dietary practices as 'evidence keeps mounting that certain food factors in current dietary habits may be linked with health problems as diverse as heart disease, tooth decay, obesity, and some types of cancer' ..."[8/] Thus, it is important for consumers that FDA quickly establish the appropriateness of the use of health messages relating to improved oral health from consumption of certain Xylitol-containing foods as part of the total dietary pattern.

6/ Id.

7/ See 52 Fed. Reg. 28443, 28444 (Aug. 4, 1987).

8/ Id. at 28444.

II. THERE IS A SCIENTIFIC CONSENSUS TO SUPPORT A HEALTH MESSAGE CONCERNING THE POSITIVE RELATIONSHIP BETWEEN CONSUMPTION OF CERTAIN XYLITOL-CONTAINING FOODS AND IMPROVED ORAL HEALTH

Under FDA's criteria for health claims in the reproposal, there is a scientific consensus in support of the use of a health message relating to the use of Xylitol for its non-cariogenic and cariostatic properties. Xylitol has been demonstrated through well-designed publicly-available studies to combat the cariogenic effects of fermentable sweeteners when included even as a small part of the daily diet. Certain food products containing Xylitol can play an essential role in fulfilling the desire for sweets while, at the same time, providing a significant oral health benefit. The use of such foods in reducing the incidence of dental caries is particularly important in view of the recent safety issues raised in connection with fluoridated water treatments.[9/] Therefore, health claims relating to the proper role of sugar alcohols such as Xylitol in the diet should be permitted in

9/ See 55 Fed. Reg. 6836, 6837 (Feb. 27, 1990) (National Toxicology Program announcement of peer review of cancer bioassay (NTP Technical Report No. 393) which produced positive results (osteocarsomas) in rats exposed to sodium fluoride).

order to assist Americans to moderate the consumption of fermentable sweeteners and, thus, to improve oral health.[10/]

The 1988 University of Michigan Symposium "Caries Prevention With Xylitol" established a scientific consensus that sugar-free chewing gum containing Xylitol provides a cariostatic benefit, aiding in the prevention of caries and reducing the incidence of new caries. This consensus is supported by numerous in vitro and in vivo studies involving Xylitol which were conducted in Europe and which we describe below. In addition to sugar-free chewing gum, other Xylitol sweetened sugar-free confections such as tablets, chocolate, and hard and soft candies have been associated with reductions in the incidence of new caries.

In addition to the Michigan Symposium, the report, "Sweeteners in Foods, Nutritional Quality - Toxicological Risk" resulting from a dental seminar held in Norway in November, 1988, presents another consensus view of the significance of sweetener alternatives, particularly Xylitol, in combatting

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10/ This scientific consensus, and the clear support it provides for making health claims, is summarized in the attached recent address by Makinen, K.K., "Future Scientific and Regulatory Issues Relating to Health Claims: Dietary Prevention of Dental Caries by Xylitol," presented at the Tufts University Symposium on Health Messages, Boston, Mass. (March 29, 1990).

dental caries. In particular, there was a consensus among the Nordic medical, dental, and regulatory groups that polyols, especially Xylitol, impair the growth of caries-inducing bacteria on teeth. The report therefore recommended the use of alternative sweeteners, and in particular Xylitol, in chewing gum, boiled sweets and cough lozenges. There is, thus, a consensus among the U.S. and international dental community that there is a need for alternative sweeteners and that Xylitol can assist substantially in the reduction of caries.11/

The consensus relating to the non-cariogenic and cariostatic effects of Xylitol is based on a multitude of international studies. The first significant study relating to the caries-related effects of Xylitol was the Turku Sugar Study,12/ which involved the total substitution of dietary sucrose with Xylitol or fructose for a period of two years. The development of new caries was monitored at regular intervals by counting the increase in the number of decayed,

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11/ See also Panel on Dietary Sugars, Committee on Medical Aspects of Food Policy, Department of Health, United Kingdom, Dietary Sugars and Human Disease 19 (1989) (comprehensive review of dietary sugar and human disease concluded that substitution of sugars by alternative sweeteners could substantially reduce caries development).

12/ Scheinin, A., Makinen K.K. and Ylitalo K., Turku Sugar Studies V. Final Report on the Effect of Sucrose, Fructose and Xylitol Diets on the Caries Incidence in Man Acta Odont. Scand., 33., suppl. 70:67 (1975).

missing, or filled tooth surfaces (DMFS). The results indicated a virtual absence of new caries in the Xylitol group. Subsequent studies with Xylitol have evaluated the efficacy of the partial substitution of daily fermentable sugar intake with Xylitol. The first of the partial substitution studies was the Turku Chewing Gum Study.13/ That study involved the daily consumption of 6.25 grams of Xylitol. The results indicated a -1.0 DMFS during the 12-month study period. The negative DMFS suggested that incipient lesions (D1 caries) were being remineralized during the study.

Stimulated by these promising results, the World Health Organization commissioned a number of long-term field trials as part of its oral health program. The aim of these trials was to test the effectiveness and acceptability of Xylitol in field conditions in communities having different disease levels and different nutritional, social and economic environments. The World Health Organization studies were conducted in French Polynesia and Hungary.

13/ Scheinin, A. Makinen K.K., Tammisolo E., and Rekola, M., Turku Sugar Studies: XVII, Incidence of Dental Caries in Relation to One-Year Consumption of Xylitol Chewing Gum Acta Odont. Scand., 33., Supp. 70:307 (1975).

The French Polynesian study[14/] involved school children, ages 6-12, divided into two groups. The control group children received and used a fluoridated dentifrice and followed their normal diet. The Xylitol group children, likewise, received and used a fluoridated dentifrice, followed their normal diet and, in addition, consumed 20 grams of Xylitol per day. Xylitol intake was in the form of candies and chewing gum. The 32-month DMFS results were 7.1 (control) and 4.5 (Xylitol).

The Hungarian study[15/] involved school children, ages 6-11, divided into 3 groups. The control group received a fluoridated dentifrice and instruction in its use and followed a normal diet. A second group received a fluoridated dentifrice and instruction in its use, followed a normal diet and, in addition, received systematic fluoride (0.75 milligrams per day in milk or from water having a natural 1.2 ppm fluoride level). The Xylitol group received a fluoridated dentifrice and instruction in its use, followed a normal diet and, in addition, consumed 14-20 grams of Xylitol per day in

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14/ Kandelman D., Bar A., and Hefti A., Collaborative WHO Xylitol Field Study in French Polynesia I Caries Res. 22:1 (1988).

15/ Scheinin A., Banoczy J., Szoke J., Esztari I., Pienihallinen K., Scheinin U., Tiekso J., Zimmermann P., and Hadas E., Collaborative WHO Xylitol Field Studies in Hungary I. Three-year Caries Activity in Institutionalized Children Acta Odont. Scand. 43:327 (1985).

between-meal candies and chewing gum. The Xylitol intake in this study was weighted most strongly in the form of candies. Approximately 28% of the intake was in the form of compressed mints and wafers. Approximately 40-60% of the Xylitol intake was in the form of chocolate or hard candy. The 3-year study indicated DMFS results of 7.7 (control), 6.5 (systematic fluoride), and 4.2 (Xylitol).

Additional studies involving sugar-free chewing gum containing Xylitol have supported the above studies. The Montreal Chewing Gum[16/] involved school children ages 8 and 9 divided into 3 groups. The control group received oral hygiene instruction, periodic brushing control, a weekly 0.2 sodium fluoride mouth rinse, screening and restorative treatment. A second group received the same oral hygiene activity as the control group and, in addition, received 0.75 grams of Xylitol per day in the form of 3 sticks of sugar-free chewing gum which contained 15% Xylitol. Each piece of gum was chewed for 5 minutes throughout the school day. A third group received the same oral hygiene activity as the control group and, in addition, consumed 3.4 grams of Xylitol per day in the form of

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16/ Kandelman D., and Gagnon G., Clinical Results After 12 Months From a Study of the Incidence and Progression of Dental Caries in Relation to Consumption of Chewing Gum Containing Xylitol in School Preventative Programs J. Dent. Res. 66:1047 (1987).

3 sticks of sugar-free chewing gum sweetened with 65% Xylitol. The 12-month DMFS results were 3.35 (control), 1.76 (15% Xylitol), and 1.35 (65% Xylitol). Each group followed their normal daily diet.

A chewing gum study which took place in Ylivieska, Finland[17/] has yielded even more significant results regarding the efficacy of Xylitol with respect to the inhibition of new caries. The study involved Finnish school children ages 11 and 12. The total study involved parallel 2- and 3-year observations and a post-study follow up with respect to caries activity. The 2-year study was divided into four groups: a control receiving no chewing gum, and three Xylitol gum groups in which 5, 7, and 10 grams Xylitol was consumed daily in the form of 1.5, 2, or 3 pieces of sugar-free gum respectively. Both control and Xylitol groups followed their normal diets and the same caries prevention program, which included fluoridated dentifrice, fluoride tablets, topical fluoride, monthly fluoride rinse (0.2% sodium fluoride), and first molar fissure sealants prior to the trial. The measured DMFS results were 2.4 (control), 2.4 (1.5 gums), 1.6 (2 gums) and 1.0 (3 gums).

17/ Isokangas P., Alanen P., Tieskso J., and Makinen K., Xylitol Chewing Gum in Caries Prevention presented at the 65th Annual Meeting, Int'l Assoc. of Dental Research, Chicago, Ill. (1987).

The parallel 3-year Ylivieska study involved children with high caries susceptibility. The children were divided into respective control, 1.5, 2 or 3 pieces of gum per day groups. The high-risk groups were formed based upon their total caries experience at baseline examination (11 year-olds with DMFS greater than or equal to 5 and 12 year-olds with DMFS greater than or equal to 7). Each group followed their normal diet and the basic caries prevention program noted for the 2-year study. The measured DMFS results were 7.0 (control), 7.5 (1.5 gums), 3.5 (2 gums), and 1.5 (3 gums).

Each of the parallel Ylivieska studies indicated a significant improvement in oral health for the groups that chewed two or three sticks of gum per day. The measured reduction in caries incidence for the two gum groups were 33% and 50% respectively for the two and three-year studies. The measured improvement in reduced caries incidence for the three gum groups were 58% and 79% respectively for the two and three-year study period. The results of the respective studies further suggest that the frequency of Xylitol exposure can play a vital role in the protective performance of Xylitol.

The subjects who participated in the two or three-year Ylivieska studies (completed in 1984 and 1985 respectively) were re-examined in 1987 to determine possible long-term pre-

ventative effects.[18/] Caries reduction was found two or three years after the discontinuation of the use of Xylitol. The reduction in caries increment in the post-use years was 60% for the two-year users, suggesting that more pronounced caries reduction was associated with the more regular use of Xylitol. In teeth erupting during the first year of the use of Xylitol gum, the long-term preventative effect was greater than in other teeth. Several explanations are suggested: lasting effect of the microbiological changes in the mouth, bacterial colonization on newly erupted teeth by organisms other than Streptococcus mutans, and/or thorough maturation of the teeth under favorable physio-chemical circumstances. The results suggest that the value of Xylitol may be highest during periods of high dental activity, e.g., eruption of new teeth.

A recent Xylitol study[19/] evaluated the effect of three Xylitol-containing chewing gums on plaque formation and plaque response. The gums were either sweetened solely with Xylitol or sweetened with Xylitol and sorbitol in the ratio 7:2, or

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18/ Isokangas P., Tiekso J., and Makinen K., Long Term Effect of Xylitol Chewing Gum on Dental Caries Community Dent. Oral Epidemiol. 17:200 (1989).

19/ Soderling E., Makinen K., Chen C., Pape H., Loesche W., and Makinen P., Effect of Sorbitol, Xylitol and Xylitol/Sorbitol Chewing Gums on Dental Plaque presented at 66th Annual Meeting, Int'l Assoc. of Dent. Research., Montreal, Canada, (1988).

sweetened solely with sorbitol. The Xylitol and Xylitol-sorbitol chewing gum groups experienced a decrease in dental plaque of 25-30% during the 2-week period. The sorbitol sweetened gum group experienced an increase in dental plaque of about 50% during the test period. We believe these findings are of added importance in view of the recognition by the American Dental Asscciation that dental plaque is a leading cause of tooth decay.

Thus, there is a significant body of scientific evidence supporting a consensus that Xylitol does have a causative effect in assisting in the prevention of caries. When consumed as part of a normal daily diet, Xylitol-containing products, in conjunction with accepted oral hygiene practices, have been shown to result in an average of 50% fewer new caries incidence (and as high as 80%). These well-designed, publicly available studies clearly provide an adequate, substantiated basis to conclude that there is significant agreement among qualified experts as to the relationship between the consumption of certain Xylitol-containing foods and caries prevention.

III. FDA SHOULD PERMIT SUBSTANTIATED HEALTH MESSAGES FOR FOODS TO BE MADE IN AREAS OTHER THAN THOSE REVIEWED BY THE PHS COMMITTEE AND ON AN INTERIM BASIS IN THOSE AREAS SUBJECT TO COMMITTEE REVIEW

Because Xyrofin supports the broad dissemination of useful health information in food labeling to consumers, Xyrofin recommends that FDA permit the use of properly substantiated health messages in areas to be reviewed by the PHS Committee prior to final Committee action and promulgation of health messages. In addition, Xyrofin believes that the Agency, similarly, should permit properly substantiated health messages to be made in areas other than those initially selected for PHS Committee review, in areas of significant public health concern. Under this approach, manufacturers would assume the risk of making health claims prior to final Committee action or outside of the designated topics. In these circumstances, the manufacturer would bear the burden of providing adequate substantiating scientific evidence and data in the event of regulatory or enforcement action by FDA. Manufacturers making health claims consistent with final PHS approved messages would not be subject to any risk of regulatory action.

Xyrofin submits that the proper system for determination of appropriate health claims should include development of appropriate and clear substantiation criteria and reliance on

the food manufacturer/processor to comply with such criteria. Under this system, FDA could avoid pre-approval of health messages and instead impose the burden for the accuracy and truthfulness of the label on the manufacturer. FDA can then refocus its staff and budget resources to regulatory and enforcement actions against non-complying manufacturers/ processors. If a food manufacturer was concerned about the use of a particular health claim, either in the interim period prior to action by the PHS Committee or in an area not initially being considered by the PHS Committee, the manufacturer could request an informal advisory opinion from FDA on the status of the health claim. Alternatively, to the extent that a particular health claim could be addressed generically, FDA could issue a compliance policy guide addressing its use on a particular class of foods. Such a more flexible approach would address the unavoidable potential for delay in the process for development of health messages set out in the reproposed rule. Finally, American Xyrofin believes that the FDA can and should coordinate its enforcement activities with other federal (e.g., FTC) and state agencies to maximize its enforcement efforts. A coordinated enforcement effort would also result in the development and application of a consistent and uniform health claims policy throughout the country.

IV. THE SUBSTANTIATION CRITERIA SHOULD FOCUS ON THE QUALITY OF THE EVIDENCE

American Xyrofin agrees that FDA should "consider a broad array of data concerning the relationship between diet and a chronic disease..." Id. at 5181. FDA also has proposed that a health message must be "based on a totality of publicly available evidence" and that there must be "significant agreement [...] among qualified experts that the statement is supported by such evidence." Id. at 5180. American Xyrofin believes that this proposed substantiation standard is too restrictive and will impede the dissemination of useful health information to the public. Further, the standard will remove from the private sector the motivation to undertake research relating to diet and health relationships. Consequently, FDA should revise the standard to emphasize a review of the quality of the study (rather than focus on whether it is public or private) and whether it substantially supports the particular label statement in question. By adopting a substantiation standard with this focus, FDA's standard would be more consistent with the standard for advertising used by the Federal Trade Commission. See Thompson Medical Co., 104 FTC 648, 821-825 (1984), aff'd, 791 F.2d 189 (D.C. Cir. 1986), cert. denied, 107 S. Ct. 1289 (1987). Under this suggested substantiation approach, health messages are likely to be more

precise, relevant, current and, thus, meaningful to consumers.

As American Xyrofin has previously commented, FDA should recognize that food processors will only be able to justify undertaking research and development relating to diet and health relationships if the regulatory framework allows manufacturers to recapture through competitive marketing some of the expense of such research. If, however, a food processor must disclose publicly the results of all of its research as a prerequisite to FDA's determination that the research is adequate substantiation for a given health claim, and the substantiation can then be relied upon by competitors to make similar claims, there is no motivation whatsoever for the pioneer firm to assume the cost of research.[20/] Consequently, FDA should modify its proposed standard to ensure that research and innovation in the diet and health area are not unnecessarily hindered.

CONCLUSION

Xyrofin believes that FDA has taken a significant step in the right direction by going forward in an expeditious manner

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20/ See FTC Bureau of Economics, How Should Health Claims Be Regulated?: An Economic Perspective (Sept. 1989).

with a health claims reproposed rule. Xyrofin believes, however, that oral health, as a significant, recognized public health concern, ought to be included as an appropriate topic for health messages. Consequently, use of the sugar alcohol Xylitol in confectionery and other food products to assist in the reduction of dental caries should be included as an appropriate topic for review. Finally, the Agency should permit properly substantiated health messages to be made in the interim prior to final PHS Committee action and in areas other than those initially considered by the Committee for review. By taking this approach, FDA will allow the widest dissemination of useful, positive health information and encourage the food industry to continue researching the diet-health relationship.

Sincerely,

Kenneth Sandstrom
Director of Specialty Sweeteners

Enclosure

cc: Dr. Fred Shank

THERASOL (C31G COMPOUND)

A PRESENTATION & FILING

BEFORE

THE U. S. FOOD & DRUG ADMINISTRATION

RE:

"OVER-THE-COUNTER (OTC) DENTAL AND ORAL HEALTH CARE PRODUCTS FOR ANTIPLAQUE USE, SAFETY AND EFFICACY REVIEW"

DOCKET: #81N-0033

BY

E. B. MICHAELS RESEARCH ASSOCIATES, INC.

56 ROGERS AVENUE: UNIT S
MILFORD, CT., 06460
(203) 783-1542

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a: THE INTRODUCTION

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OTC DRUG REVIEW INFORMATION

INTRODUCTION

The active ingredient used in the dentifrice for control of plaque is the compound C31G®. C31G® is a combination of amphoteric quaternary ammonium inner salt surfactants described in U.S. Patent No. 4,107,328 [# 55][1] and No. 4,839, 158 [# 50]. These combinations of surfactants were discovered to have broad spectrum antimicrobial activity when used within defined pH ranges, at effective germicidal concentrations often below 100 parts per million [ppm].

Further studies have shown that C31G has antiviral activity against enveloped viruses, but with lower toxicity to mammalian cells than other surfactants with such properties.

Studies related to possible use as a cleansing agent in dentifrices indicated that C31G was cidal to all oral pathogens tested and that it had unique properties in the oral cavity related to the inhibition of adhesion of plaque to dentition.

Personal communications from Dr. Paul Keyes expressed the conviction that the use of polishing agents or abrasives in dentifrices inhibited diffusion and delivery of oral hygiene agents to the sulcus, subgingival or interproximal. We were led to consider the use of C31G solution as a liquid dentifrice in tooth brushing. This opinion was confirmed in agar diffusion studies.

Anecdotal evidence soon indicated that C31G solutions used as a liquid dentifrice in normal tooth brushing also resulted in excellent plaque and stain removal and decrease of accumulation of plaque, of stains and decreased calculus accumulation.

This decrease of soiling and redeposition on dentition is indicated to be due to the specific inhibition of binding of bacteria to hydroxyapatite by C31G in the presence of saliva. This concentration dependent inhibition of bacterial adhesion was reported from the Dental Institute of The University of Pennsylvania.

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[1] The symbol # **nn** will be used to identify the number of each document containing data, relative to safety and efficacy, presented throughout this submission. These numbers used consecutively in the index is based on the order of filing the documents in Volumes II, III and IV.and are so referred to in discussions and abstracts of the documents.

Formulations of C31G solutions with necessary excipients and sodium fluoride as an additional active ingredient were assayed for optimum concentrations of C31G. These studies resulted in the preparation of an acceptable liquid dentifrice.

The liquid dentifrice was introduced to the market in the fall of 1989 under license from E.B. Michaels Research Associates Inc. [EMBRA] by the OraTec Corporation of Herndon, Virginia.

The product, Therasol™, an Anti-Plaque Liquid Dentifrice and Oral Irrigant is marketed to the dental profession for office use and is also marketed to patients with the recommendation of and with the supervision of the dentist for home use as a liquid dentifrice.

It should be noted that Therasol, as liquid dentifrice, is used as an adjunct to a device, the toothbrush, and as such we believe could be classified as a medical device as well as a drug.

The C31G surfactants as further described in the submission are the following two compounds;

Redacted trade secret information

Surfactants in these classifications have been in wide use since the 1940's in product use resulting in intimate human exposures such as use in laundry,hsnd dishwashing, cosmetics, personal care and as excipients in drug formulations.

Estimates of the use of the use of the above classes of surfactants in the year, 1989, were about 150 million pounds [# **32**]. In this article the betaine is classified on pages 31 and 52 with amphoteric surfactants and the alkyl amine oxide as cationic surfactants, although they are both based upon quaternary nitrogen inner bonded ionic structure.

Considerable data related to the safety and efficacy of these two classes of surfactants used in C31G technology and of the C31G formulations, has been developed over the past years. This pertinate data is entered as the main part of this submission. A brief review of the course of the study will serve as an introduction to the data.

The development of C31G was a project originally designed to find substantially non - toxic substitutes for the chlorinated hydrocarbons, e.g. hexachlorophene, which had been withdrawn from the main-stream germicide market.

These had shown enough evidence of systemic toxicity to have become severely limited in cosmetic and drug use, as personal deodorants and for germicidal use in human topical applications for disinfection. This particularly limited products available for use in prevention of the transmission of disease in environments related to health care.

With knowledge that modern topical disinfection started with use of soaps and with experiences that surface active agents synergistically increased the activity of topical germicides; our projects turned then toward seeking synergism among the many synthetic surfactants that already were being used in personal care products, with no reported unfavorable toxic effects. As will be noted in the submission the project was more successful than expections, engendering early studies on toxicity and efficacy before attempting marketing.

After C31G was discovered by these efforts, *in vitro* studies confirmed its unique broad spectrum antimicrobial activity. *In vivo* studies were then initiated in numerous areas. Inital studies were conducted at Hannemann University and at Sloan Kettering Research Laboratories in Rye, N.Y. This led to product formulation and research and development work. Some was conducted within our organization, and other work with potential or realized licensees.

Some of the areas where controlled or anecdotal data became available are: oral hygiene, personal care, hair and body shampoos, surgical scrubs, wound disinfection, (including burn wounds and fungal infections), dentifrices, studies for control of bovine mastitis with teat dips, and most recently, studies related to prophylaxis of sexually transmitted diseases including HIV viruses (AIDS).

The needs of specific products for these areas resulted in a number of formulations being studied for safety and efficacy. These formulae were prepared from raw material from various sources with properties needed for the specific applications.

The compositions varied primarily in

Additional variations depended upon the purity of product from sources then available in the market.These formulations of C31G are presented in Section II.

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c: THE ADDENDA

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ADDENDA #1

RESPONSE TO THE FDA FOR ADDED DATA : 1993

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E.B. Michaels Research Associates, Inc.
56 Rogers Avenue, Unit S, Milford, CT 06460: (203) 783-1542, Fax (203) 452-9448

April 27, 1993

Dr. William E. Gilbertson
Office of OTC Drug Evaluation (HFD-810)
U.S. Food & Drug Administration
Room 201
7520 Standish Place
Rockville, MD 20855

RE: OTC Volumes 210270
to
210273

Dear Dr. Gilbertson;

In response to your request for additional information following the format provided, we have put together the chart that is enclosed herewith as our response. Hopefully, the chart is in order and satisfactorily provides the data requested.

However, if you or your associates require more, be assured that we will do our utmost to provide it.

Very sincerely yours,

Thomas S. Galla; Exec. V.P.
EBMRA, Inc.

encl.: as stated

E.B. Michaels Research Associates, Inc.
56 Rogers Avenue, Unit S, Milford, CT 06460: (203) 783-1542, Fax (203) 452-9448

SPONSOR: E.B.Michaels Research Associates, Inc. DATE: April 27,1993

Ingred. or Combo.	Claim	Country	Years	Total Doses
C-31-G	Brush "N" Rinse	USA	19 mo.	
C-31-G	Kills Aerobes	USA	4 mo.	
C-31-G	Kills Anaerobes	USA	4 mo.	
C-31-G	Kills Yeast	USA	4 mo.	
C-31-G	No Staining/Nonstaining	USA	19 mo.	Redacted confidential commercial information
C-31-G	Inhibits Bacterial and Plaque Formation to Tooth Surfaces	USA	4 mo.	
C-31-G	Pleasant Taste	USA	19 mo.	
C-31-G	Blocks Microbial Adhesion	USA	5 mo.	
C-31-G	Helps Control Bacteria and Yeast	USA	5 mo.	
C-31-G	Oral Hygiene Solution	USA	5 mo.	
C-31-G	Anti-Plaque	USA	15 mo.	
C-31-G	Oral Irrigant	USA	10 mo.	
C-31-G	Blocks Plaque Adhesion	USA	10 mo.	
C-31-G	Liquid Dentifrice	USA	10 mo.	

DOSAGE: one half to two thirds of an ounce per use.

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ADDENDA #2

CURRENT STATEMENT OF STATUS BY EBMRA, INC.

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STATEMENT BY EBMRA, INC.

EBMRA, the owner of the patents relevant to the C-31-G Technology by assignment from the inventor, Edwin B. Michaels, contracts for the production of a product called TheraSol Liquid Dentifrice in which its patented compound, C-31-G, is the active ingredient. This product is produced for EBMRA in full accordance with GLP and GMP in a USFDA approved and accredited facility. EBMRA provides all of the materials and components necessary for production to the manufacturing facility and closely monitors the production to assure Quality Control.

Under a Supply License executed between EBMRA and OraTec Corporation in Herndon, Virginia , EBMRA has granted to OraTec the exclusive right to sell and distribute TheraSol Liquid Dentifrice in the United States of America and Canada. This Supply License is unique and of special intent in that it limits the sale of TheraSol Liquid Dentifrice by OraTec to ONLY the dental profession for use by the dentist in the dental office and/or resale by the dentist to the dentist's patients for their daily home care regimen. The only exception thereto is, in the event that the dentist does not wish to directly provide the product to the patient, the privilege to the dentist to write an Rx for the patient which, when presented to OraTec by the patient will be fulfilled by OraTec directly to that patient.

Thus, presently and by this unique procedure, the ONLY source for TheraSol Liquid Dentifrice for the patients and users is their personal dentist who, based upon professional judgment and a full awareness of the patient's needs and problems, counsels and recomends TheraSol Liquid Dentifrice to the patient; no one is more capable and competent than that dentist to advise that patient.

As of January 10, 1994, OraTec has received of TheraSol Liquid Dentifrice from EBMRA for distribution to dentists and their clientele. At an assumed ½ ounce per use, there have been nearly usages of this product over the last four years by thousands of patients, each of whom, under this unique program, HAS USED THERASOL LIQUID DENTIFRICE SOLELY UPON THE RECOMMENDATION OF THEIR DENTIST AND UNDER THE CARE AND SUPERVISION OF THAT DENTIST.

To date, neither EBMRA or OraTec has had to entertain or resolve any complaint arising from the use of TheraSol Liquid Dentifrice nor has any complaint been forwarded to either of their respective product liability carriers for consideration and resolution.

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USFDA

OTC DRUG REVIEW INFORMATION

I: LABELS & PROMOTIONAL MATERIALS

A: LABELS

B: PROMOTIONAL MATERIALS

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I-A: LABELS

1: The original THERASOL label was for a four (4) ounce bottle given as a sample to the dentist and/or sold to the dentist as a package of ten (10).(Dec.: 1989)

Directions:

MORNING: Brush with a fluoridated dentifrice. Afterwards, rinse vigorously with water and then with 1 capful of TheraSol for 20 seconds.

EVENINGS: Do not use a dentifrice (which may inactivate TheraSol) Instead, dip a clean toothbrush into a capful of TheraSol and carefully brush all tooth surfaces for at least 20 seconds. *Repeat twice, rinsing* the toothbrush in clean water between each brushing with TheraSol.

Your dentist may modify these steps. Subgingival irrigation with TheraSol may be advised.

Ingredients:

Sodium Floride 0.05%, C31G* 0.3%, Water, SD Alcohol 38B 15%, Sodium Sacharin, and FDC blue No. 1.

C31G is a Citrate buffered composition of Alkyldimethylglycine and Alkyldimethylamine oxide (U.S. Patent No. 4,839,158)

TheraSol inhibits bacterial and plaque formation to tooth surfaces. ***Brush 'N' Rinse*** with **TheraSol** is an effective adjunct in both professional and home care programs.

Questions? Please call (703) 471-0377

Distributed by OraTec Corp. Specializing in the Microbiology of Periodontal Care

TheraSol TM

Brush 'N' Rinse TM

ORAL HYGIENE SOLUTION

with C31G

- Kills Aerobes
- Pleasant Taste
- Kills Anaerobes
- Fluoridated
- Kills Yeast
- No Staining

4 oz.

2: Shortly thereafter, early in 1990, the sixteen (16) ounce and thirty two (32) bottles were introduced for sale and distribution to the dentist which, respectively, had attached the labels which follow.

Directions:

TheraSol blocks microbial adhesion to tooth surfaces. It is strong enough to be used in professional programs, yet gentle enough to be used in home care programs as well. It is best applied with a brush or in pockets deeper than 4 mm by irrigation.

Brushing: Rinse with TheraSol, then dip a toothbrush into TheraSol and brush for 20 seconds using the sulcular brushing method recommended by your dentist or hygienist. Repeat. Rinse with the leftover solution.

Irrigation: TheraSol may be used with professional subgingival irrigation instruments or with home care devices such as the OraGator or Syrette if recommended by your dentist or hygienist.

Your dentist may modify these steps.

Ingredients:

Water, SD Alcohol 38B 8%, Glycerine 6%, C31G* 0.3%, Sodium Floride 0.02%, Saccharin, and FDC blue No. 1.

* C31G is a citrate buffered composition of alkyldimethylbetaine and alkyldimethylamine oxide (U.S. Patent No. 4,839,158).

Questions? Please call (703) 471-0377

© 1990 OraTec Made in U.S.A. 1090

Distributed by OraTec Corp. Specializing in the Microbiology of Periodontal Care

Fluoridated | Pleasant Taste

Blocks Microbial Adhesion

Helps Control Bacteria & Yeast

Brush 'N' Rinse TM

ANTI-PLAQUE ORAL HYGIENE SOLUTION

with C31G

16 oz.

TheraSol™

Brush 'N' Rinse ™

ANTI-PLAQUE
ORAL HYGIENE SOLUTION

Directions:

TheraSol blocks microbial adhesion to tooth surfaces. It is strong enough to be used in professional programs, yet gentle enough to be used in home care programs as well. It is best applied with a brush or in pockets deeper than 4 mm by irrigation.

Brushing: Rinse with TheraSol, then dip a toothbrush into TheraSol and brush for 20 seconds using the sulcular brushing method recommended by your dentist or hygienist. Repeat. Rinse with the leftover solution.

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* C31G is a citrate buffered composition of alkyldimethylbetaine and alkyldimethylamine oxide (U.S. Patent No. 4,839,158).

Questions? Please call
(703) 471-0377

© 1990 OraTec
Made in U.S.A. 1060

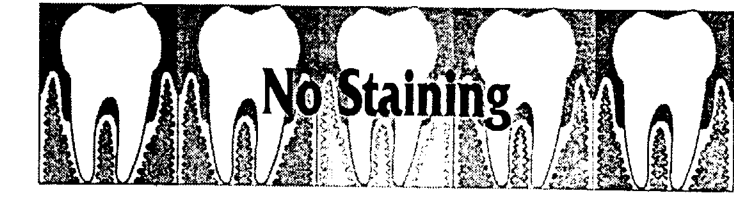
Distributed by OraTec Corp.
Specializing in the Microbiology of Periodontal Care

TheraSol™

Fluoridated

Pleasant Taste

Blocks Microbial Adhesion



Helps Control Bacteria & Yeast

Brush 'N' Rinse ™

ANTI-PLAQUE
ORAL HYGIENE SOLUTION

with C31G
32 oz.

3: In January, 1991, the 32 ounce bottle was converted to 64 ounces and the labels for the 16 and 64 ounce bottles were changed as per the labels following.

TheraSol is strong enough to be used professionally and gentle enough for use in home care programs. It is best applied with a brush. In pockets deeper than 4 mm, apply by irrigation. Do not dilute or use with toothpaste. Avoid rinsing with water afterward.

Directions:

BRUSHING: Dip a clean toothbrush into TheraSol and brush each quadrant for 15 seconds using the sulcular brushing method recommended by your dentist or hygienist. Repeat. Rinse with the leftover solution.

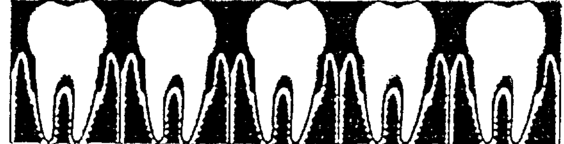
Alternatively a teaspoonful may be used as a resevoir in ones mouth for brushing. Rinse with second teaspoonful.

Your dentist may modify these procedures.

TheraSol™

LIQUID DENTIFRICE
ORAL IRRIGANT

NON-STAINING | ANTI-PLAQUE



Pleasant Taste | Fluoridated

Brush 'N' Rinse™

with C31G®: Blocks Plaque Adhesion

NET WT. 16 oz.

Directions:

IRRIGATION: In deep pockets, TheraSol may be applied with professional subgingival irrigators or at home with devices such as the OraGator or Syrette as recommended by your dentist or hygienist.

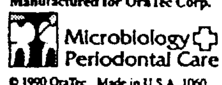
Your dentist may modify these procedures.

Ingredients:
Water, SD Alcohol 38B 8%, Glycerine 6%, C31G* 0.3%, Sodium Fluoride 0.02%, Saccharin, FDC blue No. 1. and Flavoring

* C31G is a citrate buffered composition of alkyldimethylbetaine and alkyldimethylamine oxide (U.S. Patent No. 4,839,158).

Questions? Please call
(703) 471-0377

Manufactured for OraTec Corp.



© 1990 OraTec Made in U.S.A. 1060

TheraSol is strong enough to be used professionally and gentle enough for use in home care programs. It is best applied with a brush. In pockets deeper than 4 mm, apply by irrigation. Do not dilute or use with toothpaste. Avoid rinsing with water afterward.

Directions:

BRUSHING: Dip a clean toothbrush into TheraSol and brush each quadrant for 15 seconds using the sulcular brushing method recommended by your dentist or hygienist. Repeat. Rinse with the leftover solution.

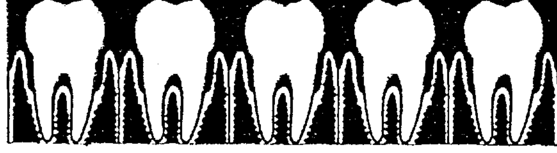
Alternatively a teaspoonful may be used as a resevoir in ones mouth for brushing. Rinse with second teaspoonful.

Your dentist may modify these procedures.

TheraSol™

LIQUID DENTIFRICE
ORAL IRRIGANT

NON-STAINING | ANTI-PLAQUE



Pleasant Taste | Fluoridated

Brush 'N' Rinse™

with C31G®: Blocks Plaque Adhesion

NET WT. 64 oz.

Directions:

IRRIGATION: In deep pockets, TheraSol may be applied with professional subgingival irrigators or at home with devices such as the OraGator or Syrette as recommended by your dentist or hygienist.

Your dentist may modify these procedures.

Ingredients:
Water, SD Alcohol 38B 8%, Glycerine 6%, C31G* 0.3%, Sodium Fluoride 0.02%, Saccharin, FDC blue No. 1. and Flavoring

* C31G is a citrate buffered composition of alkyldimethylbetaine and alkyldimethylamine oxide (U.S. Patent No. 4,839,158).

Questions? Please call
(703) 471-0377

Manufactured for OraTec Corp.

Microbiology
Periodontal Care

© 1990 OraTec Made in U.S.A. 1060

4: TOPICARE is sold and distributed primarily in 8 ounce bottles and 5 gallon containers and the labels attached following have been used since the beginning (May, 1988) with only a name change from CBL, Inc. to that of Dermalogic, a Division of EBMRA, Inc.

DIRECTIONS FOR USE

For the Body:
Apply Topicare to wet cloth or sponge. Wash and rinse.

For Hair:
Apply directly to wet hair. Lather and rinse.

INGREDIENTS

Water, C31G - a patented combination of cocobetaine and cocoamine oxide, sodium citrate, hydroxypropyl cellulose, fragrance and colorant.

WARNING

For external use only. In case of eye contact, flush thoroughly with water. If irritation occurs discontinue use.

A deodorizing, emollient cleanser for the whole body

8 fl.oz. (237 ml)

Manufactured exclusively for:
Dermalogic, Inc.
800-331-5412

CLEANSES

A gentle, pH balanced, all-purpose skin cleanser and shampoo. Excellent for cleaning perineal areas.

MOISTURIZES

Protects the skin from moisture loss and chapping. Minimizes need for creams and oils.

DEODORIZES

Provides long-lasting odor control.

PATENTED FORMULA

Contains a patented combination of active ingredients (5 U.S. patents) which moisturize and deodorize while cleaning.

DIRECTIONS FOR USE

For the Body:
Apply Topicare to wet cloth or sponge. Wash and rinse.

For Hair:
Apply directly to wet hair. Lather and rinse.

INGREDIENTS

Water, C31G - a patented combination of cocobetaine and cocoamine oxide, sodium citrate, hydroxypropyl cellulose, fragrance and colorant.

WARNING

For external use only. In case of eye contact, flush thoroughly with water. If irritation occurs discontinue use.

CLEANSES

A gentle, pH balanced, all-purpose skin cleanser and shampoo. Excellent for cleaning perineal areas.

MOISTURIZES

Protects the skin from moisture loss and chapping. Minimizes need for creams and oils.

DEODORIZES

Provides long-lasting odor control.

PATENTED FORMULA

Contains a patented combination of active ingredients (5 U.S. patents) which moisturize and deodorize while cleaning.

I-B: PROMOTIONAL MATERIALS ENCLOSED

LISTING OF ABOVE BY TITLE:

a: THERASOL TM (C-31-G)

b: C-31-G RESEARCH ABSTRACTS

c: THERASOL : INSTRUCTIONS FOR USE

d: THERASOL TM

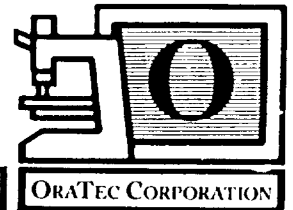
e: THE TOPIC IS QUALITY CARE

f: THE TOPICARE TM ADVANTAGE (I)

g: THE TOPICARE TM ADVANTAGE (II)

h: THE BENEFITS OF USING TOPICARE

i: TOPICARE : INSTRUCTIONS



THERASOL™ (C31-G)

TheraSol is a pleasant, mint flavored, antimicrobial solution consisting of a patented mixture (C31-G) of two synergistic surfactants: N,N-alkyl dimethyl betaine and N,N-alkyl dimethyl amine oxide.

University of Pennsylvania Dental School has conducted a number of studies on the efficacy of C31-G as an antimicrobial agent and its mechanism of action. According to these studies, several characteristics of C31-G make it an ideal agent for oral hygiene use, including:

1. Effective antimicrobial agents should both inhibit microbial colonization and kill bacteria. In tests carried out, C31-G was found to inhibit bacterial adhesion (the first step in colonization) and was also extremely potent in killing a large variety of oral microorganisms. It effectively suppressed strains of streptococci, staphylococci, actinomyces, pseudomonas, and yeasts including *Candida albicans*.

2. Many antimicrobial agents have a limited spectrum, i.e., they only kill Gram-positive or Gram-negative bacteria, or they are ineffective against yeast. In contrast, C31-G has an extremely broad spectrum of activity; indeed, a resistant microorganism has yet to be identified. This is important since it is possible to kill one or more classes of organisms only to have an overgrowth of another class.

3. Many agents are effective antimicrobials, but have toxic or unpleasant side effects, e.g. stain tooth surfaces, irritate mucosal cells, alter taste sensitivity, etc. To date, we have not identified any side effects of oral C31-G use.

Thus C31-G appears to be an extremely potent anti-microbial agent that both kills oral bacteria and prevents their colonization, has a broad spectrum of activity, including all oral bacteria and yeast tested, and has not demonstrated any toxic or unpleasant side effects.

Studies at the Universities of Maryland and Pennsylvania have shown that mouth rinsing with C31-G was efficacious in inhibiting supragingival plaque formation in humans and that it suppressed bacterial counts for up to 6 hours after rinsing. In this respect, it compared favorably to both 0.12% and 0.2% solutions of chlorhexidine.

THERASOL ... a pleasant tasting, extremely effective antimicrobial agent with no significant side effects is now available to the dental profession as a mouth rinse. *TheraSol* appears to be a beneficial adjuvant in the maintenance phase of periodontal therapy and in preventive oral hygiene procedures. It may be used as an antiplaque solution for brushing the teeth and gingival crevices and then as a mouth rinse. It may also be used as subgingival irrigant with an irrigation devise: Syrette, Viadent, Water Pik, etc.

AVAILABLE EXCLUSIVELY THROUGH THE DENTAL PROFESSION

For more information or to order, call:

1 800 368-3529

Washington, D.C. metropolitan area: **471-0377**

OraTec Corporation ... Specializing in Antimicrobial Periodontal Therapies

485 SPRING PARK PLACE, SUITE 100 HERNDON, VA 22070 (703) 471-0377

ORATEC
485 Spring Park Place, Suite 600
Herndon, Virginia 22070
Tel. (703) 471-0377
Fax (703) 471-1632

C31G

RESEARCH ABSTRACTS

$$CH_3(CH_2)_n\text{-}\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{N^{+}}}\rightarrow O^{-}$$

N,N-alkyl dimethyl amine oxide

$$CH_3(CH_2)_n\text{-}\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{N^{+}}}\text{-}CH_2\text{-}C(=O)O^{-}$$

N,N-alkyl dimethyl glycine

The topical antibacterial agent C31G is composed of two synergistic surfactants: N,N-alkyl dimethyl amine oxide and N,N-alkyl dimethyl glycine. It was invented by Edwin B. Michaels working from a background in biological macromolecules, surfactants and antimicrobial agents.

In the early 70's, there was a growing recognition that the chlorinated hydrocarbons which were often the main components of personal antiseptics were toxic. Mr. Michaels realized that many, less toxic surfactants could synergistically enhance the activity of other antimicrobial agents.

The object of his studies was to find the most synergistic combination of the mildest and least toxic synthetic surfactants. The result of his investigations led to the invention of C31G, a composition having remarkably useful properties, far beyond the original goal of finding a safe deodorant body wash. C31G was the subject of intense research by Michaels and other scientists throughout the 70's, resulting in early patents and numerous scientific publications.

When it was realized that periodontal diseases were the result of bacterial infections, it was readily apparent that there were very few antimicrobial agents that were simultaneously effective, safe and free of side effects. That realization spurred investigations into the oral applications of C31G.

C31G is available to dentistry in the form of *TheraSol*, a non-prescription oral hygiene solution distributed exclusively to and for the dental profession. Patients may obtain TheraSol directly, but only with their dentist's knowledge and approval. TheraSol contains 0.3% C31G in a vehicle of water; SD alcohol 38B 8% (a solvent for the flavoroids); glycerine 6%; sodium fluoride 0.02% (anti-caries); flavor; saccharin and FDC blue No.1.

TheraSol is recommended for use as a liquid dentifrice/oral irrigant adjunctive to professional therapy and home care programs. It has exceptional properties with regard to the removal of plaque, debris and stains from enamel and cemental surfaces and inhibits the adherence of plaque.

The foregoing statements make no claims for any therapeutic activity of TheraSol independent of its adjunctive use by professionals or as a recommended part of home care programs.

ANTI-PLAQUE / SUBSTANTIVITY . . . HUMAN IN VIVO STUDIES

Efficacy of C31G Mouthrinse on Inhibiting Supragingival Plaque Formation. *Univ. of Maryland, College of Dental Surgery; (1988) MA Siegel DDS, MS, LG DePaola DDS, GC Williams DDS, MBA*

In a 6 week clinical study, 126 subjects divided into 3 groups used either C31G, Listerine or a placebo twice daily. Oral hygiene was scored for supragingival plaque & staining. After a complete soft tissue examination, subjects were questioned for adverse reactions or unwanted side effects.

A significant improvement in plaque scores was seen with both Listerine & C31G vs. placebo. No significant difference was observed between Listerine & C31G. The stain index of both Listerine & C31G did not change from baseline. The study concluded that C31G mouthrinse was shown to be efficacious in inhibiting supragingival plaque without staining & with minimal side effects.

Clinical Study of a C31G Containing Mouthrinse: Effect on Salivary Microorganisms. *J Clin Dent 2:34-38, 1990; AM Corner, VJ Brightman, S Cooper, SL Yankell, D Malamud.*

Studies in 12 subjects revealed that C31G significantly reduced total bacterial counts, total streptococcus and *S. mutans*, in saliva samples obtained three hours after rinsing. The agent significantly inhibited glycolysis of salivary bacteria for up to 6 hours post rinsing. In the same study Listerine reduced bacterial counts for one hour, but it did not inhibit glycolysis at any time point. No staining or altered taste sensations were noted with either product. "These results suggest that C31G containing mouthrinses may be a valuable aid in oral hygiene"

Panel Studies on the Comparison of Peridex to a C31G Mouthrinse. *Unpublished data, 1989*

From a panel of 60 subjects, previously scored for rate & degree of plaque accumulation, 4 equivalent groups were selected. Group 1 used water; Group 2, a commercial mouthrinse; Group 3, Peridex; Group 4, 0.3% C31G mouthrinse.

Studies were conducted over 7 days with a prophylaxis on day 1 to remove plaque. Oral hygiene was restricted to the supplied mouthrinse. On days 2 & 3, the subjects rinsed twice daily, the morning rinse being supervised. After 48 hours, plaque was scored by Erythrosin staining.

Results: 20.4% inhibition by the commercial mouth rinse (Group 2); 43.0% inhibition by both Peridex & C31G (Groups 3&4)

ANTI-PLAQUE / SUBSTANTIVITY . . . IN VITRO STUDIES

C31G, a New Agent for Oral Use with Potent Antimicrobial and Antiadherence Properties. *AM Corner, MM Dolan, SL Yankell. & D Malamud. Antimicrobial Agents and Chemotherapy, 32:350-353, 1988.*

C31G demonstrated broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria, *Candida albicans*, cariogenic species, Capnocytophaga, Actinobacillus species, and against antibiotic resistant strains of Pseudomonas.

C31G in a mouthwash vehicle inhibited bacterial acid production as measured by inhibition of glycolysis in salivary sediment. C31G also inhibited *S. Sobrinus* adherence to nichrome wire. C31G was more effective in these assays than any other commercial formulation tested and was as effective as chlorhexidine.

C31G, A new Agent for Oral Use. I. Antiglycolytic tests. *AM Corner, MM Dolan, D Malamud, SL Yankell, U. of Penn., School of Dental Medicine. J. Dent. Res. 65, Abstract #947 (1986)*

In studies with radiolabeled *S. sanguis*, C31G inhibited bacterial adhesion to hydroxyapatite in the presence of saliva in a dose dependent manner. In the absence of saliva, the inhibition was significantly lower. These findings suggest that C31G will interfere with the ability of bacteria to colonize dentition and form adherent plaque.

In an artificial plaque system (Dolan et al.), 0.5% C31G and its vehicle were compared with 0.5% chlorhexidine digluconate (positive control) and water (negative control). C31G & chlorhexidine maintained pH over 7 hours, while the vehicle alone and water showed significant drops within 3 hours.

C31G, A New Agent for Oral Use. II. Antibacterial Activity. *D Malamud, AM Corner, MM Dolan, BF Hammond, S Saunders & SL Yankell. J Dent Res. 65: Abstract #949 (1986)*

Agar diffusion studies were undertaken on *Streptococcus sanguis*, *Actinomyces viscosus*, *Bacteroides intermedius*, *Capnocytophaga sputigena*, and *Actinobacillus actinomycetemcomitans*, by measuring zones of inhibition. C31G was as effective as chlorhexidine at all concentrations tested in both plaque adherence studies and growth inhibition. C31G was significantly better than Listerine and Fluorigard in preventing plaque adherence & inhibiting growth.

Binding Studies on the Antimicrobial Surfactant C31G. *AM Corner, D Malamud, & SL Yankell. J Dent Res. 65, Abstract #407, p.771. (1986)*

In vitro studies assessed the binding of ^{14}C labelled C31G to *Streptococcus*, *Actinomyces*, *Actinobacillus*, *Candida*, dentine, hydroxyapatite, glass beads & ground teeth. C31G binding to bacterial and yeast cells correlated with the antimicrobial MIC values.

ANTIMICROBIAL ACTIVITY . . . STUDIES IN ANIMAL MODELS

A Burned Mouse Model to Evaluate Antipseudomonas Activity of Topical Agents. *DD Stieritz, A Bondi, D McDermott, & EB Michaels. J Antimicrob Chemother 9:133-140; (1982)*

C31G was used to develop a burned mouse model to evaluate anti-infective topical agents. Results: the model was relevant to previously reported animal models & that in vitro systems such as agar diffusion studies may not be significant.

C31G was more effective than topical sprays and in the same range of efficacy as topical creams incorporating effective systemic antimicrobial agents.

Controlled Wound Healing Repair in Guinea Pigs, Using Antimicrobials That Alter Fibroplasia. *AJ Kenyon, SG Hamilton, DM Douglas. Am J Vet Res 47:96-101; (1986)*

Guinea pig wounds inoculated with *Staphylococcus aureus* and then treated with antimicrobials for 10 minutes after inoculation had the following viable bacterial counts 24 hours after treatment: Alcide gel reduced mean number of recoverable organisms from 2.7×10^2 to 1.9×10^6; C31G reduced the number to 1.6×10^3.

Although similar with respect to inhibition of bacterial growth, the response of guinea pig wounds to C31G or Alcide was different when evaluated for wound tensile strength. From 7 to 16 days after surgery, C31G greatly increased wound strength compared to Alcide.

The rate of collagen synthesis in wounds treated with these antimicrobials corresponded with breaking strengths. The data indicated that Alcide-treated wounds had greatly reduced collagen synthesis when compared to controls.. As indicated by ^{14}C-labeled proline uptake, chlorhexidine-treated wounds had high amounts of collagen synthesis and also had wounds that were inflamed and tended to gap more than those treated with Alcide or C31G.

Effect of C31G, an Antimicrobial Surfactant, on Healing of Incised Guinea Pig Wounds. *EB Michaels, EC Hahn, AJ Kenyon. Am J Vet Res. 44:1378-1381; (1983)*

C31G promoted healing of infected and non-infected wounds in guinea pigs. Histological examinations of wounds treated with C31G revealed an increased rate of wound closure associated with a decrease in inflammation and an increase in fibroblast infiltration and epithelialization. Seemingly C31G increased the protein cross-linking of fibrin in clots containing fibronectin.

TOXICITY STUDIES

Mice and Rabbit Models for Oral and Percaneous Absorption and Disposition of Amphoteric Surfactant C31G. *EB Michaels, EC Hahn, AJ Kenyon. Am J Vet Res. 44:1977-1983; (1983)*

Absorption of radio-labelled [^{3}H]C31G at an oral dosage level of 0.21 g/kg of body weight was followed in mice, and absorption through skin was followed within a 10-fold exposure time and concentration was followed in both mice and rabbits. Excretion of C31G after oral or dermal dosing was predominantly renal at higher dosage levels, whereas fecal excretion dominated at the lowest levels. Dermal transport in the rabbit was less than one-fourth of that in the mouse.

Acute Oral Toxicity Study in Beagle Dogs. *International Research and Development Company. Unpublished data; (1977)*

In beagle dogs C31G was administered once by oral lavage at concentrations of 3.125, 6.25, 12.5, 25, and 50 ml/kg of body weight. Within 4 hours after administration of the agent, some of the dogs experienced tachycardia, emesis, salivation, hypoactivity, tremors, and respiratory congestion. All dogs were essentially normal at 72 hours. None of the dogs succumbed during the study period. Autopsy findings revealed no gross findings that could be attributed to administration of C31G.

Oral LD_{50} Evaluation for C31G 3.0% Liquid. *BAYVET Division of Miles Laboratory, Inc. Merriam, Kansas, Unpublished data; (1983)*

A 3.0% solution of C31G was administered to forty Sprague-Dawley rats by esophageal intubation. (3000 and 6000 mgs of agent per kg. of body weight). During the 14 day post treatment period, the rats gained weight. After 14 days of observation the rats were killed and autopsied. Abnormal necropsy findings included slight intestinal hemorrhage, slight liver discoloration, and slight to severe lung congestion. Under the conditions of this study C31G was determined to have an LD_{50} of greater than 6000 mg. per kg of body weight.

Rat Acute Oral Toxicity. *Stillmeadow, Inc. Mission City, TX. Unpublished data; (1976)*

In Long Evans rats the LD_{50} for C31G was calculated to be 15.89 g/kg of body weight.

Toxicological Evaluation of Surfactant C31G. *Sloan-Kettering Institute; (1976)*

The LD_{50} for intraperitoneal injection of C31G in a Swiss-Webster mice model was 0.28 g/kg of body weight. The oral LD_{50} occurred at a level of 2.20 g/kg of body weight in similar mice. Animals that survived did not develop abnormalities attributable to the oral administration of C31G.

Long term administration of low levels in the rodent's water supply (2.5 ml of a 13% solution per 500 ml of water or 65 mg % active ingredient) did not cause a change in behavior, reproduction, or growth rates of parent or offspring mice.

C31G applied topically to mice each day for 8 days at the level of 0.1 ml (104 mg per mouse in 8 days) produced no signs of irritation or subsequent abnormalities.

Twenty-four hour patch tests were made in 5 guinea pigs using a 13% solution of C31G. No alteration was detectable on direct observation in areas when the patches were applied directly to clipped skin.

In guinea pigs, incised wounds experimentally infected with strains of Staphlococcus aureus were treated with C31G. These wounds healed at rates comparable or better than isotonic saline-treated wounds. The antimicrobial activity of C31G in healing wounds was extremely significant in view of the rapid wound closure with no subsequent adverse reactions.

ORATEC
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TheraSol

Instructions for Use

Alkyl dimethyl amine oxide and alkyl dimethyl glycine (C31G) have been proven effective against a wide range of microorganisms associated with periodontal diseases. It's lack of side effects makes it a suitable choice for use in both professional and home care programs.

Because of its unique formulation, TheraSol liquid dentifrice may be administered in a wide variety of ways. Best results are obtained with routine home use. One cautionary note ... detergents (typically sodium laurel sulfate) incorporated into commercial toothpastes and mouthwashes for their sudsing effect may act as antagonists to C31G. Avoid using these products for at least 1 hour before and after using TheraSol.

PROFESSIONAL APPLICATIONS

General Dental — Use as a pre-treatment & post-operative antiseptic and breath freshener. Rinse vigorously with 1 ounce for 15-30 seconds.

Periodontal — May be used as a pre or post-root planing irrigant. Post irrigation should be performed full mouth using 100-200 cc's. In pockets >4 mm, delivery to the apical third of the pocket is more effective when a sub-gingival cannula used with a professional irrigation system.

Oral Surgery & Implants — Irrigation of surgical sites may be performed using bulb syringes or professional irrigators. Quantity depends on severity of wound. See studies re wound healing in abstracts.

Orthodontics — Brushing and/or rinsing with 1 ounce after orthodontic procedures is recommended.

HOME CARE

These are general recommendations. The specific instructions of your dentist or dental hygienist may differ and should take precedence.

Orthodontics — Use TheraSol as a liquid dentifrice before retiring & on awakening. Be sure the brush is clean and free of toothpaste/mouthwash residues. Dip the brush into 1/2 oz. (1 tablespoon) of TheraSol and brush the teeth using a modified Bass or sulcular technique. Dip again for each arch. Expectorate. Rinse 30 sec. with another 1/2 oz.

Periodontics:

Pockets <= 4 mm: Rinse with 1/2 oz. (1 tablespoon) after brushing after breakfast, lunch, & before retiring. Brush using the above instuctions for orthodontic patients.

Pockets >= 4 mm: Same as above. In addition, before retiring, irrigate sug-gingivally with 2-3 oz.'s using a Water Pik or Viadent irrigator equipped with a sub-gingival adaptor (OraGator, Max-i-Probe, Pocket Tip Adaptor) or a Luer Syrette.

TheraSol Liquid Dentifrice is distributed solely by OraTec and available exclusively through the dental profession. For more information on TheraSol or our other professional & home care products, call or fax the numbers above.

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The TopiCare™ Advantage

"TopiCare immediately solved a severe body odor problem which had seemed insurmountable. We are very pleased with the results!"

D. Douglass, R.N., D.N.—
Meridian Healthcare

"TopiCare has *reduced odor* substantially and leaves our nurses' hands both *clean* and *soft*."

M. Knox, R.N., Director of Staff Development for a Large Nursing Home Chain

"TopiCare is a new breakthrough in skin care. It cleans effectively, eliminates incontinence-related odor, and softens the skin."

S. Thompson, R.N., M.A.—
Meridian Healthcare

"Since using TopiCare, odors are gone. My staff loves it. TopiCare thoroughly and gently cleanses without robbing the skin of essential moisture. No dry, cracked skin due to a harsh soap."

A. Hague, PHA., Administrator—
Community Care
Nursing & Geriatric Center

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dermalogics a division of EBMRA, Inc.

11 Lewis Road • Trumbull, CT 06611 • (800) 331-5412 • (203) 261-5606 • FAX (203) 452-9448

PRESENTS

The TopiCare™ Advantage

A Patented Compound which, "ALL IN ONE", is:

* NATURALLY "CLEANSING"
* NATURALLY "DEODORIZING"
* NATURALLY "EMOLLIENT"
* NATURALLY "CONDITIONING"

****

TOPICARE is thoroughly "cleansing" while, at the same time, being gentle to your skin; it is "deodorizing" simply because it destroys the bacteria which cause odor but without blocking your pores and interfering with normal skin function; "emollient" because it enhances the smoothness and suppleness of your skin; and, "conditioning" because it assists the skin to retain moisture, thus, inhibiting dryness, redness, itchiness, flaking and irritation. In most cases, the combination of these actions eliminates the need for a "cabinet full" of additional products for skin care as well as the further expenditure of your time for their utilization.

"FOR THE SKIN YOU'LL LOVE TO LIVE IN"

TOPICARE, but please don't take our word for this, ... "try it" and learn for yourself that you'll "like it".

****

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BENEFITS OF USING TOPICARE

IMPROVED QUALITY OF CARE.

TopiCare can improve the overall quality of care a facility provides.

1. Less time goes to resident "maintenance." More time is devoted to caring. Staff also have fewer products to carry around.

2. Residents have less odor, and a better appearance.

3. Nursing staff who use TopiCare report significant improvements in the condition of their own hands. Chapping, redness and scaling disappear. Their skin is smoother, softer, and more supple, even with repeated daily use.

Easy to Use.

TopiCare is easy to use. Apply it to hands, damp washcloth or sponge. Wash and rinse. TopiCare is gentle enough to be applied directly to resident skin or hair. Because it has no artificial sudsing agents, users don't become "soapy" and require multiple rinses.

REDUCED COSTS.

TopiCare is a cost-effective product for today's modern long-term care facility. It saves three ways:

1. Staff Expense.

   Nursing aides spend less time bathing and "maintaining." Fewer staff can do more. Directors of Nursing spend less time solving odor problems.

2. Administrative Time.

   Families are happier with resident care. Administrators and DONs together spend less time answering odor and skin-care related complaints. Discharge planners, families and inspectors see fewer incidents of odor and skin-related problems.

   There are fewer products to inventory and track. Administrators spend less time ordering and re-ordering.

3. Product Costs.

   Using TopiCare costs less than buying separate deodorants,

TOPICARE : INSTRUCTIONS FOR THE OPTIMUM RESULT

IN THE SHOWER : FOR HAIR & BODY:

1: Wet the hair and body thoroughly and then either turn away or step away from the direct stream of water.

2: Place a dab of TOPICARE, about the size of more than a quarter but less than a half dollar, in the palm of your hand and apply it your wet hair and scalp; then rub gently to generate lather and then more briskly to clean. If necessary or desired, rinse very lightly and repeat. When finished, allow the suds to remain in the hair while attending to cleansing the remaining body.

3: Apply about one half ounce (about a tablespoon) of TOPICARE to a bath sponge thoroughly wetted; then, using the sponge as you would a cake of soap, apply suds to the entire body. Pay special attention to areas needing special care, such as underarms, perineal or places of rashes or irritation; if needful or desirable, rinse lightly and resuds the area.

4: Rinse the hair and body thoroughly.

IN THE BATHTUB : FOR HAIR & BODY:

1: Proceed as described above and washing the hair first.

2: Continue by washing the body using a sponge to which TOPICARE has been applied as directed above and using the sponge as you would a washcloth or cake of soap.

3: Rinse body and hair thoroughly, either by showering or otherwise, as per your preference.

* If the bathtub is equipped with a Jacuzzi or other type of Whirlpool, it is suggested that the whirlpool be used prior to bathing and the use of TOPICARE as the presence of the TOPICARE will produce too much foaming by the aeration of the water. However, one teaspoon of TOPICARE can be used as a water treatment and which would produce a minimum of foaming.

** The use of cotton wash cloths is not recommended for lathering will be inhibited as TOPICARE is absorbed by the cotton and, in addition, the TOPICARE will act to remove soap or detergent residues left after laundering of the wash cloth. However, paper or non-woven wash cloths, which are disposable, may be used.

II: QUANTITIES & INGREDIENTS

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QUANTITY OF ACTIVE INGREDIENT **C31G Liquid Dentifrice-TheraSol™**

Each kilogram of TheraSol contains 3 grams of the C31G active ingredients buffered with citric acid monohydrate to yield a pH of of about 4.85 and 0.3% ai as follows;

Ingredient	
SDA-38B Alcohol USP[2]	
Glycerine USP	
Sodium Saccharin USP	
Sodium Flouride USP	
FD&C Blue #1	

[1] The Citric Acid is not considered an active ingredient, but it provides a protonating agent for buffering action.

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SUNMARY OF DATA: In Vitro Antimicrobial Activity C31G § V

I Bacteria

ORGANISM	NO.	TEST	pH	TYPES	LABORATORY	PPM	MIN
ACTINOBACILLUS: ACTINOMYCETUM COMETANS	1	MIC	7	-		20	20^{HRS}
ACTINOMYCES VISCOSUS	1	MIC	7	+		8	"
BACTEROIDES INTERMEDIUS	1	MIC	7	- ANAER		8	"
CAPNOCYTOPHAGA SPUTOGENA	1	MIC	5.5	- ANEAR		15	"
CORYNEBACTERIUM ACNE	1	MIC	~ 7 AGAR	+ ANAER		13	11^{DAYS}
B. CEREUS	1	MCC	~ 5	+ SP		13	5
B. CEREUS	2	MCC	7	+ SP		25- 2500	15
B. SUBTILLIS	1	MCC	~ 5	+ SP		1300	1
DIPTHEROIDS	1	MCC	~ 5	+		13	5
E. COLI	4	MCC	~ 5	-		13	1
E. COLI	3	MCC	7	-		25- 2500	15
HELIOBACTER PYLORI	1	MIC	1	- ANAER		4	72^{HRS}
HERELLEA VAGINICOLA	1	MCC	~ 5	-		130	1
K. PNEUMONIAE	1X	MCC	~ 5	-		13	5
K. PNEUMONIAE	2	MCC	7	-		25	15
K. OXYTOCA	1	MCC	7	-		250	5
MIMA POLYMORPHA	1	MCC	~ 5	-		0.3	1

LEGEND FOLLOWS AS TABLE 9

Table 5

BACTERIA CONTINUED

ORGANISM	NO.	TEST	PH	TYPES	LABORATORY	PPM	MIN
PROVIDENSIA STUARTI	1	MCC	~ 5	–		130	10
PROTEUS MIRABILIS	1	MCC	~ 5	–		1300	10
P. MORGANIE	2	MCC	~ 5	–		130	5
P. VULGARIS	1	MCC	~ 5	–		13	1
PSEUDOMONAS AEROGINES	1	MCC	~ 5	–		13	5
PS.AERUGINOSA	22	MCC	~ 5	–		130	5
PS.AERUGINOSA PARENT CELL	4	MCC	~ 5	– PARENT CELL		130	5
PS.AERUGINOSA DAUGHTER CELL	12	MCC	~ 5	– DAUGHTER CELL		130	5
PS.AERUGINOSA	5	MIC	5.5	–		550 AVE	20 HRS
PS.TYPHIMURIUM	1	MCC	~ 5	–		13	1
SALMONELLA TYPHIMURIUM	1	MCC	~ 5	–		13	24 HRS
SALMONELLA TYPHIMURIUM	5	MIC	~ 5	–		13	1
SERRATIA MARCESENS	1	MCC	~ 5	–		130	5
SHIGELLA SONNIE	1	MCC	~ 5	–		13	1
STAPHYLOCOCCUS AUREUS	5	MCC	~ 5	+		1.3-13	1
STAPHYLOCOCCUS AUREUS	2	MCC	7	+		25	1
STAPHYLOCOCCUS AUREUS	1	MCC	8	+		>130	16

Table 6

BACTERIA CONTINUED

ORGANISM	NO.	TEST	PH	TYPES	LABORATORY	PPM	MIN
STAPHYLOCOCCUS AUREUS	1	MCC	~ 5	+		< 10	2
STAPHYLOCOCCUS AUREUS	2	MIC	~ 7 AGAR	+		26	48^{HRS}
STAPHYLOCOCCUS AUREUS	1	MIC	7	+		20	20^{HRS}
STAPHYLOCOCCUS EPIDERMIDIS	1	MCC	~ 5	+		13	5
STAPHYLOCOCCUS EPIDERMIDIS	3	MCC	7	+		25	1
STREPTOCOCCUS MUTANS	1	MIC	7	+		15	20^{HRS}
STREPTOCOCCUS SANGUIS	1	MIC	7	+		15	20^{HRS}
STREPTOCOCCUS SOBRINUS	1	MIC	7	+		15	20^{HRS}
STREPTOCOCCUS DISGALACTAE	2	MCC	7	+		25	1
STREPTOCOCCUS UBERIS	1	MCC	7	+		25	1
STREPTOCOCCUS OVRIG	1	MCC	7	+		25	1
B. STREPTOCOCCUS GROUP A	1	MCC	~ 5	+		13	1
B.STREPTOCOCCUS GROUP D	1	MCC	~ 5	+		13	1

Table 7

II YEASTS AND FUNGI

ORGANISM	NO	TEST	PH	TYPES	LABORATORY	PPM	MIN
CANDIDA ALBICANS	1	MCC	~ 5	+ YEAST		13	1
CANDIDA ALBICANS	2	MIC	7	+ YEAST		300	10 DAYS
CANDIDA TROPICALIS	1	MIC	7	+ YEAST		300	10 DAYS
CRYPTOCOCCUS NEOFORMANS	1	MIC	~ 5	DERMATO-PHYTE		13000	7 DAYS
EPODEMOPHYTON FLOCCOSUM	1	MIC	~ 5	DERMATO-PHYTE		< 130	7DAYS
TRYCOPHYTON RUBRUM	1	MIC	~ 5	DERMATO-PHYTE		130	7 DAYS
TRYCOPHYTON MENTAGROPHYTES	1	MIC	~ 5	DERMATO-PHYTE		130	7 DAYS
TRYCOPHYTON MENTAGROPHYTES	1	MIC	7	DERMATO-PHYTE		600	10 DAYS
TRYCOPHYTON INTERDIGITALE	1	MIC	7	DERMATO-PHYTE		600	10 DAYS
TRYCOPHYTON GALLINAE	1	MIC	7	DERMATO-PHYTE		600	10 DAYS
MICROSPORUM GYPSEUM	1	MIC	7	DERMATO-PHYTE		600	10 DAYS
MICROSPORUM AUDOUINII	1	MIC	7	DERMATO-PHYTE		600	10 DAYS
MICROSPORUM GYPSEUM	1	MIC	~ 5	DERMATO-PHYTE		130	7 DAYS
SACCHAROMYCES CEREVISIAE	1	MIC	~ 5	YEAST		1300	7 DAYS
SACCHAROMYCES CEREVISIAE	1	MIC	7	YEAST		300	10 DAYS
ASPERGILLUS FLAVUS	1	MIC	7	RE: AFLATOXIN		3000	5 DAYS
ASPERGILLUS 7FUMIGATUS	1	MIC	7	PATHO-GENIC SP		600	5 DAYS

Table 8

Legend

The Organisms described are tabled by species in sections for bacterium and fungi. The number of strains of a species tested is under **No.** and the type of **Test**, minimum inhibitory concentration**[MIC]** or minimum cidal concentration **[MCC]**, is in column 3. In column 4, **pH** refers to the pH of the broth or solvent in which the organism is exposed to the agent. The next column **Types** have descriptions of the organism abbreviated as [+] or [−] for gram + or gram −. Organisms are aerobic unless noted here as [anaer] for anaerobic. [Sp] indicates a sporulating organism.

As most organisms tested are pathogenic and are antibiotic resistent strains from clinical isolates, this is not noted. The column **ppm** denotes parts per million of the agent and **Min** in the next column denotes the exposure time in minutes, of the organism to the agent in the solvent or broth, before plating; unless otherwise noted in superscript.

The full names of the Laboratories used in the above table are listed below and the references containing the data follow in the next column.

Abbreviation	Institution	Reference
UPenn Dent		No. 53.
Boots		No. 60.
Hann. U.		No. 57.
Kemi		No. 58.
U Hosp Boston U		No. 61.
Bioassay Systems		No. 31.

The complete protocol used for determination of the MCC in the data from Hahnemann University is reported in Reference No. 57. This protocol defines the basic relationship between the effect of the agent on the organism, eliminating environmental artifacts such as pH or nutrients in broths or agar and permitts assay of efficacy based on defined exposure times. Efficacy, is later studied in *vivo* or using protocols to mimic *in vivo* use relating to specific formulations of the agent for various applications.

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III: ANIMAL SAFETY DATA

A: INDIVIDUAL ACTIVE COMPONENTS

1: Controlled Studies

2: Partially Controlled or Uncontrolled Studies

B: COMBINATIONS OF THE INDIVIDUAL ACTIVE COMPONENTS

1: Controlled Studies

2: Partially Controlled or Uncontrolled Studies

C: FINISHED DRUG PRODUCT

1: Controlled Studies

2: Partially Controlled or Uncontrolled Studies

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USFDA OTC REVIEW DOCKET : #8IN-0033		III: ANIMAL SAFETY DATA			
A:INDIVIDUAL ACTIVE COMPONENTS		COMBINATIONS OF B: INDIVIDUAL ACTIVE COMPONENTS		C: FINISHED DRUG PRODUCT	
1 1:	2:	1:	2:	1:	2:
CONTROLLED STUDIES	PARTIALLY CONTROLLED/ UNCONTROLLED STUDIES	CONTROLLED STUDIES	PARTIALLY CONTROLLED/ UNCONTROLLED STUDIES	CONTROLLED STUDIES	PARTIALLY CONTROLLED/ UNCONTROLLED STUDIES
# 1 # 2 # 3 # 4 # 5 # 6 # 7 # 8 # 9 #10 #11 #12 #13 #14 #15	NONE	#16 #17 #18 #19 #20 #21 #22 #23 #24 #25 #26 #27 #28 #29 #30 #31 #65 #66 #67	NONE	#37	NONE

TABLE : All 85 Numbered References were considered relevant to these Columnar Headings and, if applicable to any, were entered by Number in the Appropriate Column.

E.B.MICHAELS RESEARCH ASSOCIATES, INC., MILFORD, CT., USA

SECTION III: Animal Safety Studies.

DISCUSSIONS OF ANIMAL SAFETY DATA

The data presented in the Tables of Animal Safety indicate the lowering of toxicity by formulation of components into C31G with the following order of toxicity:

C31G < alkyl betaine < alkyl amine oxide < T-Alkyl amine < alkyltrimethyl ammonium salts < benzalkonium chlorides.

Acute oral toxicity varies from 2.2 g/kg for C31G to ~1.0 g/kg for components and as low as 200 m/kg for quaternary ammonium salts. The I.P. LD50 for C31G is at 280 mg/kg whereas Quaternary germicides are as low as 36 mg/kg (i.e. 8 times more toxic than C31G).

Of importance in the tables are the chronic toxicity data from Reference numbers[#] 8, 11 and 15 and related metabolic studies in #s 6, 7, and 12 which with 22 will be treated in Section **VI** by further discussions related to the long term safety of C31G and TheraSol Liquid Dentifrices.

The structural relationships between the Alkyltrimethyl ammonium salts and the alkyldimethyl glycines are shown in the #11 abstract and the further interrelationships appear in #s 5, 10, 13, 14, 33, and 83.

We have included references on eye irritation that cover a wide range of results and presented some difficulties in early studies. The problem was illustrated in early data shown in # 15 where study of an aberrant batch of C31G indicated that either an excess of free amine and/or amine oxide increased dermal toxicity. As potential licensees felt constrained to formulate products with components from their usual suppliers C31G studies were at times conducted with non-standard formulations of C31G. Mucosal and dermal toxicity is most sensitive to an excess of free tertiary amines.

The data in # 24 and 25 compared to some early data illustrates this problem and solution. Of interest regarding mucosal toxicity is the two #s 23 and 24 which rate the same batch of C31G as a mild transient irritant in dogs and a severe eye irritant in rabbits. This may be related to an idiosyncratic hypersensitivity of rabbit platelets to amines.

31 reports on the absence of a mutagenic response in the Ames protocol using type II C31G which contains components of varied alkyl chain lengths.

Two papers related both to efficacy and to safety are discussed in # 27 on wound healing, and 28 on anti-inflammatory activity.

The relation of wound healing to periodontal disease was of interest to Dr Paul Keyes as it coincided with his view of periodontal disease as an infected wound.

The quaternary germicides such as chlorhexidine, CTAB, BAC etc. delay wound healing as these compounds precipitate serum proteins which seal capillaries resulting in inhibition of wound healing by blocking blood circulation to the edge of the wound. The effects on blood by various surfactants are demonstrated in this paper.

Inhibition of inflammation by a germicidal agent is a unique advantage in safety as well as efficacy. Reference to anti-inflamtory effects are shown in # **28.**

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IV: HUMAN SAFETY DATA

A: INDIVIDUAL ACTIVE COMPONENTS.

1: Controlled Studies.
2: Partially Controlled or Uncontrolled Studies.
3: Documented Case Reports.
4: Pertinent Marketing Experiences that may Influence a Determination as to the Safety of Each Individual Active Component.
5: Pertinent Medical & Scientific Literature.

B: COMBINATIONS OF THE INDIVIDUAL ACTIVE COMPONENTS.

1: Controlled Studies.
2: Partially Controlled or Uncontrolled Studies.
3: Documented Case Reports.
4: Pertinent Marketing Experiences that may Influence a Determination as to the Safety of Combinations of the Individual Active Components.
5: Pertinent Medical & Scientific Literature.

C: FINISHED DRUG PRODUCT.

1: Controlled Studies.
2: Partially Controlled or Uncontrolled Studies.
3: Documented Case Reports.
4: Pertinent Marketing Experiences that may Influence a Determinationas to the Safety of the Finished Drug Product.
5: Pertinent Medical & Scientific Literature.

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DOCKET : #81N-0033

IV : HUMAN SAFETY DATA

A: INDIVIDUAL ACTIVE COMPONENTS					B: COMBINATIONS OF INDIVIDUAL ACTIVE COMPONENTS					C: FINISHED DRUG PRODUCT				
1:	2:	3:	4:	5:	1:	2:	3:	4:	5:	1:	2:	3:	4:	5:
CONTROLLED STUDIES	PART. CONTROLLED UNCONTROLLED	DOCUMENTED CASES	MARKETING EXPERIENCES	PERTINENT LITERATURE	CONTROLLED STUDIES	PART. CONTROLLED UNCONTROLLED	DOCUMENTED CASES	MARKETING EXPERIENCES	PERTINENT LITERATURE	CONTROLLED STUDIES	PART. CONTROLLED UNCONTROLLED	DOCUMENTED CASES	MARKETING EXPERIENCES	PERTINENT LITERATURE
#6 #7	NONE	NONE	NONE	# 5 # 8 # 9 #10 #11 #13 #14 #32 #33	NONE	NONE	NONE	NONE	NONE	#35 #36 #37 #38 #39 #40 #41 #42 #43 #44 #76 #77 #80 #81 #82	NONE	#46 #47 #48 #49	#34 #45 ALSO : BOOK I : Appendex 2	#50 #51 #54 #55 #61 #83 #84 #85

TABLE: All 85 Numbered References were considered relevant to these Columnar Headings and, if applicable to any, were entered by Number in the Appropriate Column.

E.B. MICHAELS RESEARCH ASSOCIATES, INC., MILFORD, CT., USA.

SECTION 1V ; HUMAN SAFETY DATA.

DISCUSSION

The initial report in this discussion presents the statistics available on the manufacture and distribution of the two active ingredients of C31G. 210 million pounds per year of the amine oxides are used as foam boosters and cleansing agents in surfactant formulation closely related to human contact. The largest amount is used in residential hand dishwashing compounds, and secondary uses in laundry products, hair shampoos, and drugs, such as Hibiscrub™, a 4% chlorhexidine germicidal hand scrub which contains 2% lauramine oxide and non-ionic surfactants. The use in hand dishwashing compounds with the propensity for oral intake of soap residues and dermal contact in hot water at significant concentration probably gives rise to the greatest risk of systemic absorption. It is likely that this potential exposure led to the extensive studies by Proctor & Gamble's laboratories reported in Section III above.

The alkyl betaines are widely used in body cleansers and shampoos making use of their unique properties as detoxifying agents when added to the anionic fatty acid soaps and surfactants. This detoxifying effect is shown on both mucosal and dermal toxicity, #13, trade literature etc. # 44 where C31G is studied as a replacement for the health care personnel hand wash which was formulated with an alkyl betaine and fatty acid soap and considered at that time the mildest soap in Sweden.

A major factor in judging the safety of these compounds is the biodegradability as this effects behavior of products in sewage and waste disposal. This is a most important factor in human health related to environmental conditions..

Of related importance is the stability of the compounds after formulation into the drug product. These factors are noted in the MSDS reports in # 2,and 33, 35, and 83.

The remaining reports involving human safety data cover the clinical studies which vary from the short exposures of the range finding studies at the University of Pennsylvania to the 6 week study at the University of Maryland and to the 6 month study reported from Moscow. These data are supplemented by the continued experiences shown in the TheraSol usage reports which clearly indicate that acute and chronic toxicity is absent and C31G presents a less toxic alternative to the quaternary ammonium salts.

The experiences of use of C31G in personal care products at concentrations more than 15 times that of TheraSol over the past decade lends more confidence to use of C31G in the liquid dentifrice. This discussion is continued in Section VI.

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36[2]: Abstract: Clinical Studies on a C31G Containing Mouthrinse. Journal of Dental Research, vol. 66, 1987. Corner, A. M. *et al.*

C31G (Type I) mouthrinse was used in a clinical study in a range of 0.00%, 0.05%, 0.1%, 0.2%, and 0.5% on 12 adult subjects at 3-day intervals. Although primarily a range finding study the subjects were monitored for adverse effects. After supplying a stimulated saliva sample for baseline, participants then rinsed with C31G mouthrinse at one of the above concentrations. Subjects then provided saliva samples at 10 minutes, 1, 3, and 6 hours post-rinsing. Each sample was examined for total bacterial colony counts, for proportion of selected species, and for inhibition of glycolysis. The participants were monitored for adverse effects during and after the study.

In conclusion, 0.2% C31G was the lowest effective concentration of mouthrinse causing a significant reduction in the number of oral microbes, and a significant inhibition of salivary glycolysis. ***No adverse effects were exhibited at any concentration studied.*** **[the complete report appears under Reference 77: in § V]**

82[2]: C31G Progress Report: Clinical Studies., Malamud, D. , Dental Institute, University of Penn., Phil. PA, Nov. 1987 Unpublished Data.

This is a continuation of data relate to the clinical range study reported below (J. Clinical Dentistry)

77[2]: Clinical Study of a C31G Containing Mouthrinse: Effect on Salivary Microorganisms. Journal of Clinical Dentistry, 2, pp. 34-38. Corner, A. M. *et al.*

Twelve subjects were exposed at two day intervls to four different concentrations of C31G up to 0.5% concentrations. *No adverse effects were shown in any subject during or after the study. [See § V. Efficacy, for complete Abstract]*

77[2]; Panel Studies on the Comparison of Peridex® to a C31G Mouth Rinse. Unpublished data, E.B.Michaels, 1989.

Using a panel of 60 subjects previous scored for plaque and stain production,of four groups. one group, n = 15 were exposed to C31G III formulated mouth rinse, and two groups exposed to positive controls, commercial mouthrinses and one to a water mouthrinse. The subjects used the mouthrinses as the sole method of oral hygiene for seven days after prophylaxis on day one. Scoring was by erythrosin staining.

No subjects showed adverse effects. The full abstract also appears under Section V., Efficacy.

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50[2]: U.S. PATENT No. 4,839,158 (1989) E.B.Michaels Process and Composition for Oral Hygiene.

Covering C31G compositions with alkyl betaines and alkyl amine oxides for use in dentifrices, mouth rinse and irrigation formulations, to control plaque, gingivitis, and bacterial adhesion to dentition.

Some 60 volunteer subjects were treated under the supervision of dentists with various formulations of C31G for studies of use in mouthwash, toothpaste and irrigation for control of oral pathology. During these studies no significant deleterious effects were reported.

V: EFFICACY DATA

A: INDIVIDUAL ACTIVE COMPONENTS.

1: Controlled Studies.
2: Partially Controlled or Uncontrolled Studies.
3: Documented Case Reports.
4: Pertinent Marketing Experiences that may Influence a Determination on the Efficacy of Each Individual Active Component.
5: Pertinent Medical & Scientific Literature.

B: COMBINATIONS OF THE INDIVIDUAL ACTIVE COMPONENTS.

1: Controlled Studies.
2: Partially Controlled or Uncontrolled Studies.
3: Documented Case Reports.
4: Pertinent Marketing Experiences that may Influence a Determination on the Efficacy of Combinations of the Individual Active Components.
5: Pertinent Medical & Scientific Literature.

C: FINISHED DRUG PRODUCT.

1: Controlled Studies.
2: Partially Controlled or Uncontrolled Studies.
3: Documented Case Reports.
4: Pertinent Marketing Experiences that may Influence a Determination on the Efficacy of the Finished Drug Product.
5: Pertinent Medical & Scientific Literature.

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USFDA OTC REVIEW DOCKET : #8IN-0033					V : EFFICACY DATA									
A:INDIVIDUAL ACTIVE COMPONENTS					COMBINATIONS OF B: INDIVIDUAL ACTIVE COMPONENTS					C: FINISHED DRUG PRODUCT				
1:	2:	3:	4:	5:	1:	2:	3:	4:	5:	1:	2:	3:	4:	5:
CONTROLLED STUDIES	PART. CONTROLLED UNCONTROLLED	DOCUMENTED CASES	MARKETING EXPERIENCES	PERTINENT LITERATURE	CONTROLLED STUDIES	PART. CONTROLLED UNCONTROLLED	DOCUMENTED CASES	MARKETING EXPERIENCES	PERTINENT LITERATURE	CONTROLLED STUDIES	PART. CONTROLLED UNCONTROLLED	DOCUMENTED CASES	MARKETING EXPERIENCES	PERTINENT LITERATURE
NONE	NONE	NONE	NONE	#32 #33	#26 #27 #28 #35	NONE	NONE	NONE	NONE	# # 36 67 37 68 38 69 39 70 40 71 41 72 42 73 52 74 53 75 56 76 57 77 58 78 59 79 60 80 62 81 63 82 64 65 66	NONE	#46 #47 #48 #49	#34 #45 ALSO : BOOK I : Appendex 2	#50 #51 #54 #55 #61 #83 #84 #85

TABLE: All 85 Numbered References were considered relevant to these Columnar Headings and, if applicable to any, were entered by Number in the Appropriate Column.

E.B. MICHAELS RESEARCH ASSOCIATES, INC., MILFORD, CT., USA.

SECTION V. EFFICACY DATA

DISCUSSION

The data collected on the individual components of C31G confirm the synergistic effects of the combination of components as to efficacy. This synergism has also shown by these components in the decrease of dermal and mucosal toxicity provided in the safety data above. Studies of eukaryotic mammalian cell toxicity in reference # 63 show that cell lysis occurs at lower concentrations by either component than by the C31G. This further illustrates such synergism.

The data in the Introductory Tables 6-9 on in vitro antimicrobial activity and the data in Table of # 53, illustrate the wide range of drug resistant organisms which are sensitive to C31G at low levels of exposure.

The #51 is a study of the germicide C_{13} alkyldimethylamine oxide showing an E.Coli strain becomes resistant when exposed to sub-inhibitory concentrations. To date we have not experienced such drug resistance to C31G using both standard cultures or drug resistant strains of Ps. aeruginosa or other organisms..

A characteristic of C31G further manifested is the equivalence in concentrations found between the MIC and the MCC when studied under like conditions. This decreases the probablity of survival of resistant strains.

The limitations of the antimicrobial activity of betaines are shown in # 54 and of other individual componentsin #53 and #55.

The highest sensitivity of gram negative organisms occur in the pH range of about 5.0. Such limitations are hardly unique as all agents have variable activity as a function of pH as well as other conditions. The quaternary germicide, CTAB [# 5], shows an optimum activity toward organisms at the pH of 8 and sharply decreases below the pH of 7. Even aqueous chlorine solutions has orders of magnitude higher efficacy at pH 7 than st higher pH. The effect of pH is of singular importance when studying new compounds in vitro as well under actual use conditions.

C31G is used generally in infection control and therapy between 0.2-0.5 % in a final application concentration. Here the volume applied has adequate buffering to control gram negative as well as gram positive organisms with no apparent overgrowth of resistant species. This has been shown in the long period of use in both personal care and oral hygiene products.

In # 67 which describes the studies related to the development of C31G bovine teat dips for the control of mastitis, a protocol was used which allowed precise study of exposure time for determining the optimum formlations of C31G for this application.

§ V. Discussion, continued.

After inoculation of the sterile teat with the pathogen and exposure to C31G the treated teat was rinsed with a neutralizing solution [Letheen Broth] and the aliquot plated for determination of surviving CFU/ml. In preliminary studies a 3% solution of C31G used at exposure times of 10 to 120 seconds showed no variation of efficacy with time. One minute exposures were used throughout the study.

The references in this Section trace the controlled studies of C31G relevant to oral hygiene,beginning with KemaNobel in Sweden and through the developments in the investigations at the University of Pennsylvania, School of Dental Medicine.. Much of this work is summarized in # **53**.

However a significant finding related to the control of plaque deposition and staining is reported separately in #**79.** Here the inhibition of bacterial adhesion was revealed as a concentration dependent, specific inhibition of binding of the bacteria to hydroxy apatite, only in the presence of saliva. This may be related to the successful control of staining by C31G in TheraSol Liquid Dentifrice, used without polishing agents.

Clinical studies reported include four references; # **77** a range finding study with comparison to Listerine™, #**78** a study with Peridex® and one other commercial mouthrinse re plaque and stain control, # **80** a six week study related to plaque and stain control with Listerine as a positive control and # **81** a six month treatment clinical, studying the effect of TheraSol as a Liquid Dentifrice in treatment of gingivitis, of Type I and of Type II advanced periodontal disease. All of the above clinical trials manifested positive results.

§ V: A. Individual Active Components

51: Immunochemical changes in the Outer Membrane E. Coli Cells Adapted to Amine Oxides, Ceskoslov. FARM. 41, 1992,(9-10),299-302: Bukovsky,M., et. al.[Czech with Eng. Abstract.

he antimicrobial active methyldodecyldimethylamine oxide, and 1-dodecylpiperidin oxide were used to obtain resistant strains of E. Coli by stepwise cultivation in sub-inhibitory concentrations.

Resistant strains were obtained and were obtained with changes in the chemical composition of the outer membranes and also by different antigenic reactions. The resistance by these strains to these "active" compounds is defined by immunodiffusion and immunoelectrophoretic means is by different antigenic reactions related to limitation of sites of action on the outer cytoplasmic membrane.

52[2]: C31G, a New Agent for Oral Use.I. Antiglycolic Tests., Abstract, J. Dental Research (1986),65, 948. A.M. Corner, et al., Abstract. [This data re. individual components is included below in ref# 53.

53: C31G, a New Agent for Oral Use with Potent Antimicrobial and Anti-adherence Properties: Antimicro. Agents Chemother.,12, 350-3, (1988), Corner,A-M, et al. School of Dental Medicine, Un. Penn.

A study of glycolysis is presented here using a protocol to show the drop of pH of salivary sediment due to glucose metabolism by salivary bacteria, when inhibited by the presence of C31G or its components, alkyl dimethylbetaine [ADB] or alkyldimethylamine oxide [ADMAO]. The following data is presented as percent inhibition of glycolysis comparing water to concentrations of 0.5% of C31G or its components. The data is presented as the percent decrease of the drop in pH of water alone to the pH in the presence of the agents. This is reported in the Table below.

Percent Inhibition of Glycolysis

AGENT	*2 hrs*	*3 hrs*	*4 hrs*	*5 hrs*	*6 hr*	*7 hrs*
C31G	*100*	*87*	*75*	*35*	*50*	*33*
ADB	*80*	*33*	*23*	*11*	*22*	*23*
ADMAO	*80*	*33*	*23*	*11*	*17*	*10*

Note the increase of efficacy in the combination of active ingredients over the individual components of C31G. See also § B 53:

55: U.S. Patent No. 4,107,328 (1978) E.B.Michaels.
Antimicrobial Compositions and Methods for Utilizing the Same Employing Mixtures of Amines

Covering C31G compositions using alkyl betaines and alkyl amine oxides which are antimicrobial agents for topical use as deodorants and improving wound healing. In Examples numbered 15 and 16 data is reported on the Minimum Cidal Concentration[MCC] of C31G formulations comprising a pH controlled alkyl betaine[ADMB] with alkyldimethylamine oxide[ADMAO] and compared to the MCC of their components separately. The data in μg/ml is shown in the Table below.

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61: In Vitro Inhibition of Helicobacter Pylori by Surface Active Compounds, Gastroenterology, 1991, 100, AGA Abstr. A40, Cave, T.R. et.al. Un. Hosp. Boston Un. Med. Center.

H. pylori a anaerobe closely associated with peptic ulcers was grown at 37°in a micro-aerobic environment. The MIC of this organism was investigated with three major groups of bile salts, and three synthetic compounds for their inhibition of growth of H. pylori, using blood agar plates. The MIC were investigated over the range of 1-100 mmols/liter.

The agents studied were chenodeoxycholate[**CDC**], deoxycholate[**DC**], taurocholate[**TC**],ursodeoxycholate[**UC**], **CHAPS** a non-denaturing surfactant, **C31G** an amphoteric surfactant, and glutaraldehyde. The MIC in mmols/l follow;

CDC	DC	TC	UC	CHAPS	C31G
20	10	10	50	1.0	1.0

Glutaraldehyde had an MIC of 50 mmols/L, 50 x that of C31G, indicating a possible reason for occasional transmission of H. pylori by endoscopy. The use of C31G at 10 mmols/l as mouthwash is noted.

55[2]: U.S. Patent No. 4,107,328 (1978) E.B.Michaels.
Antimicrobial Compositions and Methods for Utilizing the Same Employing Mixtures of Amines

Covering C31G compositions using alkyl betaines and alkyl amine oxides which are antimicrobial agents for topical use as deodorants and improving wound healing.

**50[2]: U.S. PATENT No. 4,839,158 (1989) E.B.Michaels
Process and Composition for Oral Hygiene.**

Covering C31G compositions with alkyl betaines and alkyl amine oxides for use in dentifrices, mouth rinse and irrigation formulations, to control plaque, gingivitis, and bacterial adhesion to dentition.

The patents are introduced as partially controlled studies with data relevant to efficacy in oral hygiene and topical use in human subjects in significant numbers. This data gave evidence of the safety of the use of the combination of the ingredients without adverse effects.

62: Antiviral Activity of C31G , Presentation at the VII International Conference on Aids, 1991, Florence ,Italy; Corner, A-M, Malamud D; Biosyn Inc. Phil., PA.

HIV 111b or HSV-1 were incubated with graded levels of C31G [Type I] for 2 to 45 minutes , and then serial two fold dilutions were applied to either CEM cells or monkey kidney cells. HIV tiers were estimated by inhibition of syncytia formation or by p24 Elisa. The HIV titers (PFU) were calculated 36 hours post infection.

Toxicity to mammalian cells of C31G was studied on CEM, and SUP T-1 cells as well and antimicrobial activity was determined for a group of pathogenic organisms related to sexually transmitted disease. Comparisons were made with Nonoxynol 9 [N-9].

Results: Exposure of HIV to C31G for 2 to 5 minutes resulted in a greater than 3 log reduction in viral titer an concentrations of 0.025 % [250 ppm]. C31G at 0.03 % or higher resulted in a 7-8 log reduction of HSV titers.

C31G showed significantly less toxicity to mammalian cells than N-9. with exposure of SUPL-1 cells in 10 days exposure at 10 ppm to N-9 or C31G cell growth was 100% greater in the presence of C31G than N-9. The results were duplicated in a 5 day study with CEM cells.

The comparative MIC of the agents are shown below;

	N.GONORRHEA	*T.PALLDIUM*	*S.SANGUIS*	*E.COLI*	*S.AUREUS*	*C.ALBICANS*
N-9	*30 ppm*	*150 ppm*	*2000 ppm*	*>10M ppm*	*>10M ppm*	*>10Mppm*
C31G	*15 ppm*	*80 ppm*	*15 ppm*	*80 ppm*	*20 ppm*	*40 ppm*

In all cases the MCC of C31G was identical to the MIC.

65: A Burned Mouse Model to Evaluate Anti-Pseudomonas Activity of Topical Agents, J. Antimicro. Chemother., 9, 133-140, (1982), Stieritz,D.D., et al.

C31G at 2.6% actives as a liquid or gel was compared with various liquid or cream anti-infective agents used clinically as topical anti-infective agents on burned wounds. The liquids were used as a spray, and the creams or gels spread on infected burn wounds with a spatula. Studies were performed on CF-1 female mice burned over 10% of the body and inoculated with a pathogenic strain of Ps. aeruginosa.

Statistical studies include relating the log concentration (ln) of the infecting inoculum to mortality (M) and to the mean time of death (MTD) showed good correlation; in ln of the concentration to M the correlation coefficient (r) was 0.877, $p < 0.00001$ and with MTD r was 0.714, $p < 0.001$.

Of the sprays which included benzalkonium chloride, Iodophor and silver nitrate at clinical concentrations only C31G spray reduced mortality from the control level.

In the creams Gentamicin Ointment and Iodophor Cream showed no significant efficacy from the control while the other creams or gels, Gentamicin, Benzoyl Peroxide, C31G, Mafenide acetate, and Silver sulfanilamide showed significant decrease of mortality, $p < 0.001$.

Comparison of anti-Pseudomonas activity between in vivo mortality data and in vitro agar diffusion data showed no correlation.

66: Evaluation of a Surfactant Mixture C31G as a Teat Dip by a Modified Excised Teat Model; J. Dairy Science,<u>67</u>,421 (1983), Amin,M.M., et al.

C31G at three concentrations, using an accepted *in vitro* model was compared to both positive and negative controls for antimicrobial activity against two Gram positive and a gram negative organism, *S. aureus*, *S. fecalis* and *E. coli*. The positive control was a 1% iodophor. The results follow, reporting the log reduction from the control;

	Iodophor		C31G	
	1%	3%	1.5%	0.75%
Staph. aureus	3.26	3.53	1.92	1.79
Strep. fecalis	2.16	2.89	1.74	1.54
E. coli	2.83	3.16	1.70	1.59

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73: Inhibition of *Actinomyces* and *Streptococci* Salivary Sediment by mouthrinses., Dolan, M.M., *et al.*, Abstract, Journal of Dental Research, (1986). Presentation Int. Assoc. Dent. Research, Netherlands.

The protocol consisted of preparation of a concentrated, 3/1 salivary sediment. Mouthrinses were diluted 1/1 with the saliva sediment for periods of time of 30 seconds to 5 minutes. then cultured on selective media; Mitis Salivarius agar (Difco) and Actinomyces selective media (Man. Clin. Micro. 469, 1985). After 24 hours growth was rated absent, limited or full.

Growth of species was absent or limited after the limited time of exposure to C31G or chlorhexidine mouth rinses. Marketed mouthrinses such as Scope and Listerine decreased growth of both species, but Viadent was effective only against Actinomyces after extended exposure. Flucriguard and water controls were inactive.

36[2]: Abstract: Clinical Studies on a C31G Containing Mouthrinse. Journal of Dental Research, vol. 66, 1987. Corner, A. M. et *al.See 77•: below.*

74: Binding Studies on the Antimicrobial Surfactant C31G.J. of Dental Research 65, 407 (1986) A.M.Corner, et al.,

A possible mechanism of action of the agent C31G was studied by measuring the binding of ^{14}C labeled C31G to oral organisms and to some organic and inorganic materials.
Bound and free label sampling was separated by filtration. 20-hour cultures of *Streptococcus, Actinomyces, Actinobacillus* and *Candida,* washed and re-suspended in buffer were incubated in labeled C31G for five minutes. Binding curves plotted for total and bound C31G gave the % bound for each organism. Binding plateaued at 4.5 µmols C31G and at this concentration ranged from 0.5ols to 1.5 µmols bound / 10^7 cells.

The % binding was correlated to the minimum inhibitory concentration of the organisms tested. Significantly lower binding was shown for other materials tested; dentine, hydroxy-apatite, glass beads and ground teeth.

52[2]: C31G, a New Agent for Oral Use.I. Antiglycolic Tests., Abstract,J. Dental Research (1986),65, 948. A.M. Corner, et al.

The efficacy of C31G, its components (cocamine oxide and cocobetaine), a positive control (chlorhexidine digluconate) and two commercially available mouthwashes were compared using an accepted plaque model for anti-glycolic activity. The protocol uses agar tipped glass slides coated with 3 X concentrated salivary sediment. Glycolytic activity is expressed as change of pH after exposure to the positive control or water, a negative control, and subsequent incubation.

O.5% C31G or 0.5% chlorhexidine maintained pH over a seven hour period. C31G maintained this ability to inhibit glycolysis when formulated into mouthwash, where the vehicle or water alone should drops in pH below 5 within 3 hours. The mixture of C31G [illegible]

After 24 hours growth was rated absent, limited or full.

75: C31G, a New Agent for Oral Uses. II.- Anti-bacterial Activity, Malamud,D., et al., Abstract, J. Dental Research, 65, 949 (1986).

The purpose of this study was to evaluate prevention of plaque adherence using *Strep. mutans* and inhibition of bacterial growth by agar diffusion studies using a representative group of oral pathogens.

The anti-plaque studies used nichrome wire mesh dipped in test compounds for 10 seconds and then incubated at 37°C in 5% sucrose Trypticase Soy Broth. Mean weight of accumulated plaque was then assessed.

Agar diffusion studies were undertaken on *Streptococcus sanguis, Actinomyces viscous, Bacteroides intermedius, Capnocytophaga sputigena* and *Actinobacillus actinomycetemcomitans* by measuring zones of inhibition.

C31G was as effective as chlorhexidine at all concentrations tested in both assays, in preventing plaque adherence and inhibiting growth, and both were significantly better than Flouriguard and Listerine in the two assays.

77[2]: Clinical Study of a C31G Containing Mouthrinse: Effect on Salivary Microorganisms. Journal of Clinical Dentistry, 2, pp. 34-38. Corner, A. M. *et al.*

C31G (Type I) mouthrinse was used in a clinical study in a range of 0.00%, 0.05%, 0.1%, 0.2%, and 0.5% on 12 adult subjects at 3-day intervals. AT each visit they were given one of the increasing concentrations of C31G, a placebo (the vehicle), or Listerine®, Listerine was administered on the first visit and increasing concentrations of the C31G mouthrinse, starting with the placebo.

Saliva was collected at various times, 10 minutes, 1,2,3 and 6 hours after rinsing and assayed for presence of selected species of salivary bacteria and for inhibition of glycolysis

Two day intervals were maintained between visits. Although primarily a range finding study the subjects were monitored for adverse effects.

Results are reported with significant values of $p<0.05$ as follows:

A significant reduction of salivary bacteria, ~80%, when compared to the placebo, was shown by all concentrations of C31G from 0.1 to 0.5 and by Listerine one hour after rinsing. A significant improvement is shown by the C31G mouthrinse over Listerine at 2 and 3 hours after rinsing. The C31G mouthrinse showed a significant inhibition of glycolysis for up to 6 hours after rinsing, with no inhibition shown by either Listerine or the placebo.

There was no visible staining of teeth or tissues by C31G mouthrinse in these studies, no oral mucosa irritation, and no loss of taste sensation, concluding that C31G-containing mouthrinses may be a valuable aid in oral hygiene.

53[2]: C31G, a New Agent for Oral Use with Potent Antimicrobial and Anti-adherence Properties: Antimicro. Agents Chemother., 12, 350-3, (1988), Corner, A-M, et al. School of Dental Medicine, Un. Penn.

C31G was evaluated for properties related to use in oral hygiene, including a review of safety and efficacy data from the literature. The efficacy studies used standard methods for assay of antimicrobial activities by minimum inhibitory concentration (MIC), the pH dependance of the MIC, inhibition of glycolysis by a salivary sediment method where glycolysis is measured by decrease in pH, and inhibition of *Streptococcus* strains to wire mesh.

MIC studies conducted at pHs of 5.5 and 7.3 indicated that among the 16 organisms tested gram positive organisms and yeasts were least sensitive to pH and gram negative organisms lost sensitivity above pH 5.5. as shown below.

TABLE 1. MICs of C31G

Organism and Strain	*MIC in ppm*	
	pH 7.3	*pH 5.5*

Actinomyces viscosus T4	*8.0*	*8.0*
Lactobacilli casei 27216	*4.0*	*NT[a]*
Staph. aureus	*20.0*	*20.0*
Strep. mutans KPSK2	*15.0*	*15.0*
Strep. sanguis M5	*15.0*	*15.0*
Strep. sobrinus	*15.0*	*12.0*
Actinbac. acetinomycetemcomitans	*20.0*	*NT*
Bacteriodes intermedius	*8.0*	*NT*
Capnocytophagasputigena	*30.0*	*15.0*
E. coli C600	*2000.*	*80.0*
Ps. aeruginosa		
PsA1[b]	*1000.*	*120.*
PsA5[c]	*>2000.*	*1000.*
P3177	*>2000.*	*120.*
P3179	*>2000.*	*500.*
P3180	*>2000.*	*1000.*
Ps. cepacia PsA6[d]	*60.0*	*30.0*
Ps. sp. PsA2[e]	*100.0*	*50.0*
Candida albicans	*40.0*	*40.0*

Notes;

a Not tested

b Resistant to carbenicilin, cefotaxime, and trimethoprim-sulphamethoxazole.

c Resistant to ampicillin, cefazolin, cefataxime, chloramphenicol,and trimethaprim-sulphamethoxazole.

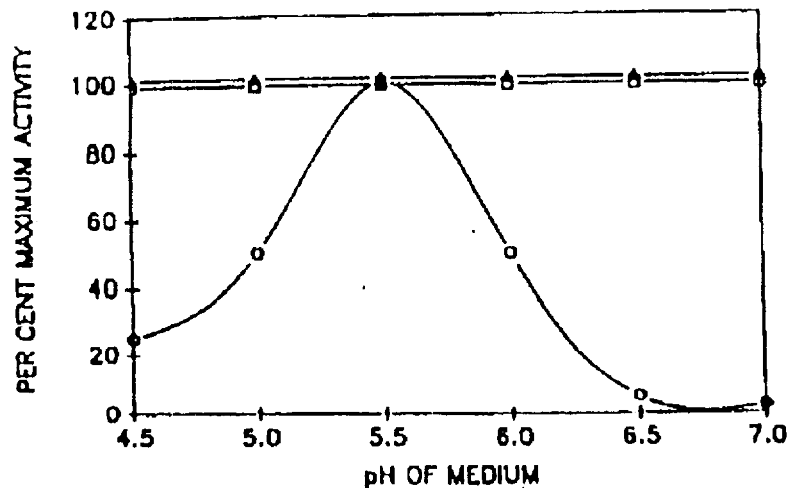
d Resistant to amphicillin, carbenicillin, ticarcillin, cefazolin, cefuroxime, cefatoxime, gentamicin, tobramycin, amikacin, netilmicin, and trimethoprin-sulphamethoxazole.

e Resistant to ampicillin, carbenicillin, ticarcillin, cefazolin, cefuroxime, cefotaxime, chloramphenicol, and trimethoprim-sulphamethoxazole.

Included in these MIC studies was a study which determined the MIC of E. Coli and two gram-positive organisms A. viscosus and Candida albicans,at four pH levels; 4.5, 5.5, 6.5 and 7.0. The maximum activity determined occured at the pH of 5.5 and dropped to 20% of the maximum at 4.5 and to 0 % between 6.5 and 7.0. The gram-positive organisms maintained 100% activity throughout the pH range studied.

A. viscosus and C. albicans were equally susceptible at pHs including 4.5-7.3. The optimum pH for E. coli sensitivity was 5.5.

This is illustrated in the following graph.



Legend: Effect of pH on the MCC; E. Coli (○), A.viscosus(Δ), and C. albicans (□) were grown in the presence of concentrations of C31G ranging from 0.0001 to 0.05% at the indicated pHs. Results are expressed as percentages of maximum activity.

In this same range the authors studied the binding of radio-active C31G to E.Coli (J. Dental Res.1986,65 Abstract No.407; Reference;79:). Here the maximum binding was found to be identical to the maximum activity and the binding followed the identical curve. This is presumed to be related to the mode of action of C31G as the optimum pH was 5.5. The graph is shown with the abstract in Reference **79**:.

Both C31G and chlorhexidine inhibited glycolysis with no drop in pH for 7 hours after exposure to solutions at 0.5%, with the negative control, water, showing a pH decrease of 3.0 units in this time. 0.05% C31G shows no significant pH drop for up to 5 hours. A comparison with the commercial mouthrinses, Viadent, Flouriguard and Listerine showed that chlorhexidine and C31G were the most effective in inhibiting glycolysis, as indicated in the graph of figures 4. and figure 6.

It is also shown here that at 0.05%. C31G, had much greater inhibition of glycolysis than its components, the alkyl betaine or the alkyl amine oxide tested alone. C31G and chlorhexidine were equally effective in inhibiting bacterial adherence. At low concentrations of C31G where growth was still observed in the tubes no adherence was observed on the wire indicating a true anti-adherence effect and not bare wires due to the bactericidal effect.

The diverse properties of C31G suggest that it has applications as an agent to control microbial colonization in potential human and animal infection sites.

79: **Inhibition of Bacterial Adhesion., Malamud,D.,et al. Presentation at the Meeting American Assoc. for Dental Research, March 1986.** *(This was part of this presentation and in the manuscript but omitted from the published paper [53■ above] to conserve space.)*

These studies of bacterial adherence used a model system with hydroxyapatite beads (**HA**) and radioactive *S. sanguis*. To distinguish between specific and non-specific binding assays were carried on with **HA** and saliva treated HA (**SHA**) in the presence or absence of C31G.

C31G has a very significant effect in inhibiting specific binding of S. sanguis to ***SHA****, reducing binding from 6% in the absence of C31G to 0.5% in the presence of 5% C31G. In the absence of saliva the effect of C31G on non-specific binding is minimal. The effect of C31G on bacterial adherence shows a dose response effect over the concentration range of 0.5-5 %.*

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82: Evaluation of the Interfacial Properties of a New Potent Antimicrobial Surfactant C31G; Boll. Chim. Pharmaceutico, 1991, 110, 234-238, Unlü, N. et. al

The surface activity of C31G a mixture of alkyl betaines and alkyl amine oxides were evaluated to determine the relationship between its antimicrobial effectiveness and its physical properties. Determined were; surface tension measurements at a range of temperatures, critical micelle concentrations [CMC], and the interfacial and thermodynamic parameters of C31G calculated from the surface tension data.

Results: Interfacial tension at the air water interface is reported as a function of log molar concentration at the temperatures of 10, 20, 25, 30, 40 50 and 60 degrees C..
The CMC and thermodynamic data were determined from this surface tension data. The CMC for C31G reaches a minimum at ~45°C and is reported in the range of 0.5 mmols/L, below that of benzalkonium chloride.
The surface tension at 0.005% is also reported as 25% lower than this ionic quaternary germicide. The free energy of C31G is also reported.
These physical properties are correlated to the minimum cidal concentrations of the germicides.

83: Primary Biodegradation of Amine Oxides and Quaternary Ammonium Amphiphiles; Folia Microbiol. 38, (1), 43-8; 1993 (Eng.) Cupkova, V., et. al..

The four compound in this study presents the biodegradation of compounds classified as "soft" antimicrobial but with an amide group more resistant to hydrolytic processes. Previous studies had shown that the alkyl amine oxides were readily biodegradable [3]. The last paper by Cupkova et.al., in these references, indicates that the alkyl amine oxides are more readily biodegradable than quaternary ammonium salt germicides. This study was undertaken to determine if the amide linkage in the amido amine oxide affects this relationship.

Two compound types, amine oxides[B and C] and quaternary germicides[A and D], based upon amido derivatives of lauric acid were studied using a potentiometric titration with sodium diphenylborate as titrant and ion selective electrodes for primary degradation, as well as chemical oxygen demand [COD], for total degradationt.

In compounds under study for structure-primary degradation relationship the ester linkage was shown as the linkage most readily subject to hydrolysis, during primary degradation. One compound [D] a bisdodecyldimethyl-3- methylaza-1,5-pentane diammonium bromide was totally hydrolyzed within twenty minutes of exposure. Primary degradation of the other amide compounds was slower. However compound D was not completely biodegradable and is not considered biocompatible.

It is concluded that although Quats are more effective germicides the amine oxides are more convenient as antimicrobials at higher concentrations because of their better biodegradability

The lauramine oxide at 30 ppm was completely degraded within 144 hrs.

84: Amine Oxides, American Perfumer and Cosmetics,84,37-39,(1969, Marsh,B.E.

A review article covering the broad range of properties of aliphatic amine oxides related to use in cosmetics, VXKV structure related to dermal substantivity, physical and colloidal chemistry, surface activity, synthesis, specifications of commercial products and formulation of personal care products. At this time amine oxides were produced at 25-50% concentrations, often with isopropanol added to the aqueous solution to provide a convenient low viscosity liquid for dispensing in formulation of products.

Data on stability indicate a limit at 75°C for acceptable stability with appreciable decomposition at 100°C to the tertiary amine. The non-ionic state persists to a pH of 6.5, with cationic properties becoming evident at lower pH.

Toxicity of the concentrated product is reported at 2-6 gms per kilo for LD50s, and but mildly irritating to the skin and eyes 2% active, while being non-irritating at 1%.

85: Thermal Decomposition of Dimethyllauramine Oxide, JAOCS, 41, 329-331,(1964); Shulman, G.P. and Link W.E..

The principal reaction of the decomposition of the lauramine oxide is deoxygenation to the tertiary amine, with some formation of 1-dodecene. Here the rates of decomposition of the amine oxide is determined between 80 - 100°C. Comment is made regarding the rate dependency on the dissociation of the hydrate of the amine oxide. The study was conducted A) by gas chromatography or B) by immersion in constant temperature bath an titration. There is no significant decomposition below 80°^C.

The rate constant for decomposition decreases from 1.07 0.104 between 100° and 80° C. As indicated elsewhere[4] decomposition is negligible in more dilute solution. Note that the hydrate ADMOX 14-86 is reported (Ethyl Corp.) as having greater stability than other grades of ADAO.

---

[4] JAOCS,55,(7),268(1963),Hoh,G.L.K.,et.al
JACS,85,1263-1268(5)(1963),Sahyun,M.R.V. and Cram, D.J.

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pages of trade

secret and/or

confidential

commercial

information

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VI: A SUMMARY

A Summary of the Data and Views Setting Forth the Medical Rationale and Purpose (or lack thereof) for the Drug and its Ingredients and the Scientific Basis (or lack thereof) for the Conclusion that the Drug and its Ingredients have been Proven Safe and Effective for the Intended Use. If there is an Absence of Controlled Studies in the Material Submitted, an Explanation as to Why such Studies are not Considered Necessary Must Be Included.

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SECTION VI; SUMMARY DISCUSSION and COMMENTARY

The purpose of this summary is to provide evidence that C31G deserves the classification of a generally regarded as safe drug [GRAS] based upon the data and history of usage available on C31G and the components of C31G, their structure and the related studies reported in peer reviewed journals.

The foregoing abstracts and discussions cover most of the controlled data available. The following summary of material above will be cogent to to this presentation.

One group of compounds, the alkyl trimethyl ammonium salts e.g. CTAB, cetyl trimethyl ammonium bromide:

$$\left[CH_3(CH_2)_{15} - \overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{N}}}} - CH_3 \right]^+ \quad Br^-$$

has a structure analagous to that of the cetyl betaine used in TheraSol. The difference comprising only the replacing of the terminal($N^+ - CH^3$, X^-) group of the quaternary salt by the glycine group $N^+ -CH_2\ COO^-$. the negative charge of the carboxylate forming an inner bond with the quaternary nitrogen in the betaine, instead of the ionic bond with the external ion, of bromine or chlorine, etc. as shown below. In this respect the betaine is the analogue of CTAB and a homologue of the amino-acid, betaine [trimethyl glycine], widely occuring in animals and plants. The structure of CTAB follows:

$$CH_3(CH_2)_{15} - \overset{\displaystyle CH_3\ +}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{N}}}} - CH_2 - \overset{\displaystyle {}^-O}{\overset{|}{C}}{=}O$$

There is a lack of long term chronic toxicity studies on the alkyl betaines, but ample sub acute chronic studies. However this compound is a derivative of the amino acid, betaine, or trimethyl glycine, present in both the animal and plant kingdom and also an intermediate in the conversion of choline to glycine[5]. Choline is a quaternary ammmonium ammonium base, also found in many plants and animals which forms salts homolagous to the quaternary ammonium germicides. It is also a constituent of lecithin, an edible surfactant.

---

[5]Biochemistry with Clinical Correlations; Devlin,T.M.,ed.1982, John Wiley & Sons, N.Y; pg 578.

The structure of betaine follows:

$$CH_3 - \overset{CH_3}{\underset{CH_3}{N^+}} - CH_2 - \overset{O^-}{C}=O$$

The structure of choline as the chloride follows:

$$[\ H_2OH - CH_2 - \overset{CH_3}{\underset{CH_3}{N}} - CH_3\ ]^+\ Cl^-$$

Trimethyl amine oxide is a common compound occuring as a renaturant in blood and tissue of marine animals in conjunction with betaine, and in the detoxication of tertiary amines in metabolic processes [# 9]. The structure of the alkyl amine oxides are represented as:

$$R - CH_2 - \overset{CH_3}{\underset{CH_3}{N^+}} :\ddot{\underset{..}{O}}:^- \quad \text{or} \quad R - CH_2 - \overset{CH_3}{\underset{CH_3}{N}} \rightarrow O$$

The ionic structure above was shown by Linus Pauling[6] to be based upon electron sharing of coordinate bonds which converts this group to a quaternary moiety that readily forms hydrates and salts. It is non-ionic above the pH of 7 becoming more cationic at decreasing pH.

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[6] The Nature of the Chemical Bond, Pauling, L (3rd ED.1960), Pg 7-10. Cornell Un. Press, Ithaca,N.Y.

Including oral, dermal and mucosal toxicity, the comparative increase, of toxicity of various C31G related agents studied, as shown in animal safety studies, are:

C31G < alkyl betaine < alkyl amine oxide < T-Alkyl amine < alkyltrimethyl ammonium salts < benzalkonium chlorides.

The order of efficacy is somewhat different than the above order in that C31G rivals benzalkonium chloride in antimicrobial activity. It is unlikely that the mode of C31G action is based upon the same type reactions as benzalkonium chloride.

In addition to the C31G 110 day chonic oral toxicity reported in # 15, there is a two year toxicological study on rats [oral] and mice [dermal] using varied dosages up to an effect level to define the carcinogenic potential of a commercial cocamine oxide [ADAO].

Long term toxicological studies of monoquaternary alkyl and benzyl quaternary ammonium germicides are available in # 11 [pgs 534-ff and appendices 3,4], as is the alkyltrimethyl ammonium chloride shown in the summary Animal Safety Table 1.

A number of these studies carried out for 5 weeks to 2 years indicated no increase in neoplasms relative to controls. Mucosal toxicity limited study to about 12.5 mg/ kg, however an alkylbenzyltrimethyl ammonium chloride and a benzethonium chloride were studied at concentrations up to 0.5% for two years with significant mortality only at the 0.5% level. Here histopathology also showed no neoplasms greater than the control level.The 2 year toxicological study on ADAO at an effect level showed no increase in neoplasms.

The absence of the induction of mutations in the Salmonella /Microsome assay with C31G, # 31 and with both dodecyl and tetradecyl ADAO reported in #10, which includes studies of in vitro embryonic cell transformation, shows the absence of embryo transformation by these compounds.

It should be clear from this data that the substitution of long chain alkyl groups to the above noted trimethyl quaternary nitrogen terminal polar groups is unlikely to introduce carcinogenic properties to compounds widely occurring in animals as normal recycling or detoxifying metabolic processes.

This is perhaps more evident in C31G formulations in that the combination of the alkyl betaine with the alkyl dimethylamine oxide further decreases toxicity significantly from that of the components of C31G.

Actual experience, controlled or partially controlled shows dermal exposure to large populations over a decade and oral exposure during the past four to five years in the TheraSol Usage Reports [#s **45**, **46**, **47**, **48**, **49**] and clinical studies over shorter time periods, but clearly indicating low or absent dermal and mucosal toxicity.

The combination of extraordinary safety and efficacy of TheraSol as a liquid dentifrice and as an oral irrigatant in dental offices provides a degree of oral prophylaxis not surpassed by any other oral hygiene product available for patient use. Whereas there are considerable benefits available in the use of C31G as a mouthrinse, use of C31G in tooth brushing, makes a significant contribution to decreasing of both subgingival and supragingival plaque. This is evidenced clearly in the report from Moscow, # **81**. The indication has been obtained that these studies will be extended in a clinical trial to start within a few months.

Five reports noted in this dossier are related to toxicological properties of C31G and the related compounds demonstrating the disposition of the absorbed dose. These reports include # **22** on C31G and the data on ADAO as DDAO appears in #s 7 and **8**. Data on the alkyd trimethyl ammonium germicide CTAB is shown in # **12** and a referenced paper[7].

All studies show that a major portion of absorbed activity is excreted in the first 24 hours, that a major portion of this is excreted in the urine. A significantly higher percentage of the administered oral or dermal dose is shown by both DDAO and C31G. In oral dosage at levels of 100 mg/kg or more, 70 % of C31G appears as systemic absorption and 65% of DDAO is so absorb. Although major amounts of absorbed DDAO are excreted as expired CO_2, this obvious has no effect on potential carcinogenicity.

Oral dosage in CTAB reported in # **12** as limited by mucosal toxicity, and was used here at 0.8% with 3.2% of the administered dose absorbed systemically and 48% excreted within 48 hours bile and urine. In the noted Hughes reference the using a dose of 15 mg/kg I.P. the disposition of the absorbed dose was the same. The reference in of the 2 year feeding studies shown for the alkyltrimethyl ammonium germicide from # **11, above,** indicates a safe dosage at the 2% level in water which also indicate no toxicity after 2 years. It is likely that the 0.5% limitation on aqueous oral dosing is limited to benzalkonium chlorides and the like.

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[7]Molecular Weight as a Factor in the Excretion of Monoquaternary Ammonium Cations in the Bile of Rat,Rabbit and Guinea Pig. Hughes,R D et.al., Biochem.J.(1973)136, 967-978.

MICRODENT 200

...CONFIDENTIAL

WhiteHill Oral Technologies, Inc.

IRA HILL, PH.D., CHAIRMAN
ROBERT D. WHITE, PRESIDENT
DALE G. BROWN, PH.D., DIRECTOR R&D

FDAB
June 17, 1991
201-295-8000

Division of OTC Drug Evaluation (HFD-210)
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857

Re: (1) 21 CFR Part 356 [Docket No. 81 N-0033] Over-The-Counter Dental and Oral Health Care Drug Products for Antiplaque Use, Safety and Efficacy Review; and

(2) FDA Request for Data and Information as set forth in Federal Register Vol. 55, No. 182, Wed. Sept. 19, 1990, pp. 38560 thru 38562.

A. ACTION:

In response to the referenced FDA request for data, WhiteHill Oral Technologies, Inc., WhiteHill, hereby submits data and information relating to the marketing of an active ingredient comprising a mixture of a poloxamer 407 and simethicone generally marketed under the trademarks MICRODENT™, ULTRAMINT™ and OMNiiDENT™ in the products, Take-5™, OMNii® Plaque Fighter and OMNii® Nighttime Spray with relevant antiplaque claims, such as "PLAQUE FIGHTER", "fight plaque buildup", "disrupt plaque formation" and "makes teeth so slick-plaque won't stick. See Exhibit I which shows:

a. four distinct versions of the brand Take 5, i.e.: Take 5 PLAQUE FIGHTER™ with MICRODENT 200™, Smoker's Take 5 Plaque and Stain Fighter™ with ULTRAMINT 200™, Take 5 For Dentures™ with MICRODENT 300™, and Take 5 For Braces™ with MICRODENT 400™, and

b. two OMNii® products with OMNiiDENT™.

WhiteHill requests the Agency review the enclosed marketing data and information.

WhiteHill further requests the Agency find pursuant to the provisions of 21 USC 321(p)(2) that the active ingredient generally described as MICRODENT™, ULTRAMINT™ and OMNiiDENT™, has been:

a. marketed to a material extent, i.e. approximately of various versions of the Take 5 brand product containing MICRODENT™ or ULTRAMINT™ were sold to date, at retail in the U.S. nationally by over 280 retail accounts, (See Exhibit II), thru food, drug mass merchandise, convenience, warehouse, department stores and specialty shops using various

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retail programs as set forth in Exhibit III including "clip strips", display trays, "hook programs", "shelf talkers" etc. See Exhibit III and with national advertising in magazines such as McCalls, See Exhibit IV, and

b. marketed for a material time, i.e. from Nov. 1986 thru the present. See Exhibit II, III, and IV.

Thus, WhiteHill submits this active ingredient has been used to a material extent and for a material time as these terms are described in Weinberger v. Hynson, Westcott & Dunning, Inc. 412 U.S. 609, 628-32 (1973); Weinberger v. Bentex Pharmaceuticals, 412 U.S. 645, 652-54 (1973); Premo Pharmaceutical Laboratories, Inc. v. United States, 629 F.2d 795, 803 (2nd Cir. 1980); United States v. Articles of Drug..Promise Toothpaste..624 F. Supp. 776, 779-86 (N.D. Ill. 1985).

WhiteHill further submits that this active ingredient was marketed and advertised in national magazines such as McCalls with a relevant indication of an antiplaque claim, i.e. "PLAQUE FIGHTER", "FIGHTS PLAQUE", "cleans and reduces the materials that form plaque", FIGHTS PLAQUE BUILDUP", "disrupts plaque formation" and "makes teeth so slick, plaque won't stick". See Exhibit IV.

WhiteHill further requests that, in view of the marketing data and information enclosed herewith, the Agency find that the "PLAQUE FIGHTER" active ingredient generally described as MICRODENT™, ULTRAMINT™ and OMNiiDENT™ is eligible for: review under the OTC drug review procedures and the submission herein qualifies as a response to be considered by the FDA in their request for data and information as set forth in the Federal Register Vol. 55 No. 182, Wed. Sept. 19, 1990, pp. 38560 thru 38562.

B. STATEMENT OF GROUNDS:

In accordance with the provisions of 21 U.S.C. 321(p)(2) the active ingredient comprising a mixture of poloxamer 407 and simethicone, generally described as MICRODENT™, ULTRAMINT™ and OMNiiDENT™ has been marketed in various versions of the spray products Take-5™, OMNii PLAQUE FIGHTER and OMNii NIGHTTIME SPRAY, (see Exhibit I) to a material extent (see Exhibit II) for a material time (see Exhibit II) using various merchandising programs in various channels of distribution, See Exhibit III; while nationally advertising various relevant antiplaque claims, i.e. "PLAQUE FIGHTER" etc. (see Exhibit IV).

CONCLUSION

WhiteHill has requested the FDA review the data and information submitted herewith and find:

1. that the active ingredient generally marketed as MICRODENT™ and ULTRAMINT™ has been marketed to a material extent and marketed

for a material time; per 21. USC 321(p)(2), and

2. that the active ingredient, under trademarks including: MICRODENT™, ULTRAMINT™ and OMNiiDENT™ has been marketed to a material extent and for a material time with a relevant plaque claim, per the requirement set forth in Federal Register Vol. 55 No. 182, Wed. Sept. 19, 1990 page 38562.

C. CERTIFICATION:

The undersigned certifies, that, to the best knowledge and belief of the undersigned, this submission of marketing data and information includes representative marketing data and information on which WhiteHill relies to qualify under the FDA Sept. 19, 1990 call for data.

Review of this marketing data and information has been requested. A favorable determination is requested that the active ingredient generally described as MICRODENT™, ULTRAMENT™ and OMNiiDENT™:

1. qualifies under 21 USC 321(p)(2), and

2. is eligible for review according to the FDA Sept. 19, 1990 call for data: the Federal Register Vol. 55 No. 182 Wed. Sept. 19, 1990.

Your review and favorable determination will be appreciated.

Sincerely,

WhiteHill Oral Technologies, Inc.
Robert D. White
President, Chief Marketing Officer

RDW:gw
Encl.

WhiteHill Oral Technologies, Inc.

IRA HILL, PH.D., CHAIRMAN
ROBERT D. WHITE, PRESIDENT
DALE G. BROWN, PH.D.,DIRECTOR R&D

June 17, 1991
201-295-8000

Division of OTC Drug Evaluation (HFD-210)
Center for Drug Evaluation & Research
Food & Drug Administration
5600 Fishers Lane
Rockville, MD 20857

Re: (1) 21 CFR Part 356 [Docket No. 81 N-0033] Over-the-Counter Dental and Oral Health Care Drug Products for Antiplaque Use, Safety and Efficacy Review; and
(2) FDA Request for Data & Information as set forth in Federal Register Vol. 55. No. 182, Wed. Sept. 19, 1990, pp. 38560 thru 38562.

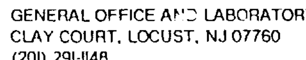
INTRODUCTION

The concept of classifying an article as a "new drug" or "old drug" (GRAS/E) is used in the regulation of over-the-counter ("OTC") drugs as well as prescription drugs. The OTC Drug Review is a process whereby FDA's Advisory Panels recommend GRAS/E marketing status for various OTC ingredients rather than requiring the products containing these ingredients to go through the new drug application ("NDA") process.

Thus, the present OTC drug review and call for data of Sept. 19, 1990 is primarily an ingredient review (as distinguished from a "product") that should culminate in the development of a monograph for plaque and gingivitis related oral health products. During this review, the active ingredients are studied and a determination is made as to whether a given active ingredient is generally recognized as safe and effective ("GRAS/E") and thus an "old drug". If so, this active ingredient is included in the proposed monograph for plaque and gingivitis OTC health care drug products.

A. ACTION: In response to the referenced FDA call-for-data and per the format set forth in OTC DRUG REVIEW INFORMATION, 21 CFR CH. 1 (4-1-90 Edition) WhiteHill Oral Technologies, Inc. (WhiteHill) hereby submits studies and information relating to a plaque fighter, active ingredient comprising a mixture of a nonionic surfactant, such as poloxamer 407, and a polydimethylsiloxane, such as simethicone, generally marketed under the trademarks MICRODENT™, ULTRAMINT™ and OMNiiDENT™.

WhiteHill hereby requests the Agency review the enclosed studies and information (as outlined in the attached Table of Contents per 21 CFR 330.10 copy attached) and further supplemental



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studies and information to be filed in the future relating to this active ingredient.

B. STATEMENT OF GROUNDS:

In accordance with the provisions of 21 CFR 330.10(a)(4), WhiteHill has engaged in a number of adequate and well controlled studies beyond that in the literature to demonstrate the safety and effectiveness of the active ingredient as a plaque fighter.

These studies and the relevant literature are detailed in the enclosures submitted herewith.

In addition to the clinical studies reported herein, WhiteHill plans to perform further studies to demonstrate the safety and effectiveness of this plaque fighter active ingredient in various oral health care drug products.

CONCLUSION

WhiteHill requests the FDA review the studies and information submitted herewith. Upon review of these studies and information, WhiteHill requests that the FDA find that the active ingredient meets the provisions of 21 CFR 330.10(a)(4) and is safe and effective as a plaque fighter, suitable for use in various oral health drug products as described in detail in the enclosures submitted herewith.

C. CERTIFICATION:

The undersigned certifies, that, to the best knowledge and belief of the undersigned, this submission includes all current information and views on which WhiteHill relies, and that it includes representative data and information known to WhiteHill which are unfavorable to the request for GRAS/E status of the active ingredient.

Your review of these studies and information and favorable determination will be appreciated.

Sincerely,

WhiteHill Oral Technologies, Inc.
Ira D. Hill, Chairman,
Chief Scientific Officer

IDH:gw
Encl.

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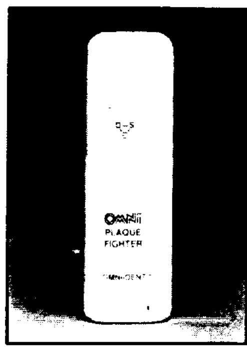
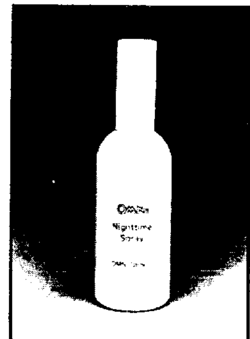
VI. Summary of Data and Views Setting Forth Medical Rationale and Purpose for Drug

DISRUPT PLAQUE FORMATION frequently, and you CONTROL GUM DISEASE.

INTRODUCING . . .





. . . recommended by your DENTIST as a supplement to your present oral hygiene . . . for the disruption of plaque throughout the day.

OMNII® PLAQUE FIGHTER
OMNII® NIGHTTIME SPRAY
with
OMNIIDENT™

- Has been clinically proven to fight plaque buildup, when used several times throughout the day, after meals, snacks, coffee breaks, etc.
- Is not a substitute for brushing, but it is an effective alternative to **not brushing** after every meal, snack, coffee break etc.
- Reduces the debris normally found in the mouth throughout the day, while leaving a clean just-brushed feeling and a freshened breath that lasts for hours.
- Nighttime Spray fights the **cause** of "morning breath" as it occurs . . . **throughout the night!**

"Makes teeth so slick — Plaque won't stick"

U.S. Patent 4,950,479, other Pats. Pend.
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OMNII PLAQUE FIGHTING SPRAYS

Your oral health office has made available to you the latest and only plaque fighting mouth sprays. These state-of-the-art plaque fighting sprays are designed to do the following:

1) Stop bad breath
2) Stop plaque build-up
3) Stop denture odor
4) Help control periodontal disease
5) Protect your teeth and gums

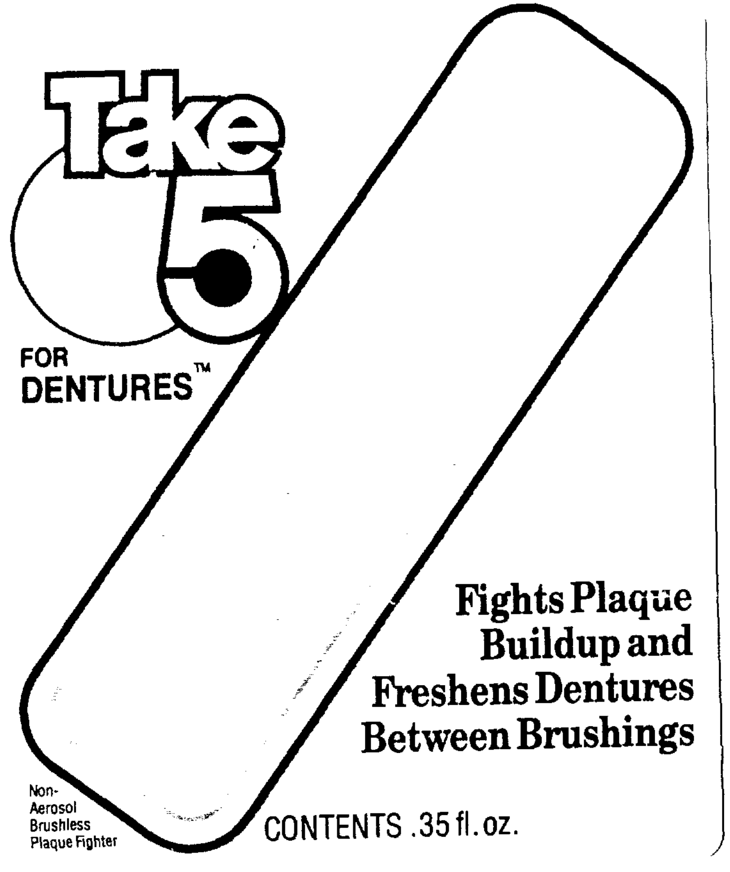
These sprays have been made available by your doctor direct from the manufacturer to save you **time** and **money.** They are to be used throughout the day and night. The sprays are to be utilized with brushing and flossing and especially after meals and snacks when you are unable to brush and floss.

The OMNII PLAQUE FIGHTER SPRAY, conveniently sized for your pocket or purse, is to be used 4 to 5 times a day. OMNII NIGHTTIME SPRAY is to be used at night before retiring and in the morning after rinsing to prevent morning breath!!!

The sprays are available in a natural, refreshing flavor and at professional strength.

If you have any questions about this spray, please ask your doctor or a member of the staff.

(813) 576-9100 / (800) 284-4123
1-800-643-3639



DT5

Tamper resistant package. Use only if package is sealed.

Take 5 for Dentures* is a Brushless Dentifrice* that fights **PLAQUE AND DENTURE BREATH** where it starts, by helping remove food particles, staining substances and other materials that get trapped under and around dentures.

Regular use of **Take 5 for Dentures** after meals, snacks, coffee breaks, smoking etc gives your mouth that "clean, just brushed" feeling without removing dentures. Partial dentures feel fresh, clean & comfortable when sprayed with **Take 5 for Dentures** before replacing in mouth. Convenient, shatterproof package fits pocket or purse. Approximately one month s supply.

DIRECTIONS

Hold near lips, pump 2 or 3 sprays into mouth onto tongue. Rub tongue over dentures, gums and surfaces of mouth. Swallow. Use regularly as a supplement to current oral hygiene. **Avoid spraying in eyes. KEEP OUT OF REACH OF CHILDREN.**

INGREDIENTS

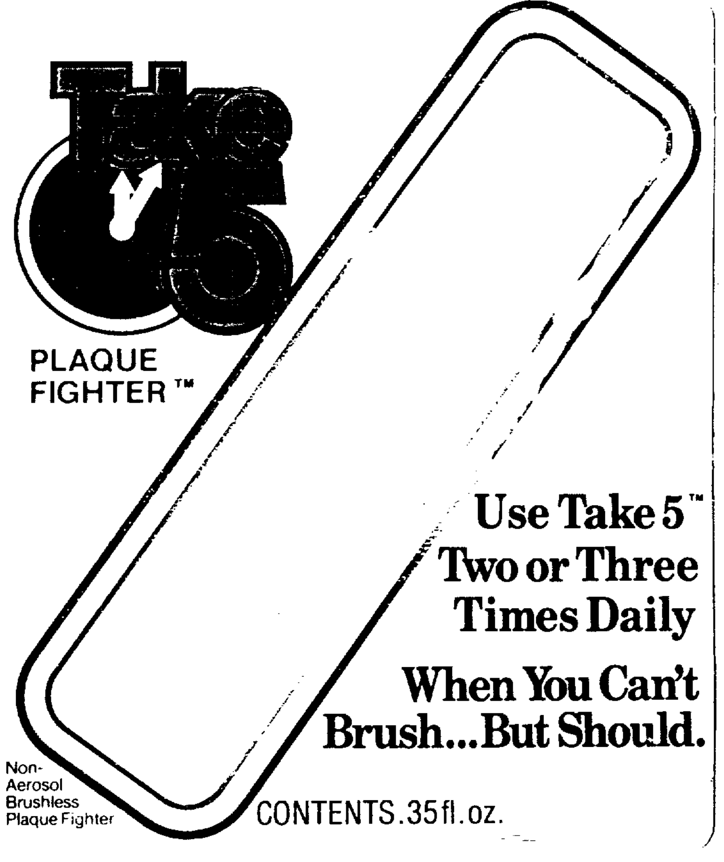
Microdent 300* is a Brushless Dentifrice* base of alcohol SD38B, deionized water, sorbitol, glycerine, flavor, saccharin, methyl-cellulose and FD & C Blue 1 and Yellow 10.

Microdent 300 is a trademark for polaxamer 407-simethicone based proprietary denture cleaner and mouth conditioner. **Take 5 for Dentures***, Microdent 300* and Brushless Dentifrice* are trademarks of

PROOF OF PURCHASE

Princeton
Pharmaceutical Inc., Div. of CLI,
Paterson, NJ 07501, Patents Pending

1988 Princeton Pharmaceutical, Inc.



T5-PF

Tamper resistant package. Use only if package is sealed.

Take 5 Plaque Fighter™ is a Brushless Dentifrice™ in a non-aerosol spray. Use regularly after meals, snacks, coffee breaks, smoking etc. . . "when you can't brush . . . but should" Regular use of **Take 5 Plaque Fighter™** cleans teeth, helps to reduce the accumulation of food particles, staining substances, and reduces the amount of materials that form plaque. It gives your mouth "that clean, just brushed feeling". . . IN SECONDS! Over 100 measured sprays, approximately a one month supply. Convenient shatterproof package fits pocket or purse.

DIRECTIONS

Hold near lips, pump 2 or 3 sprays into mouth onto tongue. Rub tongue over teeth, gums and surfaces of mouth. Swallow. Use regularly "when you can't brush . . . but should". **Take 5 Plaque Fighter™ is to be used throughout the day as a regular supplement to current oral hygiene. Avoid spraying in eyes. KEEP OUT OF REACH OF CHILDREN, EXCEPT UNDER ADULT SUPERVISION.**

INGREDIENTS: *Microdent 200™ in a Brushless Dentifrice™ base of alcohol SD38B, deionized water, sorbitol, glycerine, flavor, saccharin, methyl-cellulose and D & C Red 33.

*Microdent 200™ is a trademark for a poloxamer 407-simethicone based proprietary mouth conditioner

Take 5 Plaque Fighter™, Microdent 200™ and Brushless Dentifrice™ are trademarks of

PROOF OF PURCHASE

0 50729 80001 6

Princeton Pharmaceutical Inc.,
Paterson, NJ 07501, Patents Pending

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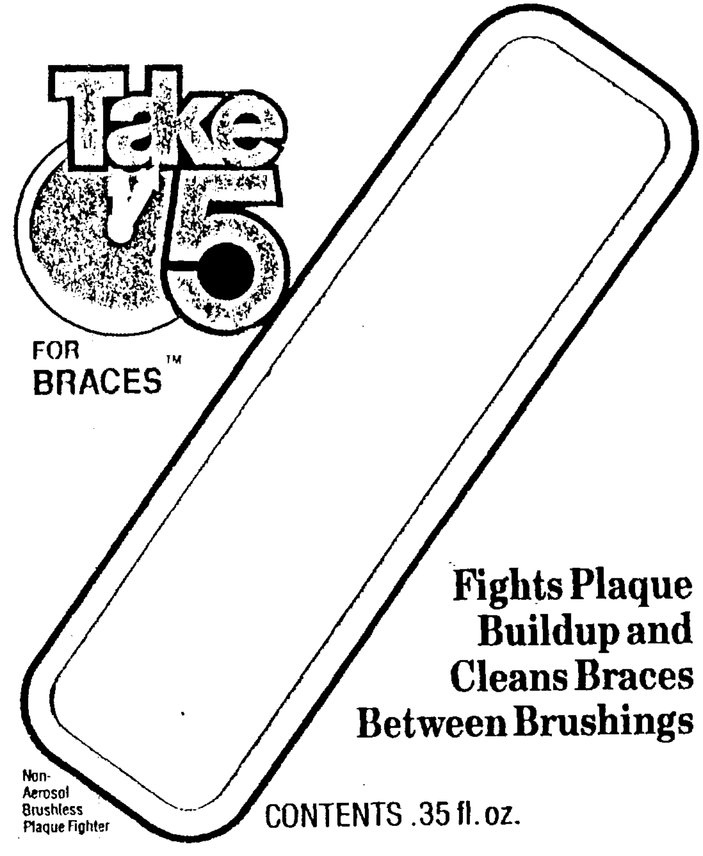

BT5

Tamper resistant package. Use only if package is sealed.

Take 5 for Braces™ is a Brushless Dentifrice™ in a nonaerosol spray with sugar free sweetness of **XYLITOL.** Cleans under, around and between braces. **Regular** use of **Take 5 for Braces** cleans breath & reduces:

- Accumulation of food particles,
- Odor causing materials,
- Staining substances, and
- The materials that form **PLAQUE.**

Three sprays of **Take 5 for Braces** with MICRODENT 400™ fights Braces Breath. Approximately one month's supply. Convenient shatterproof package fits pocket or purse.

DIRECTIONS

Hold near lips, pump 2 or 3 sprays into mouth onto tongue. Rub tongue over braces, teeth and mouth surfaces. Swallow. Use **regularly** throughout the day as a supplement to current oral hygiene. **Avoid spraying in eyes. Keep out of reach of small children, except under adult supervision.**

INGREDIENTS

Microdent 400™ in a Brushless Dentifrice™ base of deionized water, alcohol SD38B, xylitol, sorbitol, glycerine, flavor, methylcellulose, saccharin and FD & C Red 4 and Yellow 6

*Microdent 400™ is a trademark for a poloxamer 407-simethicone based proprietary braces cleaner and mouth conditioner. **Take 5 for Braces**™, Microdent 400™ and Brushless Dentifrice™ are trademarks of

Princeton Pharmaceutical Inc.,
Paterson, NJ 07501, Patents Pending

PROOF OF PURCHASE

0 50729 80004 7

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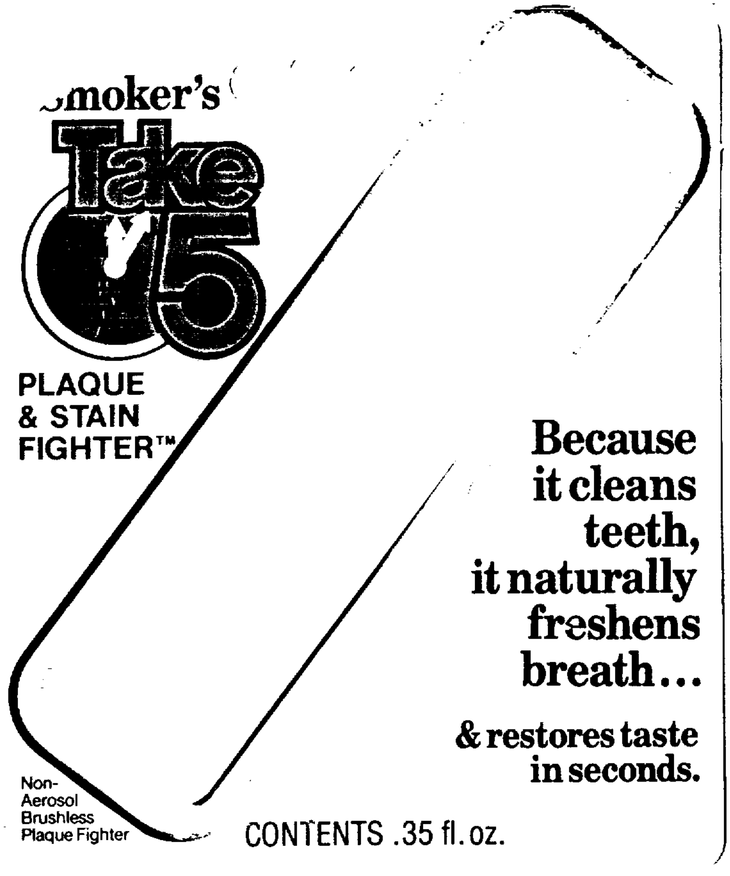

ST5-PSF

Tamper resistant package. Use only if package is sealed.

Smoker's Take 5 Plaque & Stain Fighter™ with Ultramint 200™ is a Brushless Dentifrice™ in a non-aerosol spray. **Smokers Take 5™** cleans teeth, naturally freshens breath . . . and restores taste in seconds. Regular use of **Smoker's Take 5™** cleans breath and reduces:

- Accumulation of smoke particles
- Odor causing materials
- Staining substances and
- The materials that form plaque.

Three sprays of **Smoker's Take 5™** with Ultramint 200™ are more effective than an *entire* package of mints or breath deodorant mints. Approximately one month's supply. Convenient, shatterproof package fits pocket or purse.

DIRECTIONS:
Hold near lips, pump 2 or 3 sprays into mouth onto tongue. Rub tongue over teeth, gums and surfaces of mouth. Swallow. Use regularly after you smoke as a supplement to current oral hygiene. **Avoid spraying in eyes. KEEP OUT OF REACH OF CHILDREN.**

INGREDIENTS: Ultramint 200™ in a Brushless Dentifrice™ base of alcohol SD38B, deionized water, sorbitol, glycerine, flavor, saccharin, methyl-cellulose and FD & C Blue 1.

*Ultramint 200™ is a trademark for a poloxamer 407-simethicone based proprietary smoke particle/stain remover mouth conditioner.
Smoker's Take 5 Plaque and Stain Fighter,™ Ultramint 200™ and Brushless Dentifrice™ are trademarks of
Princeton Pharmaceutical Inc.,
Paterson, NJ 07501, Patents Pending

PROOF-OF-PURCHASE

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Dentifrice spray fights plaque

For all those times when you can't brush after meals, you can do the next best thing: use a dentifrice spray.

If that sounds like a new product category, you're right. Entrepreneurs Ira Hill and Bob White have developed "Take 5", a mouth spray that they claim fights plaque, based on surfactant ingredients that actually interrupt the formation of plaque on teeth.

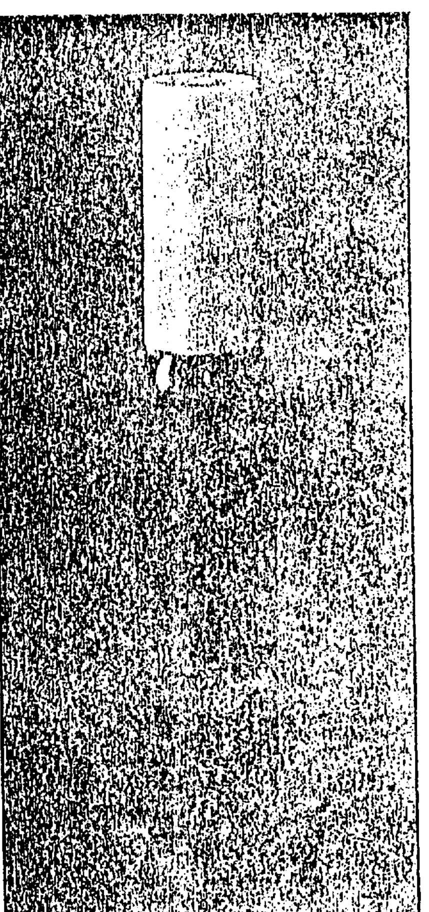
While it won't remove plaque—only abrasion will do that—it is a substantial improvement in oral hygiene, claims Hill. He says the product should be used anytime one can't brush, but should: after meals, smoking, etc.

Hill is director of research, and White is president of Princeton Pharmaceuticals, Inc., which they founded expressly to market Take 5. They stress that the product is not a breath freshener, but a "mouth cleaner," although, observed Hill, a clean mouth smells good.

According to him, studies indicate that sloughed off cells from the mouth interior break down and cause mouth odor. Take 5's surfactants will clean all cellular debris in the mouth, thus the mouth will smell fresher. At the same time, plaque buildup will be slowed considerably. But they want to be sure that the consumer doesn't confuse this with breath fresheners.

Because "our whole positioning is that this is not a breath freshener but a mouth cleaner," Hill said the product was formulated to be slightly viscous, just enough to give it a "slower moving bubble" as the bottle is tilted. While it isn't so viscous as to be syrupy, it does look richer, more concentrated, more businesslike. The product does not contain fluoride.

"We did a consumer acceptance test in Texas," Hill told AEROSOL AGE, and 80% of the respondents gave us the top three boxes on a six point test." The top three boxes on the consumer acceptance survey were: 6: Definitely will buy, 5: Probably will buy and 4: Likely to buy. "We had all the right demographics," he added.



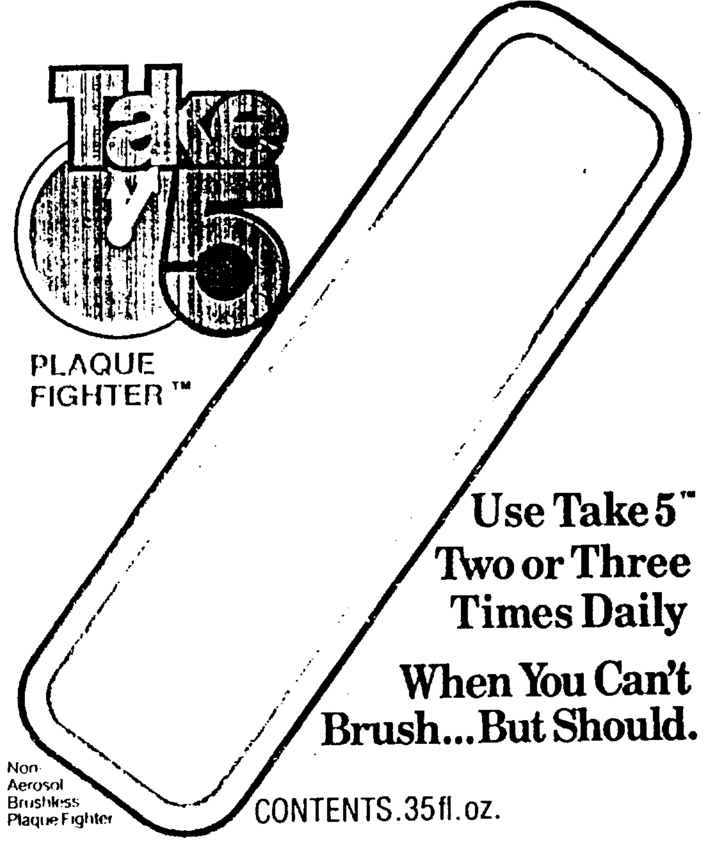
As of press time, two major supermarket chains in the Northeast not only have agreed to carry Take 5, but both will do price point tests to determine an appropriate retail price.

Take 5 is unusual in another way: the spray pump is crimped onto a PET* plastic bottle supplied by Wheaton. Hill said the .35 oz plastic bottle, much like its larger cousins now ubiquitously sold for soft drinks, was chosen for three specific reasons. It is crystal clear, it has the requisite strength ("You can't have a bottle shatter in the purse or pocket.") and PET is particularly resistant to flavor transmission. The see-through bottle allows the purchaser to distinguish this product from the traditional breath fresheners in their aluminum tubes. Pharmasol fills the product for Princeton.

Princeton Pharmaceuticals is a division of Convenience Light, Inc. or CLI, of which White is president as well. CLI is a major factor in the disposable flashlight business.

For more information, write Princeton Pharmaceuticals, Div. CLI, 70 Spruce Street, Paterson, NJ 07501, or contact Ira Hill, Clay Court, Locust, NJ 07760.

*Polyethylene terephthalate



T5-PF

Tamper resistant package. Use only if package is sealed.

Take 5 Plaque Fighter™ is a Brushless Dentifrice™ in a non-aerosol spray. Use regularly after meals, snacks, coffee breaks, smoking etc. . . "when you can't brush . . . but should" Regular use of **Take 5 Plaque Fighter™** cleans teeth, helps to reduce the accumulation of food particles, staining substances, and reduces the amount of materials that form plaque. It gives your mouth "that clean, just brushed feeling". . . IN SECONDS! Over 100 measured sprays, approximately a one month supply. Convenient shatterproof package fits pocket or purse.

DIRECTIONS

Hold near lips, pump 2 or 3 sprays into mouth onto tongue. Rub tongue over teeth, gums and surfaces of mouth. Swallow. Use regularly "when you can't brush . . . but should". **Take 5 Plaque Fighter™ is to be used throughout the day as a regular supplement to current oral hygiene. Avoid spraying in eyes. KEEP OUT OF REACH OF CHILDREN, EXCEPT UNDER ADULT SUPERVISION.**

INGREDIENTS: *Microdent 200™ in a Brushless Dentifrice™ base of alcohol SD38B, deionized water, sorbitol, glycerine, flavor, saccharin, methyl-cellulose and D & C Red 33.

*Microdent 200™ is a trademark for a poloxamer 407-simethicone based proprietary mouth conditioner.

Take 5 Plaque Fighter™, Microdent 200™ and Brushless Dentifrice™ are trademarks of

Princeton Pharmaceutical Inc.,
Paterson, NJ 07501, Patents Pending

PROOF OF PURCHASE

0 50729 80001 6

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ST5-PSF

Tamper resistant package. Use only if package is sealed.

PLAQUE & STAIN FIGHTER™

Smoker's Take 5 Plaque & Stain Fighter™ with Ultramint 200™ is a Brushless Dentifrice™ in a non-aerosol spray. **Smokers Take 5™** cleans teeth, naturally freshens breath . . . and restores taste in seconds. Regular use of **Smoker's Take 5™** cleans breath and reduces:

- o Accumulation of smoke particles
- o Odor causing materials
- o Staining substances and
- o The materials that form plaque.

Three sprays of Smoker's Take 5™ with Ultramint 200™ are more effective than an *entire* package of mints or breath deodorant mints. Approximately one month's supply. Convenient, shatterproof package fits pocket or purse.

DIRECTIONS:
Hold near lips, pump 2 or 3 sprays into mouth onto tongue. Rub tongue over teeth, gums and surfaces of mouth. Swallow. Use regularly after you smoke as a supplement to current oral hygiene. Avoid spraying in eyes. KEEP OUT OF REACH OF CHILDREN.

INGREDIENTS: Ultramint 200™ in a Brushless Dentifrice™ base of alcohol SD38B, deionized water, sorbitol, glycerine, flavor, saccharin, methyl-cellulose and FD & C Blue 1.

*Ultramint 200™ is a trademark for a poloxamer 407-simethicone based proprietary smoke particle/stain remover mouth conditioner.

Smoker's Take 5 Plaque and Stain Fighter™ Ultramint 200™ and Brushless Dentifrice™ are trademarks of

Princeton Pharmaceutical Inc.,
Paterson, NJ 07501, Patents Pending

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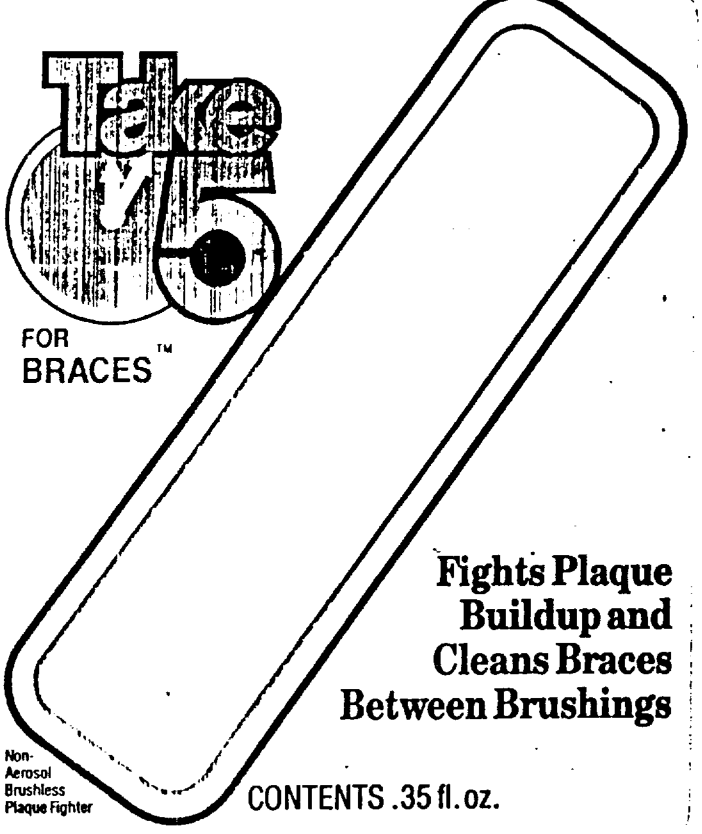

Fights Plaque Buildup and Cleans Braces Between Brushings

CONTENTS .35 fl. oz.

BT5

Tamper resistant package. Use only if package is sealed.

Take 5 for **Braces**™ is a Brushless Dentifrice™ in a nonaerosol spray with sugar free sweetness of **XYLITOL.** Cleans under, around and between braces. **Regular** use of **Take 5 for** Braces cleans breath & reduces:
Accumulation of food particles,
Odor causing materials,
Staining substances, and
The materials that form **PLAQUE.**

Three sprays of Take 5 **for Braces** with MICRODENT 400™ fights Braces Breath. Approximately one month's supply. Convenient shatterproof package fits pocket or purse.

DIRECTIONS

Hold near lips, pump 2 or 3 sprays into mouth onto tongue. Rub tongue over braces, teeth and mouth surfaces. Swallow. Use regularly throughout the day as a supplement to current oral hygiene. **Avoid spraying in eyes. Keep out of reach of small children, except under adult supervision.**

INGREDIENTS

Microdent 400™ in a Brushless Dentifrice™ base of deionized water, alcohol SD38B, xylitol, sorbitol, glycerine, flavor, methylcellulose, saccharin and FD & C Red 4 and Yellow 6.

PROOF OF PURCHASE

0 50729 80004 7

*Microdent 400™ is a trademark for a poloxamer 407-simethicone based proprietary braces cleaner and mouth conditioner. **Take 5 for Braces**™, Microdent 400™ and Brushless Dentifrice™ are trademarks of

Princeton Pharmaceutical Inc.,
Paterson, NJ 07501, Patents Pending

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DT5

Tamper resistant package. Use only if package is sealed.

Take 5 for Dentures™ is a Brushless Dentifrice™ that fights **PLAQUE AND DENTURE BREATH** where it starts, by helping remove food particles, staining substances and other materials that get trapped under and around dentures.

Regular use of **Take 5 for Dentures** after meals, snacks, coffee breaks, smoking etc. gives your mouth that "clean, just brushed" feeling without removing dentures. Partial dentures feel fresh, clean & comfortable when sprayed with **Take 5 for Dentures** before replacing in mouth. Convenient, shatterproof package fits pocket or purse. Approximately one month's supply.

DIRECTIONS

Hold near lips, pump 2 or 3 sprays into mouth onto tongue. Rub tongue over dentures, gums and surfaces of mouth. Swallow. Use regularly as a supplement to current oral hygiene. **Avoid spraying in eyes. KEEP OUT OF REACH OF CHILDREN.**

INGREDIENTS

Microdent 300™ is a Brushless Dentifrice™ base of alcohol SD38B, deionized water, sorbitol, glycerine, flavor, saccharin, methyl-cellulose and FD & C Blue 1 and Yellow 10

*Microdent 300™ is a trademark for polaxamer 407 simethicone based proprietary denture cleaner and mouth conditioner. **Take 5 for Dentures**™, Microdent 300™ and Brushless Dentifrice™ are trademarks of

PROOF OF PURCHASE

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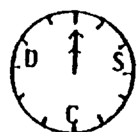
PROFESSIONAL CONCENTRATION



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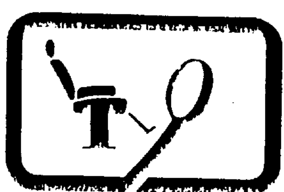


PLAQUE FIGHTER™

with

OMNiiDENT™

18mL

BACK LABEL

This OMNii® PROFESSIONAL SPRAY fits pocket or purse and can be used throughout the day.

Distributed by OMNii INTERNATIONAL,
St. Petersburg, FL 33702, a division of
Dunhall Pharmaceutical, Inc.,
Gravette, AR 72736
©Dunhall Pharmaceutical, Inc., 1991
US Pat. 4,950,497. Other pats. pending.
OMNii® is a registered trademark

- -

OMNii® PLAQUE FIGHTER™
with
OMNiiDENT™

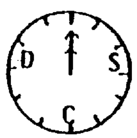
works throughout the day to help prevent plaque and keep a cleaner, fresher mouth.

ACTIVE INGREDIENT: 1.2% OMNiiDENT™
Also contains: deionized water, alcohol, sorbitol, glycerin, flavor, sodium saccharin, hydroxypropylmethylcellulose

DIRECTIONS: Use 4 or 5 times daily, preferably after meals, snacks, coffee breaks, smoking, etc. Hold near lips, pump 3 or 4 sprays into mouth onto tongue. Rub tongue over all teeth, gums and surfaces of mouth for at least 20 seconds. SWALLOW. Follow regular oral hygiene program recommended by your dentist.

OMNiiDENT™ is a trademark for a poloxamer 407/simethicone emulsion.

PROFESSIONAL
CONCENTRATION



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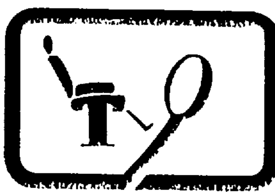

NIGHTTIME
PLAQUE FIGHTER
SPRAY™
with

OMNiiDENT-1000™

35mL

<u>BACK LABEL</u>

OMNii® NIGHTTIME
PLAQUE FIGHTER SPRAY™
with
OMNiiDENT-1000™

when used according to directions, works through the night to help fight morning breath. OMNiiDENT-1000™ cleans and coats the teeth, gums and soft tissue to help disrupt bacterial action including plaque formation which may be associated with morning breath.

DIRECTIONS: Locate on night stand, use just before retiring and shortly after waking. Hold near lips, pump three or four sprays into mouth, onto tongue. Rub tongue over all teeth and gum surfaces for at least 20 seconds. SWALLOW. Follow regular oral hygiene program recommended by your dentist.

ACTIVE INGREDIENT: 2.5% OMNiiDENT-1000™
Also contains: deionized water, alcohol, sorbitol, glycerin, flavor, sodium saccharin, hydroxylpropylmethylcellulose

OMNiiDENT-1000™ is a trademark for an emulsion of poloxamer 407/dimethicone.
US Patent 4,950,497. Other patents pending

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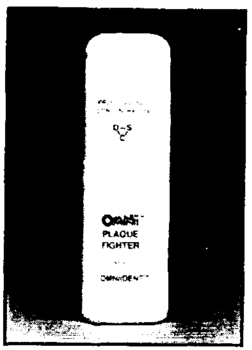
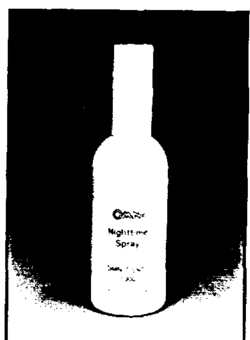
Gravette, AR 72736
©Dunhall Pharmaceutical, Inc., 1991

OMNII-MED™
PERIO-MED™
OMNII PLAQUE FIGHTER

DISRUPT PLAQUE FORMATION frequently, and you CONTROL GUM DISEASE.

INTRODUCING . . .





. . . recommended by your DENTIST as a supplement to your present oral hygiene . . . for the disruption of plaque throughout the day.

OMNII® PLAQUE FIGHTER
OMNII® NIGHTTIME SPRAY
with
OMNIIDENT™

— Has been clinically proven to fight plaque buildup, when used several times throughout the day, after meals, snacks, coffee breaks, etc.

— Is not a substitute for brushing, but it is an effective alternative to **not brushing** after every meal, snack, coffee break etc.

— Reduces the debris normally found in the mouth throughout the day, while leaving a clean just-brushed feeling and a freshened breath that lasts for hours.

— Nighttime Spray fights the **cause** of "morning breath" as it occurs . . . **throughout the night!**

"Makes teeth so slick — Plaque won't stick"

U.S. Patent 4,950,479, other Pats. Pend.

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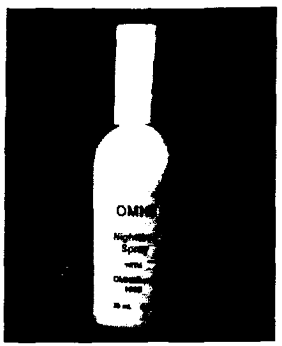
OMNII® INTERNATIONAL
1 (800) 643-3639



For those tens of millions of people suffering from "morning breath," SCOPE® is recommended ... "first thing in the morning".

THE OMNII® CHALLENGE:

instead of waiting until "first thing in the morning," OMNII® recommends people try fighting the **cause** of "morning breath" as it occurs ... **throughout the night!**

OMNII® Nighttime Spray, with OMNIIDENT 1000™ , is a more concentrated version of the OMNII Plaque Fighter with OMNIIDENT™ .

OMNII® Nighttime Spray does not work by "perfuming" the breath the morning after; rather, it is taken **before retiring** for the evening and **works throughout the night,** cleaning and coating mouth surfaces with OMNIIDENT 1000™ .

By cleaning debris from the oral cavity prior to retiring, and altering the surface energy of teeth, gums and mucosa, OMNIIDENT 1000™ helps disrupt the bacterial action associated with the shutdown of saliva flow during sleep.

OMNII® Nighttime Spray is preferably located on the NIGHTSTAND so it is readily available before retiring and after rising.

OMNII® Nighttime Spray, with OMNIIDENT 1000™ , contains approximately 12 times the surfactant concentration of SCOPE® , on a % weight basis. This higher surfactant level makes OMNII® Nighttime Spray particularly effective for secondary use in the morning upon rising, to help clean the debris remaining and to coat mouth surfaces. Such secondary use, "first thing in the morning", prepares mouth surfaces for brushing and improves the efficacy of brushing with a dentifrice.

OMNII® PLAQUE FIGHTER
with
OMNIIDENT™

fits pocket or purse and is as convenient to use as a package of mints.

For healthier teeth and gums, get into the OMNII PLAQUE FIGHTER habit...you will feel the difference and your dentist will see the difference.

OMNII® PLAQUE FIGHTER
with
OMNIIDENT™
Professional Concentration

is to be used under the strict control of the dentist and their professional staff.

U.S. Patent 4,950,479, other Pats. Pend.

© OMNII INTERNATIONAL 1990

Expect Positive Results Not Miracles

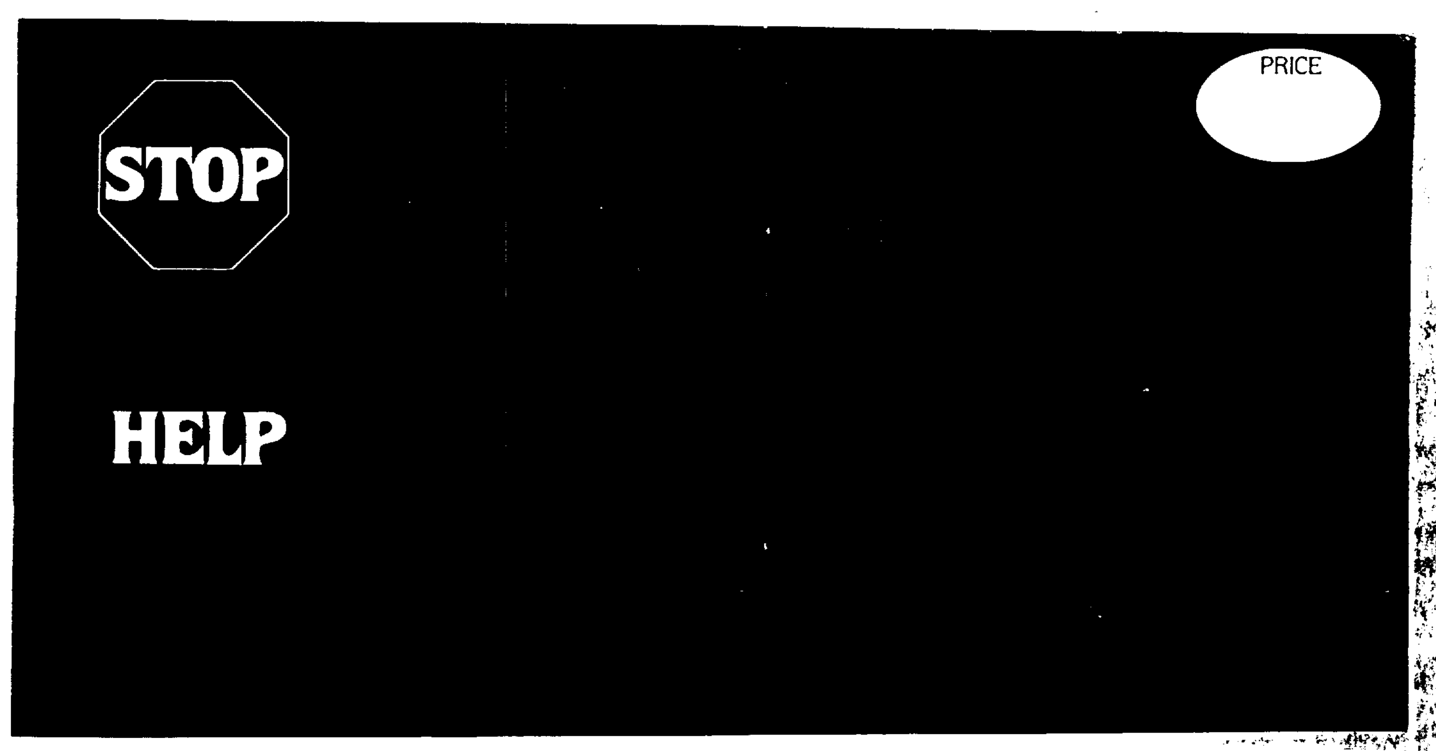
OMNII® INTERNATIONAL
1 (800) 643-3639
1 (800) 284-4123

NEW REFRESHING *NEW*

PLAQUE FIGHTING MOUTH SPRAY

1-800-284-4123
1-800-643-3639

NEW *NEW*



Makes Teeth So Slick That Plaque Won't Stick

CONFIDENTIAL

II. QUANTITIES OF ACTIVES

II. QUANTITIES OF ACTIVES

T A B L E   O F   C O N T E N T S

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II. QUANTITIES OF ACTIVE INGREDIENTS

A. INTRODUCTION

The antiplaque drug ingredient under review comprises a patented mixture of two raw materials; a nonionic surfactant, such as poloxamer 407, intimately mixed with a polydimethylsiloxane, such as simethicone.

Nomenclature: For purposes of ease of identification, one of the trademarks for this mixture of raw materials comprising the active ingredient, **MICRODENT™**, will be used as its most common denominator, although the same active ingredient is given other trademarks, such as OMNiiDENT™ (see Section I).

> Should the Panel prefer to adopt a "generic" (ie, non-trade) name for the active ingredient, WhiteHill invites the Panel to construct one suitable for the purpose. The choices are numerous, eg.,
>
> poloxamer/polydimethylsiloxane
> poloxamer-dimethicone
> poloxamethicone, etc.
>
> WhiteHill believes a hyphenated or "slash" descriptor is more communicative of the fact that the active is comprised of an intimate mixture of two dissimilar molecular species (each species in turn comprised of a mixture of molecular weight distributions). Single word descriptors, while simple and neat in appearance may inaccurately imply single molecular species properties.

Suitable nonionic surfactants, including poloxamer 407, widely approved by the FDA for use in foods, oral care products and drugs, are available commercially from BASF under the tradename PLURONIC. For example, PLURONIC F-127 is particularly well suited for this antiplaque drug. Suitable commercial nonionic surfactants are described below [B. (5)].

Suitable polydimethylsiloxanes, including simethicone, are available in both food grades and drug grades with wide FDA approval for a variety of ingested applications including OTC antacids and as defoamers for food preparations. For example, "D.C. 200 FLUID" or a silica containing emulsion, "MEDICAL ANTIFOAM AF-30", are commercially available from Dow Corning. Suitable commercial polydimethylsiloxanes are described below [B. (5)].

The active ingredient, MICRODENT™, is technically described as an emulsion of a polydimethylsiloxane in or by a nonionic surfactant. This emulsion is characterized by two distinct features:

(a) cleaning activity....ideal for clearing the mouth of debris, material alba, etc., and

(b) coating/surface energy modification....ideal for laying down micro-thin "fugitive" films on tooth surfaces that can last for up to an hour or so.

This active ingredient was discovered to disrupt the accumulation of plaque when suitably introduced into the oral cavity. Such introduction can be accomplished from a wide range of oral care products including: sprays, pre-rinses, mouthwashes, gels, dentifrices, interproximal devices such as dental floss and dental stimulators and ingestibles such as chewing gums and mints as described in (I) "Facsimile Labels".

Viewed retrospectively, the disruption of plaque accumulation is consistent with a large body of literature on oral cavity cleanliness and surface energy of teeth and hard surface appliances in the mouth. This literature and various clinical studies are detailed in the appropriate sections of this filing.

As used throughout this document, PLAQUE is a soft, sticky, colorless, bacterial film or matrix that forms continuously on the teeth. The bacteria in plaque produce acids, other toxins and enzymes that can irritate the gums and lead to gingivitis and accelerate caries formation. We believe this definition is consistent with the bulk of the relevant scientific literature, especially see L. Menaker, "Biologic Basis of Caries".

This active ingredient is distinct from most antiplaque ingredients in that it achieves its efficacy via a **non-invasive mechanism**, ie, there is no antimicrobial challenge of the flora of the oral cavity; no disruptive pharmacological manifestations; and even in very high concentrations, the raw materials comprising the active ingredient, MICRODENT™, are essentially non-toxic and chemically inert.

As is evident from Section I "FACSIMILE LABELS", this active ingredient supports several antiplaque claims (eg, "prevents plaque", "reduces plaque", "fights plaque", "plaque fighter", "plaque control", "denture plaque", and "plaque-like films") in over 40 product forms in seven categories.

These products have been and are presently marketed, or will be marketed in the future, in the same dosage strength and same dosage form as defined herein. It is the manufacturers good faith belief that these products are generally recognized as safe and effective and not misbranded in accordance with the FDA's enforcement policies relating to the OTC drug review.

WhiteHill proposes certain dosage form and dosage strength limitations, and product formulation parameters resulting therefrom in [II. F.] below.

While not directly relevant to the Panel's deliberations concerning safety and efficacy, it may be of interest to note that the unique plaque fighting properties of this polydimethylsiloxane/nonionic surfactant emulsion is the subject of five issued U.S. Patents and several pending applications. These are included in the literature submitted herein. NOTE: the basic composition of matter patent was granted after review by the U.S. Patent Board of Appeals. We are satisfied that **MICRODENT™** meets all reasonable definitions of an "active ingredient" even though it is comprised of a mixture of two dissimilar raw materials in various ratios.

II. QUANTITIES OF ACTIVES

B. DEFINITION OF ACTIVE INGREDIENT (MICRODENT™)

An adequate definition of the active ingredient requires the definition of the raw materials from which it is assembled as well as the definition of the active itself. For the benefit of the Panel, we have also included in this section a non-exclusive list of compatible non-active ingredients and raw materials not currently used in manufacture of MICRODENT™ but which are chemically equivalent.

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pages of trade

secret and/or

confidential

commercial

information

4. COMPATIBLE "OTHER ACTIVES"

WhiteHill is sensitive to the government's long standing concern for claims proliferation and reluctance by the FDA and prior OTC panels to promulgate regulations which allow or encourage "multiple actives" which have not been adequately evaluated in combination. WhiteHill has formally proposed in a separate filing under this call for data and views that the Panel must, of necessity, address at least two issues relating to multiple actives in oral care products.

Consideration of multiple actives is necessary because (1) such claims are already widespread (ie, Fights Cavities, Fights Plaque, Controls Tartar and Kills Germs are widely used in combination) and (2) good oral health management practices demand that as many beneficial functions be included in a single appropriate vehicle as possible.

The greatest single detractor to efficacious preventative oral procedures can be categorized as "lack of patient compliance". Few consumers, even under the active care of a dentist, will brush twice a day, much less the "after every meal" compliance recommended by most dental educators. Flossing suffers from an atrocious "regular flosser" rate of about 13% of the knowledgeable public. Most households "own" floss.... few use it.

Hence, it is evident that one cannot expect even motivated consumer/patients to brush, rinse or otherwise apply multiple products in order to sequentially apply oral drugs to (a) clean, (b) prevent cavities, (c) reduce plaque and/or gingivitis and (d) control tartar.

In order to promote patient compliance, there must then be given careful consideration to the simultaneous incorporation of several active ingredients, particularly where each active ingredient affects a different oral health parameter.

One of the problems of such "combination approval" is the deactivation of one active ingredient by the presence of another, or competing mechanisms of action which render the double active only an empty claim. MICRODENT™, due to the basically unreactive nature of the molecular composition and its non-invasive mode of action is uniquely suited for use in combination with active ingredients whose primary purpose is (1) anti-microbial drug delivery, (2) decalcification of tartar by chemical exchange, and (3) prevention of caries. In some product forms, eg., dental floss, MICRODENT™ is the only active ingredient known to us which effectively carries anti-gingivitis agents such as stannous fluoride and tartar control agents such as the soluble pyrophosphates and releases MICRODENT™ and the "second active" interproximally at the gum line...precisely where standard delivery forms of brushing and rinsing are deficient. To require the patient to "floss twice" is unreasonable as well as unresponsive to the overriding "compliance" issue.

Other examples abound. For the purposes of this section however, we attach a list of "other active ingredients" which are compatible with MICRODENT™.

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information

II. QUANTITIES OF ACTIVES

C. MODE OF ACTION OF ACTIVE INGREDIENT

According to the general outline for submissions under OTC Drug Review (21 CFR 330.10) a summary of the "medical rationale" is to be included under Section VI. However, it is very difficult to understandably present the supporting scientific literature, clinicals, etc in Sections II through V without a prior elaboration of the mode of action of MICRODENT™. Therefore, this petitioner has taken the liberty, hopefully of value to the Panel, to introduce the mode of action early in this document. It will be expanded and summarized in its appropriate location in Section VI as well.

1. COMPARISON OF MICRODENT™ WITH COMMON PLAQUE REDUCTION APPROACHES

As a generalization, common plaque reduction approaches can be divided into three broad categories:

(1) Cleaning of formed plaque from tooth surface by surfactants, with or without abrasion, is the predominate non-invasive mode of action. Examples include (a) abrasive containing dentifrices with surfactants (usually sodium lauryl sulfate) to lift, suspend, and emulsify foreign matter, materia alba and plaque primarily disrupted by the combined action of approximately sized abrasives (dicalcium phosphate, silica or calcium carbonate primarily) with the toothbrush bristles, and (b) mouth rinses with relatively low levels of surfactants (approx. 0.1 - 0.2% poloxamer or sodium lauryl sulfate) which when used for a period of one minute or so before brushing (with toothbrush only - - no dentifrice with abrasive, strangely enough, in most published studies) which purportedly "soften" or otherwise render the plaque more amenable to removal by the brushing which follows.

(2) Anti-microbial action to reduce the plaque-forming bacteria in the oral cavity generally or to reduce the viable organisms entrained by the plaque film, has been perhaps the most widely studied mode of action. Relatively non-specific agents such as certain phenolics or terpenoids in high percentage alcohol vehicles and natural extracts such as sanguinarine, are the most common in the US. More specific anti-microbials such triclosan have been widely used OTC in Europe.

Of the currently approved OTC oral care drugs, only Stannous Fluoride has marked anti-microbial activity against plaque forming microorganisms (primarily S. mutans). SnF_2's ability to significantly reduce plaque and gingivitis in animals and humans is well documented in the literature, although its strongly metallic/astringent taste in common glycerin formulations may reduce user compliance and cloud some clinical trials (illustrated most notably in the Wolff Study funded by the NIDR, where compliance seemed to be as low as 35% in one cell).

The OTC Panel which approved SnF_2 (convened in 1976, issued in 1985) only considered its' anti-caries properties. This petitioner's reading of the literature strongly indicates that if compliance could be improved, this anti-microbial represents the most efficacious of currently marketed OTC anti- microbials.

(3) Enzymes to break down the adhesive substances in plaque have frequently been recommended in the literature and possess a certain attractiveness from a bio-chemical mechanism perspective. While common in animal oral care preparations, this mode of action is not common in the US human products, perhaps because of regulatory considerations, but more probably due to the inherent slowness of the reaction kinetics between a dilute soluble enzyme and a solid substrate. The 30-60 second usual brushing regime is marginal at best for such enzymatic action.

<u>MICRODENT™ Mode of Action:</u> The active ingredient, MICRODENT™ has a mode of action not identical to but closely allied with (1) above, ie., Cleaning. It also possesses added functionalities discussed below. It does not, to the best of petitioners knowledge, possess any measurable anti-microbial activity, enzymatic properties, nor pharmacologic or biochemical interference effects at the concentration experienced in the oral cavity.

Simple inspection of the molecular structure of the raw materials comprising MICRODENT™ (poloxamers and simethicone) reveals none of those active sites usually responsible for the requisite toxicity of anti-biological agents nor sites for subsequent chemical reaction with non-active ingredients.

The high molecular weight, polymeric form of the raw materials further restricts their accessibility for deleterious action to soft oral tissue, (See Master files approved for Panel access and safety data summaries included under Section III-A). The molecular properties as well as the experimentally evaluated parameters in the referenced Master Files form the basis of the <u>non-invasive</u> description of MICRODENT™'s mode of action.

2. BASIS OF ACTION

MICRODENT™ interrupts, restricts the formation of, and/or otherwise interferes with the accumulation of plaque on tooth surfaces by a mode of action comprised of four interactive elements. Namely:

CLEANING the oral cavity and tooth surfaces of various substances from which oral cavity microbes can form plaque and plaque precursors.

ANTI-REDEPOSITION properties which alter the effective surface energy of the tooth surfaces, thereby reducing the ability of newly formed plaque to successfully attach to the tooth.

INFUSION of the plaque matrix or web by surface activity altering agents, causing the plaque to be less adherent to itself and more easily disrupted by normal abrasive oral hygiene procedures, and

FREQUENCY OF USE, while not a biologic or surface active property of MICRODENT™, this is an important and inherent element in its' mode of action. Use is not restricted (as it is with almost every other reported plaque fighter) by irritations of oral tissues, body burden limitations due to cumulative toxicity or "fatigue" due to taste and/or mouthfeel negatives. Use Frequency is, however, critical to the efficacy of certain product forms of MICRODENT™.

3. CLEANING

As used in this document, the cleaning function of MICRODENT™ is limited to the actions of the surfactant moiety. It does not include the physical removal afforded by the abrasive additives which may be included in a specific formulation.

The primary need for a surfactant in the oral cavity is to reduce the surface tension between all forms of debris in the cavity ard the soft or hard tissue with which it is in contact. There is a lesser need for oil/water type surfactantcy. Hence, the emulsification properties of the nonionic poloxamer family are especially suited for this task, even though the strong bipolar character of the anionic surfactant commonly used in toothpaste (sodium lauryl sulfate) has greater lifting power for oil based stains in a typical detergency test on fabric.

Section V.-C. contains the results of "in-the-mouth" evaluations of the debris removal efficacy of MICRODENT™. For purposes of this introduction, it is sufficient to note that frequent use of MICRODENT™ containing products, specifically sprays; demonstrates that the oral cavity has less debris at the end of the day. This reduction in debris includes that which can be rinsed from the mouth with multiple rinses with a strong (5%) surfactant solution as well as that which can subsequently be brushed off with a toothbrush and a 5% surfactant solution.

4. SURFACE ACTIVITY/SURFACE ENERGY OF TEETH

A common principle in the formulation of an effective detergent for fabric or hard surfaces is to include in the formula a molecular species referred to as an "anti-redeposition" agent. The purpose of this ingredient is to change the surface energy of the surface cleaned by the surfactant ingredient so that the surface has less attraction for the "dirt" released into the wash solution (hence, "anti-redeposition") or to which the hard surface might later be exposed. To be effective, the "anti-redeposition" agent must itself be attracted to the surface which it is intended to protect, and remain there for an appropriate length of time.

This principle was followed by Petitioners in developing the plaque disrupting active ingredient MICRODENT™.

Specifically, in MICRODENT™ the polydimethylsiloxane performs this function. A more complete discription of this chemistry and appropriate literature references are included in Section V.-C. For purposes of this introduction it is sufficient to explain that the normally coiled helix of the raw material, polydimethylsiloxane, is believed to be "stretched" open by the poloxamer so that the more polar oxygen atoms are available to hydrogen bond to the hard surfaces of the teeth, the pellicle and soft tissue of the mouth.

This "uncoiling" leaves the relatively inert, low-energy, dimethyl side of the siloxane chain exposed to any debris, plaque or plaque-like material which might be available to attach itself to the tooth or pellicle surface. Multiple methodologies demonstrating this property of attached siloxanes to various surfaces, including teeth, are found in the scientific literature appropriately referenced. However, it is easily perceived by the "smooth, slick" feeling of the teeth after use of a MICRODENT™ containing product. This slick effect, evidence of reduced surface energy, persists for about 30 minutes for polydimethylsiloxanes having a viscosity of 350 cs, and up to several hours for viscosities above 1000 cs.

5. USE FREQUENCY AND PATIENT COMPLIANCE

The non-invasive, non-irritating, extremely pleasant, mouthfeel and taste of the MICRODENT™ active ingredient (especially compared to almost all other active anti-plaque ingredients for the oral cavity) leads to a much greater use frequency than previously experienced. Again, the dental literature is so replete with commentary on the difficulty of obtaining adequate compliance by the consumer/patient that further commentary on WhiteHill's part would be redundant.

With a spray formulation which can be swallowed like a breath mint or breath spray, use frequency of 3 to 5 times a day by consumers is common. Data in Section V.-C. supports increased oral care frequency for every product form of MICRODENT™ tested, including MICRODENT™ DENTAL FLOSS, in a category with perhaps the longest history of noncompliance.

II. QUANTITIES OF ACTIVES

D. PHYSICAL FORM OF ACTIVE INGREDIENT

The physical form of the active ingredient MICRODENT™ is always the same, an EMULSION. The emulsion form is a particularly suitable form for oral care purposes in that it lends itself to a wide variety of formulation variables (described below as "product forms") which aid in patient compliance and fit into established habits (brushing, rinsing, spraying, massaging, flossing, gum chewing, etc.).

1. DEFINITION OF DOSAGE FORM

The dosage form of the anti-plaque active ingredient MICRODENT™, herein described, is:

> "MICRODENT™ is an EMULSION of a polydimethylsiloxane (dimethicone or simethicone) as the discontinuous phase in, or by, a block copolymer of ethylene oxide and propylene oxide (poloxamer) formulated into a product form suitable for direct introduction into the oral cavity. Whereby said EMULSION directly, or with the aid of oral cavity fluids, (saliva and succulal) can be adequately dispersed across the hard and soft surfaces of the oral cavity. Said EMULSION may have as its continuous phase either the poloxamer itself or a suitably ingestible liquid, gel, paste or solid into which the poloxamer is dissolved or dispersed, thereby retaining the EMULSION form of the active ingredient after introduction into the oral cavity.

No Other Dosage Forms Recommended: WhiteHill knows of no other dosage form of the active ingredient MICRODENT™ which would simultaneously be safe and effective.

Any solvent capable of dissolving both the poloxamer and polydimethylsiloxane would be extremely irritating to the oral cavity, grossly toxic, or both. Therefore a true SOLUTION is not included in the definition of dosage form.

Since the raw materials are high molecular weight polymers, it is obvious that a GAS as a dosage form is impossible.

Finally, SOLIDS, other than the solid emulsions herein defined, would not be expected to provide efficacy since the distribution of the polydimethylsiloxane would not be uniform.

MICRODENT™ has no known effect when ingested without adequate oral contact (ie, a solid pill or capsule) nor should it be presented in an injectable form. Hence, the definition above is presented to the Panel as the only rational dosage form which should be considered.

2. PRODUCT FORMS MEETING DEFINITION OF DOSAGE FORM

Unlike most anti-plaque ingredients which rely upon solution chemistry for their dosage form, the emulsion form is conveniently flexible across may product forms. The emulsion which contacts the surfaces of the teeth and oral cavity can be presented to the consumer in a number of PRODUCT FORMS while still retaining the EMULSION form as the active dosage form available upon contact with oral surfaces.

Upon first glance, it might appear that separating the definition of "dosage form" from that of "product form" is an exercise in semantics. However, the FDA has an established history of dividing a "product form" which the general public would define as a "liquid" into at least two distinct "dosage forms", ie, (a) Solution and (b) Emulsion.

This distinction is well justified in that the presentation of an active ingredient to (say) the gastrointestinal tissues as a Solution would be expected to produce a quite different response in both safety and efficacy than it would as an Emulsion. The same distinction holds true for active ingredients applied externally to the skin. Those presented as emulsions perform quite differently than the same ingredient presented as a solution (eg., in DMSO) or which if presented in a poorly admixed solid would not perform at all.

(a) LIQUID EMULSIONS

A product form which most of the general public would describe as a liquid can be achieved with a MICRODENT™ emulsion wherein the liquid continuous phase is water, water/ethanol mixtures or similar solutions of compatable materials. At relatively low levels of MICRODENT™ (below 3-5%) the poloxamer is primarily dissolved in the solvent (water) and serves to hold the polydimethylsiloxane in an emulsified suspension.

Examples detailed in Section I. (LABELS) range from water/ethanol mixtures suitable for spraying into the mouth to alcohol-free water mixtures suitable for mouth rinses. Since the active ingredient reaching the oral tissues is the same emulsion, these liquid product forms can be expected to have similar or identical performance parameters across the full range of water-alcohol concentrations, with or without other soluble non-actives in the formulation.

Another product form which most of the general public would describe as a liquid can be achieved with a MICRODENT™ emulsion wherein the liquid continuous phase is anhydrous. In these cases, the solvent mixture may be comprised of materials which have prior FDA approval for ingestion or use in the oral cavity. Liquid mixtures containing ingredients such as ethanol, glycerin, propylene glycol, or a low molecular weight poloxamer meet these criteria and allow for stable distribution of the active ingredient as an emulsion.

Depending upon the solvent system employed, some or all of the poloxamer may be dissolved in the solvent to maintain the emulsion dosage form. The anhydrous liquid forms have primary utility when the MICRODENT™ is utilized with another active, such as stannous fluoride, whose chemistry and/or OTC regulatory approval require anhydrous carriers.

The non-reactive nature of the polydimethylsiloxane and the poloxamer makes questions of active ingredient stability much less troublesome than active ingredients having strong potential to react or interact (eg., quaternary ammonium salts, phenolics, terpenoids, stannous fluoride, quaternary nitrogen hetercyclics such as Sanguinarine, ionic surfactants, soluble pyrophosphates, etc.) with other ingredients in the product form.

WhiteHill has never observed any liquid formulation variable which rendered the MICRODENT™ inactive as long as the EMULSION form was retained.

(B) GEL EMULSIONS

In many respects, a Gel Emulsion is a sub-set of Liquid Emulsion. However, in the general public's view, these semi-solids represent a different Product Form. All of the commentary in the preceding section apply to this form. In addition, the presence of a gelling agent further serves to stabilize the MICRODENT™ EMULSION.

The gelling agents suitable for MICRODENT™ include silica gel, modified celluloses, natural gums like xanthan and carrageenan. At higher concentrations, certain poloxamers (especially 407) are themselves gelling agents as well as a part of the active ingredient.

The LABEL Section I, previously referenced, describes gel Product Forms ranging from soft gels without abrasives, suitable for the plaque-like debris which accumulates on the soft tissue of babies and edentulous persons, to silica-loaded gels which function as abrasive toothpastes.

(C) SOLID EMULSIONS

So ubiquitous are the OTC products comprised of liquid emulsions, that the fact that emulsions exist in a primarily solid form is often overlooked.

MICRODENT™, when prepared from molten poloxamer 407 (a solid at room temperature) forms first a "melt emulsion" wherein the now liquid poloxamer is the continuous phase, emulsifying the insoluble polydimethylsiloxane in a stable fashion within itself. When the emulsion returns to near room temperature, the continuous phase solidifies, rendering the entire emulsion an apparent solid, although the emulsified polydimethylsiloxane is present as a discontinuous liquid phase.

This product form is especially useful for increasing the plaque fighting efficacy of interproximal devices, such as dental floss and interdental stimulators. Patents covering the utility of MICRODENT™ in these SOLID EMULSION delivery forms have recently issued and represent a major advance in both efficacy of plaque reduction and improvement of patient compliance or willingness to use these interproximal devices.

Clinical evaluation of MICRODENT™ DENTAL FLOSS produced evidence of the first statistically significant improvement over standard waxed dental floss for the reduction of interproximal plaque. This study is included in Section V.-C.

Clinical tests on the solid emulsion product form as incorporated into interdental stimulators are currently in design and may be underway before the Panel convenes. WhiteHill will present these clinical results to the Panel as they are completed and will petition the FDA for their inclusion into the official deliberations as a part of this response to the call for data.

(D) CHEWING GUM AND OTHER INGESTIBLES

This product form is essentially a sub-set of SOLID EMULSIONS, but since as a product form category, the general public does not include products originally designed as confections in the oral care product category, much less as beneficial plaque fighting products, these product forms are presented to the Panel in a separate section.

One of the unusual properties of the MICRODENT™ active ingredient is its ability to be incorporated into ordinary chewing gums in a fashion that is both pleasant and efficacious. Unlike the solid emulsion forms of dental floss and interdental stimulators, the patent filings on chewing gum and other ingestibles are incomplete. Hence, the disclosure of this information, is presented to the Panel under the rules of confidentiality included in the call for data, specifically so as to not violate the prior confidential disclosure rules of the US Patent Office.

When combined with gums formulated to not enhance the formation of caries (ie, sugarless), the gum presents the emulsion to the teeth and oral cavity over a lengthy period of time, with modest abrasion. Given the clinical evidence of plaque reduction presented in later Sections, which clearly highlight the beneficial effect of adequate contact and contact time, is appears highly probable that this product form of the active ingredient may well be among the most efficacious, with total dose levels no greater than that of other product forms.

When MICRODENT™ containing chewing gum is used, the "debris clearing" effect is immediately perceived and the reduction of surface energy (slickness) is thorough and long-lasting. WhiteHill recognizes that such perception of physical changes in the oral cavity does not assure a clinical result similar to that obtained with other product forms.

However, the opportunity to move the joy of children (and many adults) and the bane of practicing dentists into a form both pleasant, beneficial, and acceptable to the professional dentist is worthy of the Panel's patience as the lengthy process of evaluating all the data for many proposed ingredients proceeds.

Chewing gum clinicals are in the planning stages and may be underway by the time the Panel convenes. As a part of this filing, WhiteHill will petition the FDA to include the results of these clinicals as they are available.

OTHER INGESTIBLE product forms which carry the solid emulsion character include "breath mints" and candies. Many hard candies are solid emulsions of fats and flavor oils within the continuous phase of "glass" formed by complex sugars/carbohydrates. Mints are usually compression molded admixtures of similar ingredients.

Solid emulsions of MICRODENT™ are compatible with the compression moldable or "glassing" materials, providing excellent active ingredient release properties.

Of particularly utility are those "compressions" or "glasses" formed by non-cariogenic sugars and carbohydrates such as sorbitol, xylitol and hydrogenated glucose syrup. Mints and candies containing solid emulsions of MICRODENT™ in final concentrations similar to that of other clinically tested product forms, are quite pleasant and exhibit the same mouth clearing and teeth surface activity reduction previously discussed.

"Other Ingestibles" clinicals are in the planning stages and may be underway by the time the Panel convenes. As a part of this filing, WhiteHill will petition the FDA to include the results of these clinicals as they are available.

II. QUANTITIES OF ACTIVES

E. DOSAGE STRENGTH CONSIDERATIONS

1. DEFINITION OF DOSAGE STRENGTH

The dosage strength required for the anti-plaque active ingredient, MICRODENT™, is best defined by analogy to other cleaning and surface coating products whereby accessibility, time and localized surface contact are more adequate elements for definition than the typical mg/kg body weight definitions applied to pharmacological agents or percent by weight applied to common topical antimicrobials.

The proposed definition of dosage strength is:

> "(1) Sufficient poloxamer contacting the target tissues (either the entire oral cavity or specific areas, eg., interproximal) to effectively remove significant quantities of loosely attached, dispersable debris within the time the MICRODENT™ is normally and comfortably held in the mouth and (2) sufficient emulsified polydimethylsiloxane to effectively coat the targeted teeth and soft tissue surfaces with a micro-thin, ablative layer of polydimethylsiloxane."

On a concentration basis this definition requires a product form range from 0.4% to 4.0% in liquid and gel emulsions, and a range from 0.01 to 0.2 grams per use of interproximal device delivering a solid emulsion. More specific amounts/product form are detailed below.

2. CONCENTRATIONS OF INGREDIENT REQUIRED TO ACHIEVE OPTIMAL DOSAGE STRENGTH

(a) RATIOS OF RAW MATERIALS IN INGREDIENT

Achieving the "sufficient emulsified polydimethylsiloxane to effectively coat the targeted teeth and soft tissue surfaces" requires that the ratio of poloxamer to polydimethylsiloxane be adjusted according to the product form.

Specifically, ratios ranging from 100:1 (for rinses), to 40:1 (for sprays), to 16:1 (for gels), to 3:1 for solid emulsion (floss and interdental stimulators), to 1:1 (for chewing gum and mints) are required.

The principle here is obvious: For those product forms having little or no physical plaque disruption action (rinses and sprays), but considerable volume distribution across the oral cavity, a higher proportion of cleaning raw material is required. For those with more physical disruption, but less frequency or volume use (floss or gum), more surface energy altering raw material is needed, but less surfactant.

Hence, this Petitioner submits that OTC approval of all ratios of poloxamer to polydimethylsiloxane from 100:1 to 1:1, depending upon the product form desired is reasonable. The full range of ratios should be approved by the Panel because they: (1) fit the mode of action, (2) conform to the ratios for which clinical efficacy is demonstrated, and (3) are all equivalent in safety, as indicated by the lack of demonstrable toxic effects by either raw material comprising the active ingredient MICRODENT™.

(b) QUANTITIES OF INGREDIENT IN VARIOUS PRODUCT FORMS TO ACHIEVE DOSAGE STRENGTH

SPRAYS

(1) 0.4% to 1.2% if a spray intended to deliver 0.3 to 3.0 ml to the oral cavity when used 4 to 6 times/day. If a spray is intended for nighttime and morniing use (2 to 3 times per day, the concentrations should be 1.2% to 2.4%. This equates to daily delivery of 0.005 grams to 0.072 grams of active ingredient.

GELS

(2) 0.4% to 2.0% for a gel distributed across the teeth and/or gums twice a day. This equates to a daily delivery of 0.008 grams to 0.04 grams of active ingredient.

RINSES

(3) 1.0% to 4.0% for an expectorated rinse used twice a day. This equates to a daily delivery of 0.03 to 0.12 grams/day of active ingredient.

DENTAL FLOSS

(4) 0.01-0.04 grams/meter of dental floss used interproximally at 0.5 meters once a day. This equates to 0.005 to 0.02 grams per day of active ingredient.

GUMS AND MINTS

(5) 0.04 to 0.2 grams per stick of chewing gum or 0.04 to 0.4grams per breath mint or hard candy.

TOOTHPASTE

(6) 2.0% to 4.0% for an abrasive toothpaste expectorated after use. This equates to 0.01 to 0.02 grams per day of active ingredient ingested (2 grams/use X 2/day X 10% retained), 0.1 to 0.2 available for teeth contact.

INTERDENTAL STIMULATOR

(7) 0.005 to 0.02 grams per interdental stimulator.

PILOT CLINICAL STUDIES

PP-1986-01
PP-1986-02

PRINCIPLE SUPERVISING DENTIST:
PRINCIPLE INVESTIGATOR:
PROJECT COORDINATOR:
SPONSOR:

TEST SITE:

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PP-1986-01
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A. ABSTRACT

The Petitioner's active ingredient, MICRODENT™, was tested in a pair of double blind, parallel treatment design protocols wherein subjects discontinued normal oral hygiene for the test period, but used instead a Test Product with the brand name TAKE-5™ (containing 0.43% MICRODENT™) or a Placebo of commercially obtained breath freshener branded BINACA®.

Active and Placebo test groups of 10 each (PP-1986-01) and 13 each (PP-1986-02) used their assigned product delivered by a spray device at least 5 times per day. In one test (PP-1986-01) subjects were not given a dental prophylaxis, and were scored for plaque at Baseline and after 24 hours. In the second test, (PP-1986-02) the subjects received a dental prophylaxis and were scored after 48 hours.

In both pilot tests, the plaque accumulation with the Test Product containing MICRODENT™ was less than with the Placebo.

In the "no prophylaxis" test, the increase in mean plaque score after 24 hours of no oral hygiene was evaluated. The test group's increase was about two/thirds of the placebo group's increase.

In the "with prophylaxis" test, after 48 hours of no oral hygiene, the mean plaque score of the test group with MICRODENT™ was 9% less than the placebo group's plaque score.

Since the number of subjects in each group was small, no attempt at determining statistical significance was deemed to be warranted. There were no adverse effects on hard or soft tissue observed.

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

B. PROTOCOL BRIEF

1. INFORMATION SOUGHT

These pilot clinicals were designed to provide an indication whether the active ingredient of this petition, MICRODENT™, previously test marketed as a mouth "cleaner" and breath freshener, would demonstrate sufficient effect on plaque to be worthy of more complete clinical evaluation. The questions posed were:

(a) Does the active ingredient, MICRODENT™, delivered in a spray form, have an effect when used frequently by individuals with plaque already well established, and

(b) Does the active ingredient, MICRODENT™, delivered in a spray form, have an effect when used frequently by individuals with plaque removed before beginning use.

2. FORMULATIONS TESTED: ACTIVE vs PLACEBO

ACTIVE

The exact formulation of MICRODENT™ in the commercial product, TAKE-5™, and as used in these studies, follows:

PLACEBO

The exact placebo formula, BINACA®, is not known. However, the commercial label declares: alcohol, water, glycerin, sodium saccharin, and flavor. The propellent is hydrocarbon A-46 This placebo was deemed adequate to stimulate saliva flow and introduce alcohol and flavor oils (potential antimicrobials) to the oral cavity in quantities similar to the test product.

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

B. PROTOCOL BRIEF

1. PROTOCOL DESIGN ELEMENTS PP-1986-01

(a) Double blind, parallel treatment.
(b) Ten (10) subjects per test group.
(c) Suspension of normal oral hygiene during test.
(d) Subjects pre-screened for oral health and scored for baseline plaque index (Turesky modification, Quigley-Hein). Subjects randomly distributed into two groups based on plaque score equalization.
(e) Subjects were not given a dental prophylaxis.
(f) Subjects instructed in proper use of sprays, one self-administered use observed, then instructed to use the assigned product ad lib but at least five (5) times per day.
(g) Subjects returned to clinic at 24 hours for scoring of plaque index as in (d).

2. PROTOCOL DESIGN ELEMENTS PP-1986-02

(a) Double blind, parallel treatment.
(b) Thirteen (13) subjects per test group.
(c) Suspension of normal oral hygiene during test.
(d) Subjects pre-screened for oral health and scored for baseline plaque index (Turesky modification, Quigley-Hein). Subjects randomly distributed into two groups based on plaque score equalization.
(e) Subjects were given a through dental prophylaxis, reducing the plaque index to zero at time zero.
(f) Subjects instructed in proper use of sprays, one self-administered use observed, then instructed to use the assigned product ad lib but at least five (5) times per day.
(g) Subjects returned to the clinic at 48 hours for scoring of plaque index as in (d).

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

C. QUANTITATIVE RESULTS

These tests, with their small number of subjects per group, very short test periods, and informal protocol, were not intended to be subjected to statistical analysis. Thus, the only value is in the trend lines. That value is primarily limited to the encouragement to pursue the more rigorous tests submitted in this filing. There is some retrospective value, of course, if the trends are consistent with statistically analyzed full scale clinicals.

(1) PP-1986-01 RESULTS AND DISCUSSION

TABLE 1.

PRODUCT	No. OF SUBJECTS	MEAN BASELINE	MEAN 24 Hr	MEAN DIFFERENCE
TAKE-5	10	1.83	2.04	0.21
BINACA	10	1.78	2.10	0.31

The trend line suggests that under these use conditions, the expected rate of increase due to the cessation of oral hygiene for 24 hours was moderated somewhat by the test product containing MICRODENT™. On this small population sample, the increase in additional plaque over that at baseline by subjects using TAKE-5 can be considered to be one/third that of the increase in subjects using the placebo.

This is considered to be a surprising finding in light of the 24 hour test period chosen.

(2) PP-1986-02 RESULTS AND DISCUSSION

TABLE 2.

PRODUCT	No. OF SUBJECT	MEAN 0 Hr	MEAN 48 Hr	MEAN REDUCTION vs. BINACA	% REDUCTION vs. BINACA
TAKE-5	13	0	1.62	-0.16	9%
BINACA	13	0	1.78	--	--

Again the trend line suggests some beneficial effect by the active ingredient MICRODENT™ in reducing plaque build up. Over the 48 hour period, the actual mean difference in plaque score between the two test groups was somewhat greater than in PP-1986-01 (0.16 vs. 0.10). This may be due to starting with prophylaxis, or could be due to the longer, more numerous, exposure to the active ingredient.

CONCLUSION

The active ingredient, MICRODENT™, does reduce plaque under two rather different protocols and time frames. The extent of the reduction is not clear, nor does this test do much to suggest a mode of action. The consistency of direction for the two pilot clinicals is perhaps more relevent than the actual magnitude of the results.

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commercial

information

III. Animal Safety Data

C. Finished Drug Products

1. Controlled Studies

ABSTRACT

A gel emulsion formulated for use as a "Baby Gum and Tooth Cleanser" to remove "plaque-like film" that forms on babies gums before and during teething, was tested in its finished form.

As would be expected from the knowledge of the safety of the individual ingredients and Raw Materials comprising the active ingredient, MICRODENT™, there were no deleterious effects noted under stringent dosing procedures.

The Acute Oral Toxicity in Rats produced zero percent mortality when dosed with a single oral dose at 10.0 g/kg.

A Twenty Day Hamster Cheek Pouch Application Study placed three applications of 0.1 ml daily for five days of four consecutive weeks (total of 60 applications). After evaluation for gross and histopathological changes, the conclusion was; "Does not cause systemic physiological changes....or statistically significant deviations from histologic morphology"

FORMULATION TESTED
(In order of addition)

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Final Report Summary

DATE: May 1, 1990
CLIENT:
STUDY NO.: 90051
REFERENCE: L. DePellegrini
TEST ARTICLE: OTC Oral Healthcare Product B# 787-19, 12-27-90
TEST ARTICLE RECEIPT DATE: February 5, 1990
STUDY INTERVAL: February 22, 1990 to March 8, 1990

Acute Oral Toxicity in Rats

Method: Ten (5M:5F) albino rats, 214 - 232 g, each received a single oral dose of the test article at a dose level of ten (10) grams per kilogram bodyweight. Animals were observed for pharmacologic activity and drug toxicity 1, 3, 6, and 24 hours after treatment, and daily thereafter for a total of 14 days. Non-survivors and animals surviving the 14 day observation period were subjected to gross necropsy, with all findings noted. The test article was used as received (Sp.g. = 1.09).

LD_{50} > 10 g/kg

Dose Level (g/kg)	Sex	No. Dead/No. Dosed (M:F)	Mortality (%)
10.0	5M:5F	0/5:0/5	0

This test article is not toxic orally to rats under the conditions of this test.

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Final Report Summary

DATE: May 1, 1990
CLIENT:
STUDY NO.: 90051
REFERENCE: L. DePellegrini
TEST ARTICLE: OTC Oral Healthcare Product B# 787-19, 12/27/89
TEST ARTICLE RECEIPT DATE: February 5, 1990
STUDY INTERVAL: February 15, 1990 to March 15, 1990

Twenty (20) Day Hamster Cheek Pouch Application Study

Method

Twenty-four Golden Syrian hamsters (Ela:ENG (SYR)), male, were divided into two (2) groups of 12 animals each. The animals in the one (1) group, 71 - 119 g, each received three (3) applications of 0.1 milliliter of the test article daily in the left pouch, five (5) days per week for four (4) consecutive weeks. The right cheek pouches received no treatment. The 12 hamsters in the remaining group, 84 - 118 grams, were used as controls and received identical dosages of distilled water. Prior to each treatment each day, observations noting erythema and edema, and other effects, were made. Initial blood values were obtained from a separate group of five (5) animals. Terminal blood values were obtained from five (5) animals from each test or control group. All animals were euthanized after the twentieth dosing day. The left cheek pouches from ten (10) animals from each test or control group were submitted for histopathology. The test article was used as received.

Observations

Irritation was not observed in any test or control animal. There appears to be no significant difference between blood values obtained initially and terminally or between the terminal test and control group values. Gross necropsies revealed no apparent test related deviations.

Conclusion

This test article does not cause systemic physiological changes in hamsters, under the conditions of this test. "When applied to the epithelial surface of the left cheek pouch of male hamsters at a dosage of 0.1 ml, 3 times a day for 5 days per week for 4 weeks, the test article used in this study does not produce any significant incidence ($p = 0.05$ or less) of deviations from histologic morphology in the animals submitted for pathology evaluation[1]."

[1]Samuel W. Thompson, D.V.M., M.S., Diplomate, American College of Veterinary Pathologists

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Acute Oral Toxicity in Rats

This test was designed to determine the oral toxicity potential of the test article in rats at a dose level of ten (10) grams test article per kilogram of animal bodyweight. The method described by Hagan[1] served as a guide.

Wistar-strain, albino rats were used for this test. Animals were ordered from a suitably licensed dealer, in equal numbers of each sex, between 190 and 220 grams bodyweight, and approximately six to nine (6 to 9) weeks of age. Upon receipt, animals were carefully checked for respiratory difficulty, ocular or nasal lacrimation, dehydration, diarrhea, and general thriftiness.

Animals were acclimated for at least seven (7) days prior to test initiation. They were housed in stainless steel cages with indirect bedding, in a room with a 12 hour light/dark cycle. The room temperature was controlled, to provide for the health and comfort of the animals with an approximate range of 65° to 75° F. The humidity was also monitored. Diet consisted of Agway ProLab Rat, Mouse and Hamster 1000 Feed, as well as water, ad libitum.

Prior to test initiation, the test article's mass to volume relationship (specific gravity) was determined to facilitate volumetric dosing.

Twenty-four (24) hours prior to test initiation, the rats were reexamined for general thriftiness as described above. A group of five (5) male and five (5) female rats, of sufficient weight to assure a fasted bodyweight between 200 and 300 grams, was labelled and set aside.

The following day, after approximately 18 hours of fasting, each rat was weighed and marked with an ear clip. Individual doses, calculated on the basis of bodyweight, were administered using a stainless steel intragastric feeding needle, of sufficient bore to allow even passage of the test article. Rats were then returned to their cages, where food and water were available ad libitum. Each cage was labelled uniquely with respect to job number, test article, dose level, sex, animal number(s), and date of dosing.

Animals were observed for signs of pharmacologic activity and drug toxicity at 1, 3, 6, and 24 hours post-dosage. Observations were made at least once daily thereafter for a total of 14 days.

Animals sacrificed at the end of the 14 day observation period, as well as non-survivors, were subjected to complete gross necropsy, with all findings noted. Sacrificing was accomplished via carbon dioxide asphyxiation.

The test article was considered to be orally toxic to rats at ten (10) grams per kilogram of bodyweight if 50% or more of the animals in the test group died during the 14 day observation period.

[1] E.C. Hagan, "Acute Toxicity", Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, (The Association of Food and Drug Officials

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Twenty (20) Day
Hamster Cheek Pouch Application Study

This test was designed to determine the extent of irritation produced in the hamster cheek pouch following repeated doses of a formulation and to monitor any systemic physiological changes through clinical hematology and gross pathology.

Golden Syrian hamsters (Ela: ENG (SYR)), male, approximately 6 to 7 weeks old, were used. The animals were obtained through a suitably licensed dealer. They were checked carefully upon receipt for diarrhea, dehydration, respiratory difficulties, postural deficiencies, skin lesions and general condition.

The animals were acclimated for nine (9) days prior to test initiation. They were individually housed in stainless steel cages with indirect bedding, in a temperature controlled room where only hamsters were housed. The room had a 12 hour light/dark cycle and the room temperature was controlled to comply with Animal Welfare regulations, with an approximate range of 60° to 85° F. The humidity was also monitored. Diet consisted of Agway ProLab Rat, Mouse & Hamster 1000 Feed, as well as water, ad libitum. Each animal was individual identified by number and cage label.

Twenty-four (24) hours prior to test initiation the animals were reexamined. Any animals in poor condition were not used. Animals deemed fit for test were randomly divided into three (3) groups as follows:

Group	# of Animals	Assignment
I	5	Initial Blood Values
II	12	Test Article
III	12	Control Article (Distilled Water)

The animals assigned to Group I were then fasted. The following day, the Group I animals were anesthetized via inhalation, utilizing Halothane (U.S.P.). Blood samples were then taken from each of these animals.

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The parameters to be examined included the following[1]:

Hematology

Total erythrocyte count
Hemoglobin
Hematocrit
Total and differential leukocyte count

Blood Chemistry

Serum glutamic oxalic transaminase
Serum glutamic pyrivic transaminase
Serum alkaline phosphatase
Blood urea nitrogen
Glucose

Each animal in Groups II and III then received the following treatment. Food particles were cleared from the mucosal surface of the animal by rinsing the pouches with distilled water. Then the cheek pouches were gently everted manually and observations were recorded (see Table 10). A one (1) cc syringe was then filled with the appropriate test or control article. One-tenth of one (0.1) milliliter of the appropriate article was then applied to the mucosa of the left cheek pouch of each animal. The right cheek pouches served as untreated controls. The animals were also observed for pharmacotoxic effects.

These procedures were carried out on the animals in Groups II and III, three (3) times per day, five (5) days per week, for four (4) weeks. Individual bodyweights were recorded at test initiation, weekly thereafter and at termination.

On the last dosing day, after the third dosing procedure, the animals were fasted. The following day five (5) animals from each group were randomly chosen for terminal blood evaluations. The procedures and the parameters were as stated for the initial evaluations. All animals were then euthanized either by Halothane overdose or by carbon dioxide asphyxiation. Gross necropsies were performed on all animals with all findings noted. The left cheek pouch of each animal was removed, stapled to an index card, labelled and fixed in 10% formalin. The brain, liver, kidneys, adrenal glands, testicles and any abnormal tissues were also removed from each animal and similarly fixed. Ten (10) randomly chosen pouches from each group were submitted for histopathological evaluation by a board certified veterinary histopathologist. The remaining tissues were kept at this facility for possible analysis at the discretion of the sponsor.

[1] Brookdale Laboratories, Bloomfield, New Jersey. Due to the size of the animals, several samples were of insufficient volume to obtain values for all parameters.

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Acute Oral Toxicity in Rats

Indivisual results are presented in Table 1.

Twenty Day Hamster Cheek Pouch Application Study

The study organization is presented in Table 2. Group I animals, used for initial blood values, are presented in Table 3. Initial complete blood count values are presented in Table 4 and initial blood chemistry values are presented in Table 5. Terminal complete blood count values and blood chemistry values for Group II animals are presented in Tables 6 and 7 respectively. Terminal complete blood count values and blood chemistry values for Group III animals are presented in Tables 8 and 9 respectively. The scoring criteria used for oral mucosal reactions in the hamster is presented in Table 10. The individual results for Groups II and III are presented in Tables 11 and 12 respectively. The biophase/pathology cross reference is presented in Table 13. The pathology report is appended.

Summaries of all results are found preceding the text.

Table 1

Acute Oral Toxicity

OTC Oral Health care Product B# 787-19, 12-27-89

Dose Level: 10.0 g/kg

Animal Number and Sex	Bodyweight (grams)	Hours: 1	3	6	24	Days: 2	3	4	5	6	7	8	9	10	11	12	13	14	Bodyweight (grams)
1 M	232	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	330
2 M	220	N																N	312
3 M	226	N																N	340
4 M	228	N																N	308
5 M	224	N					NO CHANGES OBSERVED											N	330
6 F	214	N																N	270
7 F	218	N																N	254
8 F	216	N																N	260
9 F	218	N																N	264
10 F	218	N																N	258

Raw Data Page 12315

N = Normal
D = Depression
SD = Slight Depression
XD = Severe Depression
H = Hyperactivity
\+ = Animal Death

[1] Hair moist and matted
[2] Hair matted and unkempt
[3] Probable middle ear infection
[4] Diarrhea
[5] Mucoid diarrhea
[6] Appears dehydrated
[7] Convulsions
[8] Muscle tremors

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Comments: Animals #1-#10: No gross changes observed.

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Table 2

Study Organization

Twenty (20) Day Hamster Cheek Pouch Application Study

GROUP	NO. OF ANIMALS	ANIMAL NUMBERS	ASSIGNMENT
I	5	1-5	Initial Blood Values
II	12	1-12	OTC Oral Healthcare Product B# 787-19, 12-27-89
III	12	13-24	Distilled Water - Control

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Table 3

Group 1

Initial Blood Values Group

Animal No.	Sex	Fasted Initial Wgt (g)	Blood Samples Taken For: Complete Blood Count	Chemistry
1	M	82	X	X
2	M	95	X	X
3	M	102	X	X
4	M	87	X	X
5	M	94	X	X

Raw Data Page: 6260

Table 4

Group I

Initial Complete Blood Count Values

Animal No.	WBC $x10^3$	RBC $x10^6$	Hgb g%	Hct %	Polys* (PMNS)	Lymphs*	Monos*	Eosin*
1	8.6	6.40	16.1	43	34	63	2	1
2	9.1	6.50	15.9	41	41	56	2	1
3	11.5	5.10	12.0	38	39	59	1	1
4	4.4	5.68	13.3	31	32	64	2	2
5	11.6	6.50	16.2	43	33	63	3	1

* WBC % differential

WBC = White blood count
RBC = Red blood count
Hgb = Hemoglobin
Hct = Hematocrit
Polys = Polymorphonucleocytes
Lymphs = Lymphocytes
Monos = Monocytes
Eosin = Eosinophil

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Table 5

Group I

Initial Blood Chemistry Values

Animal No.	Calcium MG/DL	Phosphorus MG/DL	Glucose, Serum MG/DL	BUN MG/DL	Uric Acid MG/DL	Cholesterol MG/DL	Total Protein G/DL	Albumin G/DL
1*	12.6	8.8	217	19	2.7	160	5.8	3.9
2*	11.1	10.7	120	20	5.7	277	7.5	4.5
3**	----	8.8	110	18	2.6	250	6.2	4.4
4	11.8	7.4	124	18	2.4	186	6.3	4.0
5	12.9	8.8	159	20	1.9	163	6.1	4.1

* = Specimen was hemolyzed. Results may have been adversely affected.
** = Quantity insufficient for analysis. Tests reported were performed on a dilution.
- = Unable to obtain valid result.
BUN = Blood urea nitrogen

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Table 5
(continued)

Group I

Initial Blood Chemistry Values

Animal No.	Bilirubin, Total MG/DL	Alkaline Phosphatase IU/L	LDH IU/L	SGOT (AST) IU/L	SGPT IU/L
1*	0.2	190	314	97	84
2*	0.2	201	---	---	---
3**	0.1	224	186	48	84
4	0.2	215	113	42	50
5	0.2	222	166	59	59

* = Specimen was hemolyzed. Results may be adversely affected.
** = Quantity was not sufficient for analysis. Tests reported were performed on a dilution.
\- = Unable to obtain valid result.
LDH = Lactose dehydrogenase
SGOT = Serum glutamic oxalic transaminase
SGPT = Serum glutamic pyruvic transaminase

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Table 6

<u>Group II</u>

Terminal Complete Blood Count Values

Animal No.	WBC $x10^3$	RBC $x10^6$	Hgb g%	Hct %	Polys* (PMNS)	Lymphs*	Monos*	Eosin*
3	7.9	7.36	16.2	44	29	69	1	1
5	11.8	7.24	16.1	42	23	74	2	1
7	9.8	6.21	14.7	39	41	56	1	2
10	7.5	6.60	15.0	42	29	68	2	1
12	11.5	7.14	16.3	43	26	72	1	1

* WBC % differential
WBC = White blood count
RBC = Red blood count
Hgb = Hemoglobin
Hct = Hematocrit
Polys = Polymorphonucleocytes
Lymphs = Lymphocytes
Monos = Monocytes
Eosin = Eosinophil

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Table 7

Group II

Terminal Blood Chemistry Values

Animal No.	Calcium MG/DL	Phosphorous MG/DL	Glucose Serum MG/DL	BUN MG/DL	Uric Acid MG/DL	Cholesterol MG/DL	Total Protein G/DL	Albumin G/DL
3	13.9	9.9	121	16	0.7	154	6.1	3.7
5*	---	---	---	---	---	---	---	---
7	11.9	7.9	111	24	3.9	165	6.4	3.4
10	12.8	7.0	140	20	3.0	150	5.8	3.5
12	12.2	7.2	115	20	2.5	148	6.2	3.7

* Serum quantity insufficient to perform tests.
BUN = Blood urea nitrogen

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Table 7
(continued)

Group II

Terminal Blood Chemistry Values

Animal No.	Bilirubin, Total MG/DL	Alkaline Phosphatase IU/L	LDH IU/L	SGOT (AST) IU/L	SGPT IU/L
3	0.1	213	254	117	97
5*	---	---	---	---	---
7	0.1	230	486	141	108
10	0.2	183	164	58	70
12	0.1	180	262	76	110

* Serum quantity insufficient to perform tests.
LDH = Lactose dehydrogenase
SGOT = Serum glutamic oxalic transaminase
SGPT = Serum glutamic pyruvic transaminase

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Table 8

Group III

Terminal Complete Blood Count Values

Animal No.	WBC $x10^3$	RBC $x10^6$	Hgb g%	Hct %	Polys* (PMNS)	Lymphs*	Monos*	Eosin*
14	10.4	6.48	15.7	41	61	36	2	1
15	6.9	7.23	17.8	46	22	75	2	1
18	9.5	7.26	16.9	48	37	61	1	1
20	4.4	7.44	16.6	45	33	64	2	1
24	8.9	6.80	16.6	44	29	69	1	1

* WBC % differential
WBC = White blood count
RBC = Red blood count
Hgb = Hemoglobin
Hct = Hematocrit
Polys = Polymorphonucleocytes
Lymphs = Lymphocytes
Monos = Monocytes
Eosin = Eosinophil

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Table 9

Group III

Terminal Blood Chemistry Values

Animal No.	Calcium MG/DL	Phosphorus MG/DL	Glucose Serum MG/DL	BUN MG/DL	Uric Acid MG/DL	Cholesterol MG/DL	Total Protein G/DL	Albumin G/DL
14	12.9	6.6	100	21	2.8	131	5.7	3.5
15	12.6	7.6	110	22	1.5	155	6.0	3.6
18*	---	---	---	--	---	---	---	---
20	11.8	4.6	72	19	2.1	156	5.7	3.4
24	11.9	7.7	169	17	2.4	175	6.7	3.9

* Serum quantity insufficient to perform tests.
BUN = Blood urea nitrogen

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Table 9
(continued)

Group III

Terminal Blood Chemistry Values

Animal No.	Bilirubin, Total MG/DL	Alkaline Phosphatase IU/L	LDH IU/L	SGOT (AST) IU/L	SGPT IU/L
14	0.2	169	196	65	72
15	0.2	159	198	45	68
18*	---	---	---	--	--
20	0.2	164	97	41	75
24	0.2	164	153	53	70

* Serum quantity insufficient to perform tests.
LDH = Lactose dehydrogenase
SGOT = Serum glutamic oxalic transaminase
SGPT = Serum glutamic pyruvic transaminase

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Table 10

Scoring Criteria for Oral Mucosal Reactions in the Hamster

(Modified Draize Scale)

Erythema Formation	
Natural pink condition of mucosa	0
Well-defined erythema	1
Moderate to severe erythema	2
Severe erythema (beet redness)	3
Loss of color (blanching, blistering, sloughing of mucosa, etc.)	4
Edema Formation	
Normal condition (note folds in mucosa)	0
Slight edema (edges of area well-defined by definite raising)	1
Moderate edema (area raised approximately 1 mm)	2
Severe edema (raised more than 1 mm and extending beyond area of exposure)	3
Blistering	4

SAFETY

PATHOLOGY REPORT

HAMSTER CHEEK POUCH BUCCAL MUCOSAL IRRITATION

Study Number 90051

Introduction:

The specimens consist of the left cheek pouch of each of 20 acclimated, male Golden Syrian hamsters. Ten hamsters composed a test and control group. The study was composed of one test group (Group II) and one control group (Group III). A test article was applied at a dosage of 0.1 ml, three (3) times daily for 5 days a week for 4 weeks, to the left cheek pouch of each animal. The right cheek pouch of each hamster received no treatment. On the 29th day, approximately 18 hours after the last treatment, the animals were observed and then euthanized with carbon dioxide. The left cheek pouches were removed, stapled to index cards, labelled, fixed in 10% formalin and forwarded to the undersigned pathologist. The pathologist was not aware of the identity or nature of the test article.

Methods:

Two tissue specimens were selected from the left cheek pouch of each animal. All tissue specimens were processed on a VIP processor and were subsequently embedded in Paraplast in such a fashion that the two specimens from each animal were embedded in single tissue block. A tissue section of each of the 20 blocks of embedded tissue specimens was cut at a thickness setting of 5 microns on a rotary microtome and was mounted on a glass microscope slide. Each mounted tissue section was stained with hematoxylin and eosin,

coverslipped, and examined with a microscope by a pathologist.

Statistical evaluation ($p = 0.05$ or less) of the histopathology data, as required by the pathologist, was carried out by using Fisher's Exact Test - 1 tailed by references to published statistical tables (Thompson, S. W. and Rac, V. S., Tox. Path. 9, pp 1-18, 1981) as follows:

The Incidence For Any Abnormality Which Occurs In Treated Versus Control Animals And Is Needed To Be Significant At Probability Levels of 0.05, 0.01 & 0.001

Sample Size	Abnormality Incidence in Controls	Abnormality Incidence of Treated Needed for p at			
		Not Significant	0.5	0.1	.001
10	0/10	3/10	4/10	6/10	8/10
	1/10	5/10	6/10	7/10	9/10
	2/10	6/10	7/10	8/10	10/10
	3/10	7/10	8/10	9/10	---
	4/10	8/10	9/10	10/10	---
	5/10	9/10	10/10	---	---

The histomorphologic observations of the pathologist were entered in a Leading Edge Computer using the LABCAT software program for histopathology published by Innovative Programming Associates of Princeton, New Jersey.

Results:

All animals of Groups II and III survived the 28 day test period. No necropsy observation was reported to the pathologist, as noted in the form for each animal entitled "Individual Animal Data" which is attached to this report.

The results of the pathologist's evaluation of all microscopic tissue specimens from each animal comprising this study are tabulated on the tables of "Tabulated Animal Data", which forms part of this report. In the table of "Tabulated Animal Data", an entry was made for each tissue specimen from each animal to indicate if the microscopic anatomy of the site was evaluated as being normal (N) or abnormal in appearance. The histomorphologic observations shown on the Tabulated Animal Data Table appears in narrative form on the form for each animal entitled "Individual Animal Data".

The incidence ratios of microscopic observations, as described in the "Individual Animal Data" for each hamster are shown in the "Project Summary Table" which is attached to this report.

Discussion:

The deviations from normal histologic morphology observed in the left cheek pouch of each treated or control hamster submitted for pathology evaluation in this study are shown in the "Project Summary", "Tabulated Animal Data" and "Individual Animal Data" tables and need not be repeated here.

No deviation from normal histologic morphology occurred at an incidence ratio in the hamsters of Group II (test article) at a level which was significant ($p = 0.05$ or less) when compared to the incidence for the same observation in animals of Group III (distilled water). Therefore, all deviations from normal histologic morphology observed in the left cheek pouch of the animals of Groups II and III, which were submitted for pathology evaluation, are considered to be spontaneous in their occurrence, fortuitous in their distribution and unrelated to treatment with the test article.

Conclusion:

When applied to the epithelial surface of the left cheek pouch of male hamsters at a dosage of 0.1 ml 3 times a day for 5 days per week for 4 weeks, the test article used in this study does not produce any significant incidence ($p = 0.05$ or less) of deviations from histologic morphology in the animals submitted for pathology evaluation.

Diplomate, American College of Veterinary Pathologists

April 26, 1990

CONFIDENTIAL

IV. HUMAN SAFETY DATA

IV. Human Safety Data

A. INDIVIDUAL ACTIVE COMPONENTS

Under Section III., Letters of Authorization were enclosed to allow the Panel to access the Drug Master Files of the Raw Materials from which the active ingredient, MICRODENT™, is constructed. The Letters of Authorization from for are attached here again for the convenience of the panel.

Any relevant data on humans which the FDA has previously deemed necessary in order to previously allow the wide use range of oral and ingested products undoubtedly is included therein.

Petitioner has no additional information concerning Human Safety Data and submits that, given the long history of safe use of the Raw Materials at daily intake levels far above that which is possible with the oral care products containing MICRODENT™, no additional data is required to satisfy the requirements of GRAS.

B. COMBINATIONS OF ACTIVE COMPONENTS

This sub-section is not relevant to the active ingredient, MICRODENT™. Petitioner is not filing for combinations of actives.

C. FINISHED DRUG PRODUCTS

As detailed in Sections 0 and I, all ingredients used in the various finished drug products of this filing are taken from lists of ingredients already widely used in the category and are GRAS.

In view of the difficulty that the manufacturers of both the poloxamer and polydimethylsiloxane had in eliciting toxic responses in animals, Petitioner submits that there is no likelihood of finished drug products with MICRODENT™ demonstrating a lack of adequate safety in humans.

1. CONTROLLED STUDIES

All controlled clinicals which are presented in their entirety in Section V. and summarized in Section VI., included specifications that the examining dentists observe carefully for any deleterious effects to the oral tissue. NONE WERE FOUND!

Since the Mode of Action precludes pharmacologic or anti-microbial activity, Petitioner submits that high dose level, controlled studies for toxicity on human volunteers would be irrelevant and redundant.

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V. EFFICACY DATA

V. Efficacy Data

A. INDIVIDUAL ACTIVE COMPONENTS

There have been no clinical studies on the individual active components of MICRODENT™. As noted in the definition subsection of Section II, "Quantities of Active Ingredients", the active ingredient of this petition, MICRODENT™, is comprised of an emulsion of polydimethylsiloxane, in or by, a poloxamer.

Individual raw materials do not have the activity, efficacy or mode of action ascribed to MICRODENT™. The "Colgate Clinical Study (WHOTI-1990)" presented under Section V. C. 1. below, clearly demonstrates that neither of the raw materials alone have the plaque reducing effect of MICRODENT™.

Further, since the mode of action of MICRODENT™ requires self-administration of sprays, rinses, gels, dental floss, dental stimulators or chewing gums, etc. on a frequent and sustained basis, there is no reasonable and meaningful way to test for the efficacy of MICRODENT™ as an individual active component. It must be formulated into a finished drug product before it can be tested.

Further still, since MICRODENT™'s mode of action is non-invasive, ie, there is no known anti-microbial or anti-enzyme properties to be tested *in vitro*, there have been no laboratory tests relevant to the activity of MICRODENT™ performed by, or known to, the Petitioner.

V. Efficacy Data

B. COMBINATIONS OF INDIVIDUAL ACTIVE COMPONENTS

This petition, under the "call-for-data", addresses only the plaque reducing activity of MICRODENT™, as defined in Section II.

Section II does refer to the probable advantages to the general public of having available certain oral care products performing more than one beneficial ingredient. Such are already being sold, of course, and undoubtedly the subject of simultaneous filings with the FDA. [For example, certain fluoride (for caries) containing mouth rinses also making plaque or gingivitis claims.]

Petitioner expects to perform various tests, including clinical studies, showing the efficacy and beneficial effects of combinations of individual actives (such as sodium fluoride for anti-caries activity) with MICRODENT™ in the near future. These studies will be made available to the panel as they are completed.

Reference to a Simultaneous Filing in Support of Stannous Fluoride with MICRODENT™ as a "Carrier"

The Agency and the Panel should also be aware that the Petitioner has, in response to the "call-for-data", joined with a Co-petitioner, Dunhall Pharmaceuticals, Inc. in the filing of a response concerning the active ingredient, stannous fluoride.

That filing includes new and pertinent information concerning the clinical efficacy, and the improvement in consumer/patient acceptability and compliance obtained with active, stable, stannous fluoride containing oral care products, in which MICRODENT™ is used as a "carrier" of the stannous fluoride.

Said joint filing focuses on the "gingivitis" claims and clinical reduction of gingival bleeding attributable to the well known, anti-microbial, *in vivo,* activity of stannous fluoride.

It makes no assertions that stannous fluoride, when combined with MICRODENT™ has any additional clinical activity for the reduction of plaque other than that which is attributable to stannous fluoride alone.

Petitioner submits that, given the non-invasive, surface energy modifying, mode of action of MICRODENT™, one knowledgeable in the art and science of oral care products would not expect to see any *increase* in either the anti-gingivitis or the anti-plaque activity of stannous fluoride when combined with MICRODENT™.

Other than, perhaps, an increase in patient compliance due to improved taste and mouthfeel related to the surface energy modifying properties of MICRODENT™, the clinical effect of the combination should be no greater than for stannous fluoride alone.

Should information contrary to this current knowledge become known to Petitioner, it will be promptly submitted to the Agency for Panel's review.

Therefore, in said joint filing, MICRODENT™ is not presented as an "active ingredient", nor will stannous fluoride products claim MICRODENT™ as a "plaque reducer" under the active ingredient listing on the label or in advertizing.

Simply put, Petitioner does not intend to make, or allow licensees to make, "DOUBLE ACTIVE" claims so popular in today's marketing arena, without the benefit of appropriate and convincing clinical evidence.

V. Efficacy Data

C. FINISHED DRUG PRODUCTS: INTRODUCTION

In Section V. A. above, petitioner explained that the mode of action and the need for the subject to self-administer the active ingredient frequently to the oral cavity made it imperative that all studies on efficacy be performed with formulated (or finished) drug products and placebos which are as near in physical appearance, taste and mouthfeel as is possible.

Thus, all the new and pertinent information known to, or developed by, the Petitioner is included in this section. It also seemed appropriate to include the bulk of the literature review and copies of relevant publications in this Section V. C. 5., although it is referenced in other sections as well.

The material in this section is intended to be read with a full understanding of the "Mode of Action" discussed and documented from the literature primarily in Section VI., and an understanding of the physical-chemical properties of the active ingredient, MICRODENT™, discussed primarily in Section II.

When so viewed, Petitioner believes that the Panel will find the Efficacy data consistent with the general body of scientific knowledge and compelling toward an agreement with Petitioner that the active ingredient of this response to the "call-for-data" is, in fact, a "plaque reducing" active within the claims restrictions requested in Section VI. (Appendix I).

Petitioner recognizes that, as always, a review by experts knowledgeable in the field will prompt many questions such as:

> "What would be the result if a clinical protocol were designed to address this question in another way?" or,
>
> Would the effect be greater or smaller if the product were presented in a different formulation or physical form"? etc.

Petitioner also desires to know the answer to many such questions, and intends to address some of them even while the panel begins its deliberations. Petitioner asks both the Agency and the Panel for their approval to submit new and relevant information as it becomes available and under schedules which will not unreasonably disturb the Panel's deliberations.

Experimental pursuit of such new information as is reasonably requested by the Panel will be pursued by Petitioner as the time and resources of a small business (as defined by the Department of Commerce and FDA's Division of Small Manufacturers Assistance) will allow.

(2) SURFACE ENERGY MODIFICATION

This part of the Mode of Action is discussed at length in Section VI. It is supported by overwhelming confirmation from the literature reviewed herein. The surface energy modification phenomenon is perhaps best summed up in a quote from Glantz (1978):

> "the acquired film on low surface energy (siliconized) substrates gave new wettability data characterizing the polydimethylsiloxane layer."

Glantz further observed that the attachment of plaque to siliconized surfaces was "disorganized and loosely attached".

Petitioner has discovered in vivo clinical evidence, based on MICRODENT™, which is retrospectively consistent with Glantz' findings, even though his observations were made on surfaces to which the polydimethylsiloxane had been chemically synthesized in place and in vitro. Specifically, the Colgate Clinical Study WHOTI-1990 demonstrates that without the emulsified polydimethylsiloxane there was no plaque reducing effect.

Accomplishing this surface energy modification in a pleasant tasting, pleasant feeling, non-toxic and non-invasive manner is the important and novel contribution of the active ingredient, MICRODENT™ to the "plaque reducing" category.

(3) FREQUENCY OF USE

The third factor is the direct result of the non-invasive, non-irritating nature of the MICRODENT™ emulsion itself.

Effective MICRODENT™ product formulations are not limited to (1) those which can only be used occasionally (as with irritating, high alcohol vehicles), (2) those which have taste or astringency profiles which cause the user to disregard label instructions concerning "use time" or "frequency", or (3) those requiring use "in-the-private-bathroom" (not accessible for many or most of the working population for much of each day).

Section V. C. 4. details the results of about 3400 consumer home-use placements ranging from an oral hygiene spray to dental floss. Products containing MICRODENT™ were used with great frequency and pleasure. Even with the "most-lied-about" oral care use-frequency product category, Dental Floss, MICRODENT™ DENTAL FLOSS received much higher "intent to use" and "intent to purchase" responses than controls of standard, commercially available floss, in addition to the clinical evidence of plaque removal superiority.

Lack of compliance is a common editorial theme throughout professional dentistry publications and newsletters. It is not insignificant to note that one reason MICRODENT™ works is that otherwise unmotivated individuals are happy to use it...frequently.

V. Efficacy

C. Finished Drug Products

0. Introduction

(b) SUMMARY OF CONCLUSIONS FROM ALL CLINICALS

The four clinicals filed herein; three on oral hygiene spray formulations of the active ingredient, MICRODENT™, and one on a solid emulsion of MICRODENT™ in dental floss, demonstrate:

(1) MICRODENT™ is a consistent, moderate reducer of plaque when introduced into the oral cavity:
 (a) in a variety of forms and
 (b) under a variety of protocol designs.

(2) Statistically significant plaque reduction by ANOVA (alpha=.05) and t-tests ($p < 0.05$ up to $p < .001$), depending upon protocol design and the product formulation utilized, was achieved.

(3) The amount of plaque reduction was about 10% of the mean plaque scores. This was consistent with mechanistic studies of oral debris reduction and chemical assay of removed plaque on spent floss.

(4) The MICRODENT™ on plaque reduction effect is obtained with distinctly different protocols including: (a) suspension of normal oral hygiene procedures and (b) maintaining normal oral hygiene procedures.

(5) That, for "plaque" activity to be achieved, the complete active ingredient, MICRODENT™, must be utilized. Specifically, absence of the polydimethylsiloxane from the emulsion precludes activity.

(6) That, consistent with the physical-chemical Mode of Action proposed by Petitioner: the greater effects of MICRODENT™ are observed on those areas of tooth surface most efficiently contacted by the active ingredient.

(7) No adverse reactions to MICRODENT™, and

(8) MICRODENT™ does not exhibit an effect on gingivitis.
 (Petitioner does not propose gingivitis claims)

V. Efficacy

C. Finished Drug Products

0. Introduction

(c) SUMMARY OF ALL RELEVANT SCIENTIFIC LITERATURE

Complete Literature Reviews, Annotated Bibliographies and Copies of all the literature relied upon for this filing, which is all the relevant literature known to the Petitioner, is contained in V. C. 1. (d). [for Dental Floss] and in V. C. 5. [for Surface Free Energy and Bacterial Adhesion].

Simply put, the medical and scientific literature pertinent to the active ingredient, MICRODENT™, completely supports the proposed Mode of Action and makes the consistent results of plaque reduction seen in the four clinicals reported herein understandable on a fundamental basis. In layman's language, if one can "make the teeth so slick, the plaque won't stick", there should be a measurable reduction in plaque, in a variety of product forms, demonstrable under a number of clinical protocol strategies ....including those where normal oral hygiene is continued.

V. Efficacy Data

C. Finished Drug Products

1. CONTROLLED STUDIES

V. EFFICACY DATA

C. FINISHED DRUG PRODUCTS

1. CONTROLLED STUDIES

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PP-1986-01
PP-1986-02

(B) CLINICAL STUDY: WHOTI-1990

(C) CLINICAL STUDY: WHD-001

PILOT CLINICAL STUDIES

PP-1986-01
PP-1986-02

PRINCIPLE SUPERVISING DENTIST:
PRINCIPLE INVESTIGATOR:
PROJECT COORDINATOR:
SPONSOR:

TEST SITE:

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

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C. QUANTITATIVE RESULTS

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

A. ABSTRACT

The Petitioner's active ingredient, MICRODENT™, was tested in a pair of double blind, parallel treatment design protocols wherein subjects discontinued normal oral hygiene for the test period, but used instead a Test Product with the brand name TAKE-5™ (containing 0.43% MICRODENT™) or a Placebo of commercially obtained breath freshener branded BINACA®.

Active and Placebo test groups of 10 each (PP-1986-01) and 13 each (PP-1986-02) used their assigned product delivered by a spray device at least 5 times per day. In one test (PP-1986-01) subjects were not given a dental prophylaxis, and were scored for plaque at Baseline and after 24 hours. In the second test, (PP-1986-02) the subjects received a dental prophylaxis and were scored after 48 hours.

In both pilot tests, the plaque accumulation with the Test Product containing MICRODENT™ was less than with the Placebo.

In the "no prophylaxis" test, the increase in mean plaque score after 24 hours of no oral hygiene was evaluated. The test group's increase was about two/thirds of the placebo group's increase.

In the "with prophylaxis" test, after 48 hours of no oral hygiene, the mean plaque score of the test group with MICRODENT™ was 9% less than the placebo group's plaque score.

Since the number of subjects in each group was small, no attempt at determining statistical significance was deemed to be warranted. There were no adverse effects on hard or soft tissue observed.

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

B. PROTOCOL BRIEF

1. INFORMATION SOUGHT

These pilot clinicals were designed to provide an indication whether the active ingredient of this petition, MICRODENT™, previously test marketed as a mouth "cleaner" and breath freshener, would demonstrate sufficient effect on plaque to be worthy of more complete clinical evaluation. The questions posed were:

(a) Does the active ingredient, MICRODENT™, delivered in a spray form, have an effect when used frequently by individuals with plaque already well established, and

(b) Does the active ingredient, MICRODENT™, delivered in a spray form, have an effect when used frequently by individuals with plaque removed before beginning use.

2. FORMULATIONS TESTED: ACTIVE vs PLACEBO

ACTIVE

The exact formulation of MICRODENT™ in the commercial product, TAKE-5™, and as used in these studies, follows:

INGREDIENT	% BY WEIGHT

PLACEBO

The exact placebo formula, BINACA®, is not known. However, the commercial label declares: alcohol, water, glycerin, sodium saccharin, and flavor. The propellent is hydrocarbon A-46 This placebo was deemed adequate to stimulate saliva flow and introduce alcohol and flavor oils (potential antimicrobials) to the oral cavity in quantities similar to the test product.

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

B. PROTOCOL BRIEF

1. PROTOCOL DESIGN ELEMENTS PP-1986-01

(a) Double blind, parallel treatment.
(b) Ten (10) subjects per test group.
(c) Suspension of normal oral hygiene during test.
(d) Subjects pre-screened for oral health and scored for baseline plaque index (Turesky modification, Quigley-Hein). Subjects randomly distributed into two groups based on plaque score equalization.
(e) Subjects were not given a dental prophylaxis.
(f) Subjects instructed in proper use of sprays, one self-administered use observed,. then instructed to use the assigned product ad lib but at least five (5) times per day.
(g) Subjects returned to clinic at 24 hours for scoring of plaque index as in (d).

2. PROTOCOL DESIGN ELEMENTS PP-1986-02

(a) Double blind, parallel treatment.
(b) Thirteen (13) subjects per test group.
(c) Suspension of normal oral hygiene during test.
(d) Subjects pre-screened for oral health and scored for baseline plaque index (Turesky modification, Quigley-Hein). Subjects randomly distributed into two groups based on plaque score equalization.
(e) Subjects were given a through dental prophylaxis, reducing the plaque index to zero at time zero.
(f) Subjects instructed in proper use of sprays, one self-administered use observed, then instructed to use the assigned product ad lib but at least five (5) times per day.
(g) Subjects returned to the clinic at 48 hours for scoring of plaque index as in (d).

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

C. QUANTITATIVE RESULTS

These tests, with their small number of subjects per group, very short test periods, and informal protocol, were not intended to be subjected to statistical analysis. Thus, the only value is in the trend lines. That value is primarily limited to the encouragement to pursue the more rigorous tests submitted in this filing. There is some retrospective value, of course, if the trends are consistent with statistically analyzed full scale clinicals.

(1) PP-1986-01 RESULTS AND DISCUSSION

TABLE 1.

PRODUCT	No. OF SUBJECTS	MEAN BASELINE	MEAN 24 Hr	MEAN DIFFERENCE
TAKE-5	10	1.83	2.04	0.21
BINACA	10	1.78	2.10	0.31

The trend line suggests that under these use conditions, the expected rate of increase due to the cessation of oral hygiene for 24 hours was moderated somewhat by the test product containing MICRODENT™. On this small population sample, the increase in additional plaque over that at baseline by subjects using TAKE-5 can be considered to be one/third that of the increase in subjects using the placebo.

This is considered to be a surprising finding in light of the 24 hour test period chosen.

(2) PP-1986-02 RESULTS AND DISCUSSION

TABLE 2.

PRODUCT	No. OF SUBJECT	MEAN 0 Hr	MEAN 48 Hr	MEAN REDUCTION vs. BINACA	% REDUCTION vs. BINACA
TAKE-5	13	0	1.62	-0.16	9%
BINACA	13	0	1.78	--	--

Again the trend line suggests some beneficial effect by the active ingredient MICRODENT™ in reducing plaque build up. Over the 48 hour period, the actual mean difference in plaque score between the two test groups was somewhat greater than in PP-1986-01 (0.16 vs. 0.10). This may be due to starting with prophylaxis, or could be due to the longer, more numerous, exposure to the active ingredient.

CONCLUSION

The active ingredient, MICRODENT™, does reduce plaque under two rather different protocols and time frames. The extent of the reduction is not clear, nor does this test do much to suggest a mode of action. The consistency of direction for the two pilot clinicals is perhaps more relevent than the actual magnitude of the results.

PILOT CLINICAL STUDIES

PP-1986-01
PP-1986-02

PRINCIPLE SUPERVISING DENTIST:
PRINCIPLE INVESTIGATOR:
PROJECT COORDINATOR:
SPONSOR:

TEST SITE:

PILOT CLINICAL STUDIES
PP-1986-01
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C. QUANTITATIVE RESULTS

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

A. ABSTRACT

The Petitioner's active ingredient, MICRODENT™, was tested in a pair of double blind, parallel treatment design protocols wherein subjects discontinued normal oral hygiene for the test period, but used instead a Test Product with the brand name TAKE-5™ (containing 0.43% MICRODENT™) or a Placebo of commercially obtained breath freshener branded BINACA®.

Active and Placebo test groups of 10 each (PP-1986-01) and 13 each (PP-1986-02) used their assigned product delivered by a spray device at least 5 times per day. In one test (PP-1986-01) subjects were not given a dental prophylaxis, and were scored for plaque at Baseline and after 24 hours. In the second test, (PP-1986-02) the subjects received a dental prophylaxis and were scored after 48 hours.

In both pilot tests, the plaque accumulation with the Test Product containing MICRODENT™ was less than with the Placebo.

In the "no prophylaxis" test, the increase in mean plaque score after 24 hours of no oral hygiene was evaluated. The test group's increase was about two/thirds of the placebo group's increase.

In the "with prophylaxis" test, after 48 hours of no oral hygiene, the mean plaque score of the test group with MICRODENT™ was 9% less than the placebo group's plaque score.

Since the number of subjects in each group was small, no attempt at determining statistical significance was deemed to be warranted. There were no adverse effects on hard or soft tissue observed.

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

B. PROTOCOL BRIEF

1. INFORMATION SOUGHT

These pilot clinicals were designed to provide an indication whether the active ingredient of this petition, MICRODENT™, previously test marketed as a mouth "cleaner" and breath freshener, would demonstrate sufficient effect on plaque to be worthy of more complete clinical evaluation. The questions posed were:

(a) Does the active ingredient, MICRODENT™, delivered in a spray form, have an effect when used frequently by individuals with plaque already well established, and

(b) Does the active ingredient, MICRODENT™, delivered in a spray form, have an effect when used frequently by individuals with plaque removed before beginning use.

2. FORMULATIONS TESTED: ACTIVE vs PLACEBO

ACTIVE

The exact formulation of MICRODENT™ in the commercial product, TAKE-5™, and as used in these studies, follows:

SIMETHICONE AF-30 MEDICAL (DOW-CORNING)

PLACEBO

The exact placebo formula, BINACA®, is not known. However, the commercial label declares: alcohol, water, glycerin, sodium saccharin, and flavor. The propellent is hydrocarbon A-46 This placebo was deemed adequate to stimulate saliva flow and introduce alcohol and flavor oils (potential antimicrobials) to the oral cavity in quantities similar to the test product.

PILOT CLINICAL STUDIES
PP-1986-01
PP-1986-02

B. PROTOCOL BRIEF

1. PROTOCOL DESIGN ELEMENTS PP-1986-01

(a) Double blind, parallel treatment.
(b) Ten (10) subjects per test group.
(c) Suspension of normal oral hygiene during test.
(d) Subjects pre-screened for oral health and scored for baseline plaque index (Turesky modification, Quigley-Hein). Subjects randomly distributed into two groups based on plaque score equalization.
(e) Subjects were not given a dental prophylaxis.
(f) Subjects instructed in proper use of sprays, one self-administered use observed, then instructed to use the assigned product ad lib but at least five (5) times per day.
(g) Subjects returned to clinic at 24 hours for scoring of plaque index as in (d).

2. PROTOCOL DESIGN ELEMENTS PP-1986-02

(a) Double blind, parallel treatment.
(b) Thirteen (13) subjects per test group.
(c) Suspension of normal oral hygiene during test.
(d) Subjects pre-screened for oral health and scored for baseline plaque index (Turesky modification, Quigley-Hein). Subjects randomly distributed into two groups based on plaque score equalization.
(e) Subjects were given a through dental prophylaxis, reducing the plaque index to zero at time zero.
(f) Subjects instructed in proper use of sprays, one self-administered use observed, then instructed to use the assigned product ad lib but at least five (5) times per day.
(g) Subjects returned to the clinic at 48 hours for scoring of plaque index as in (d).

C. QUANTITATIVE RESULTS

These tests, with their small number of subjects per group, very short test periods, and informal protocol, were not intended to be subjected to statistical analysis. Thus, the only value is in the trend lines. That value is primarily limited to the encouragement to pursue the more rigorous tests submitted in this filing. There is some retrospective value, of course, if the trends are consistent with statistically analyzed full scale clinicals.

(1) PP-1986-01 RESULTS AND DISCUSSION

TABLE 1.

PRODUCT	No. OF SUBJECTS	MEAN BASELINE	MEAN 24 Hr	MEAN DIFFERENCE
TAKE-5	10	1.83	2.04	0.21
BINACA	10	1.78	2.10	0.31

The trend line suggests that under these use conditions, the expected rate of increase due to the cessation of oral hygiene for 24 hours was moderated somewhat by the test product containing MICRODENT™. On this small population sample, the increase in additional plaque over that at baseline by subjects using TAKE-5 can be considered to be one/third that of the increase in subjects using the placebo.

This is considered to be a surprising finding in light of the 24 hour test period chosen.

(2) PP-1986-02 RESULTS AND DISCUSSION

TABLE 2.

PRODUCT	No. OF SUBJECT	MEAN 0 Hr	MEAN 48 Hr	MEAN REDUCTION vs. BINACA	% REDUCTION vs. BINACA
TAKE-5	13	0	1.62	-0.16	9%
BINACA	13	0	1.78	--	--

Again the trend line suggests some beneficial effect by the active ingredient MICRODENT™ in reducing plaque build up. Over the 48 hour period, the actual mean difference in plaque score between the two test groups was somewhat greater than in PP-1986-01 (0.16 vs. 0.10). This may be due to starting with prophylaxis, or could be due to the longer, more numerous, exposure to the active ingredient.

CONCLUSION

The active ingredient, MICRODENT™, does reduce plaque under two rather different protocols and time frames. The extent of the reduction is not clear, nor does this test do much to suggest a mode of action. The consistency of direction for the two pilot clinicals is perhaps more relevent than the actual magnitude of the results.

CLINICAL STUDY

WHOTI-1990

PRINCIPLE SUPERVISING DENTIST:
PRINCIPLE INVESTIGATOR:
BIOSTATISTICIAN:
PROJECT COORDINATOR:
SCIENTIFIC SPONSOR:

TEST SITE:

CLINICAL STUDY

WHOTI-1990

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F. Complete Protocol

CLINICAL STUDY

WHOTI-1990

A. ABSTRACT

The Petitioner's active ingredient, MICRODENT™, was tested using a double blind, cross-over treatment design wherein each of the thirty-two subjects received all of the four Test Products and one Placebo; one each week in a random fashion over a contiguous five week period.

The products were delivered by a spray device five times per day over the three day test period. Subjects were instructed to refrain from normal oral hygiene during the test period and return to their normal procedures across the weekend.

Subjects received a dental prophylaxis each week, immediately before beginning a new test period. Plaque was scored on the morning of the fourth day by the Turesky modification of Quigley-Hein.

The mean Plaque Index for each Test Product was reduced about 10% compared to the Placebo. This result was statistically significant for each Test Product vs Placebo at $p < 0.05$. The Test Products did not significantly differ from each other.

When Test Product scores were combined for comparison with Placebo in an ANOVA, the significance was even greater, $p=0.0001$.

No deleterious effects on hard or soft tissues across the five week period were observed.

B. PROTOCOL BRIEF

1. Information Sought
2. Formulations Tested
3. Protocol Design Elements

CLINICAL STUDY

WHOTI-1990

B. PROTOCOL BRIEF

1. INFORMATION SOUGHT

This clinical protocol was designed to address three fundamental questions:

(a) Does the active ingredient of this petition, MICRODENT™, demonstrate a plaque reduction effect when tested over a three day period in which normal oral hygiene was suspended, and in which each subject was exposed to all the test/placebo products presented in a random fashion over a five week period?

(b) Would the active ingredient, MICRODENT™, exhibit a plaque reduction effect when compared to a Placebo containing a high level of one of the Raw Materials of the active ingredient (ie, poloxamer surfactant)?

(c) Do minor variations in the molecular weight of the polydimethylsiloxane Raw Material component of the active ingredient, MICRODENT™, affect the plaque reduction effect?

Data interpretation addressed a fourth issue:

(c) When all the Test Product cells were compared with each other and with Placebo, were any possible plaque reduction effects statistically significant, and additionally, when all Test Product cells were combined and compared with the Placebo by ANOVA techniques, how statistically significant would the Active Ingredient reduction over Placebo become?

CLINICAL STUDY

WHOTI-1990

B. PROTOCOL BRIEF

2. FORMULATIONS TESTED: ACTIVES vs PLACEBO

The exact formulations of the MICRODENT™ Test Product and Placebo follow. See sub-section F. Complete Protocol, for internal safety clearance documents for each formula.

FORMULA SUMMARY

PRODUCT	% by Wt MICRODENT™	% by Wt POLOXAMER	% by Wt POLYDIMETHYL-SILOXANE	(TYPE)
Placebo	-0-	1.50	-0-	
Test-I	1.64	1.54	0.10	AF Q7-2587
Test-II	1.64	1.54	0.10	AF (std)
Test-III	0.43	0.40	0.03	AF Q7-2587
Test-IV	0.43	0.40	0.03	AF (std)

The ratio of poloxamer to polydimethylsiloxane was 40:1 for all test products.

The polydimethylsiloxane in Test-II and Test-IV was the standard AF-30 Medical Emulsion (30% polydimethylsiloxane by weight). In Test-I and Test-III a narrower molecular weight range of similar viscosity was chosen to determine if differences in the polydimethylsiloxane raw material changed the plaque reducing effect of MICRODENT™.

The Placebo differed from the Test Products as follows:

(a) No MICRODENT™ active ingredient, as defined in this filing.

(b) Equivalent surfactant (poloxamer) as would be contributed by the highest levels of MICRODENT™ tested.

(c) Replacement of ethyl alcohol with water.

CLINICAL STUDY

WHOTI-1990

COMPLETE FORMULAS: TEST PRODUCTS AND PLACEBO

CAS #	CHEMICAL NAME	PLACEBO	TEST-I	TEST-II	TEST-III	TEST-IV

CLINICAL STUDY

WHOTI-1990

B. PROTOCOL BRIEF

3. PROTOCOL DESIGN ELEMENTS

The full text of the protocol designed by the staff follows under sub-section F. of this clinical report. However, for the convenience of the panel, we summarize the key design elements before proceeding to the results.

ELEMENTS:

(1) Double-blind, cross-over clinical trial
(2) Five week test, three day test period each week. Each subject randomly assigned one of the test products each week across the five week sequence.
(3) Four Test Products containing MICRODENT™, one Placebo containing poloxamer surfactant equal to highest level in any Test Product.
(4) Fifty (50) adult male and female subjects qualified into study, thirty-two (32) subjects per cell entered and completed study.
(5) Subjects instructed to refrain from all oral hygiene procedures during each three day study period. Returned to normal oral hygiene across weekend.
(6) Subjects pre-screened for oral health and plaque index score at baseline of at least 2.0 (Turesky modification, Quigley-Hein).
(7) After subjects stratified into five balanced groups according to baseline plaque score, each group randomly assigned to one of the mouthsprays, products reassigned weekly for the five week cross-over test sequence.
(8) All subjects received a dental prophylaxis on day one of each week.
(9) Subjects instructed to spray their mouth with assigned spray five times daily for three days. Supervised spraying at beginning of each week and just prior to evaluation.
(10) Subjects receive plaque examination (Turesky modification, Quigley-Hein) on morning of day four of each week.
(11) Informed consent, Medical Review Board, and other usual clinical ethics procedures followed Company standards.
(12) Statistical Analysis performed by a qualified statistician, independent of clinical investigator or sponsor.

C. PRINCIPLE INVESTIGATOR'S FINAL REPORT

TO:

FROM:

DATE: May 15, 1990

FINAL RESULTS

PLAQUE CLINICAL STUDY OF FIVE MOUTHSPRAYS

MOUTHSPRAYS	N	MEAN PLAQUE SCORES	±	STANDARD DEVIATION	STATISTICAL SIGNIFICANCE
Placebo	32	2.30	±	0.255	
0.1% New Simethicone	32	2.16	±	0.393	< 0.05
0.1% Old Simethicone	32	2.12	±	0.368	< 0.05
0.03% New Simethicone	32	2.11	±	0.323	< 0.05
0.03% Old Simethicone	32	2.06	±	0.306	< 0.05

- All subjects received an initial oral prophylaxis and were stratified into five balanced groups on the basis of their pre-prophylaxis plaque scores.

- Subjects reported to clinical site once a day for the use of mouthspray under supervision. Subjects were asked to refrain from all oral hygiene procedures during the study period.

- Placebo was significantly different and has a significantly higher plaque index than any of the other formulations.

- The other treatment groups were not significantly different from each other.

- A copy of evaluation of the data is attached.

D. STATISTICIAN'S REPORT AND FINDINGS

May 14, 1990

To:
From:
Subject: Analysis of Take5 Mouth Spray

Summary: Placebo has a significantly higher Plaque Index than any of the other formulations ($p < .05$). The other formulations are not significantly different from each other.

Analysis: Summary statistics are given for each of the treatment groups in table 1.

Table 1: Summary statistics by Treatment Group

Treatment	N	Mean	St. Dev.
Placebo	32	2.30	0.255
.1% New	32	2.16	0.393
.1% Old	32	2.12	0.368
.03% New	32	2.11	0.323
.03% Old	32	2.06	0.306

A randomized block design using Subject as the blocking variable was used to analyze the data. The overall ANOVA was significant ($p=.0001$). The Student-Neuman-Keuls multiple comparison procedure was used to determine which treatments were significantly different. Placebo was significantly different from all other treatments. This was the only significant difference.

OBS	ID	X0	X1	X2	X3	X4
1	01	2.07	1.96	1.89	1.89	2.04
2	03	2.73	2.27	2.23	2.50	2.17
3	05	2.37	1.61	2.02	2.07	2.24
4	08	2.74	2.43	2.60	2.62	2.93
5	09	2.37	2.28	2.50	2.61	2.30
6	10	2.58	1.65	2.23	2.00	2.35
7	13	2.02	1.78	1.94	2.06	2.31
8	17	2.07	2.18	1.84	2.13	2.09
9	18	1.92	2.08	2.07	1.69	2.19
10	20	2.72	1.98	2.50	2.42	2.86
11	23	2.30	2.25	2.39	2.02	2.14
12	25	2.14	1.90	1.50	1.72	2.32
13	27	2.04	2.46	2.35	2.27	1.98
14	28	2.36	2.07	2.50	2.27	2.16
15	30	2.96	2.82	3.00	2.66	2.86
16	33	2.33	1.83	2.33	2.46	2.27
17	35	2.25	2.29	1.70	1.95	1.63
18	36	2.25	1.73	2.10	1.60	2.13
19	37	2.07	2.29	2.07	2.43	2.10
20	38	2.29	1.77	1.90	1.96	2.23
21	39	1.96	1.37	1.15	1.63	1.44
22	41	2.52	2.19	2.44	2.25	2.17
23	42	2.30	2.45	2.16	2.64	2.39
24	43	2.22	1.91	1.61	1.59	1.52
25	44	1.98	2.11	1.91	1.80	1.77
26	45	2.15	1.96	1.78	1.70	2.20
27	46	2.45	2.02	1.93	2.25	2.54
28	47	2.50	2.31	2.17	2.08	2.33
29	50	2.13	2.06	2.37	1.87	2.00
30	54	2.06	1.81	1.92	1.92	1.01
31	55	2.35	2.46	2.54	2.13	2.39
32	56	2.30	1.77	2.05	2.23	1.93

General Linear Models Procedure
Class Level Information

Class	Levels	Values
ID	32	01 03 05 08 09 10 13 17 18 20 23 25 27 28 30 33 35 36 37 38 39 41 42 43 44 45 46 47 50 54 55 56
TRT	5	X0 X1 X2 X3 X4

Number of observations in data set = 160

General Linear Models Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	35	12.304873	0.351568	7.40	0.0001
Error	124	5.888261	0.047486		
Corrected Total	159	18.193134			

R-Square	C.V.	Root MSE	Y Mean
0.676347	10.14580	0.2179	2.147813

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ID	31	11.279854	0.363866	7.66	0.0001
TRT	4	1.025019	0.256255	5.40	0.0005

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ID	31	11.279854	0.363866	7.66	0.0001
TRT	4	1.025019	0.256255	5.40	0.0005

General Linear Models Procedure

Student-Newman-Keuls test for variable: Y

NOTE: This test controls the type I experimentwise error rate under the complete null hypothesis but not under partial null hypotheses.

Alpha= 0.05 df= 124 MSE= 0.047486

Number of Means	2	3	4	5
Critical Range	0.1078274	0.1292337	0.1418768	0.1508139

Means with the same letter are not significantly different.

SNK Grouping	Mean	N	TRT
A	2.2969	32	X0
B	2.1559	32	X4
B			
B	2.1153	32	X2
B			
B	2.1069	32	X3
B			
B	2.0641	32	X1

E. DISCUSSION AND CONCLUSIONS

CLINICAL STUDY

WHOTI-1990

E. DISCUSSION AND CONCLUSIONS

The Table in the "Final Results" and Statistician's Report just preceding this Discussion is re-stated here for the convenience of the Panel. We have used the Test Product designations corresponding to the "Formulations" sub-section B.-1. just preceding for the sake of clarity.

SPRAY	N	PLAQUE SCORE	STD. DEV	SIGNIFICANCE
Placebo	32	2.30	± 0.255	
Test-I	32	2.16	± 0.393	< 0.05
Test-II	32	2.12	± 0.368	< 0.05
Test-III	32	2.11	± 0.323	< 0.05
Test-IV	32	2.06	± 0.306	< 0.05

1. Quantitative Results:

The magnitude of plaque reduction seen in this protocol is consistent with that of other clinicals reported in this filing. That is: about 10% reduction of the mean total plaque scores.

These results are another indication of the fact that the active ingredient, MICRODENT™, reduces plaque on a consistent basis. Repetition of the effect within a cross-over design protocol, and repeating it so consistently, is clear evidence that the plaque reducing properties of MICRODENT™ is not a quirk of experimental design or manipulated selection of subjects.

2. RAMIFICATIONS OF PROTOCOL DESIGN:

The design establishes, unequivocally, a number of the mode of action, definition, concentration of MICRODENT™ and label claim statements of the Petitioner made elsewhere in this filing. Specifically:

a. The effect is not just observable on "means" from the population at large. Due to the cross-over design, each "mouth", in effect, becomes its own control. Each subject was treated with each of the Placebo/Test products.

b. **The unequivocal key finding is: The effect of MICRODENT™ cannot be attributed to surfactant cleaning alone. Even a high level of poloxamer does not produce the reduction in plaque observed in other clinicals.**

The use of a Placebo with high surfactant concentration, and the consistent, statistically significant, reduction in plaque by the MICRODENT™ containing Test Products clearly demonstrates that the mode of action put forward by the Petitioner for the panel's consideration is consistent with the facts. **MICRODENT™ works because it both cleans and modifies the surface energy of the tooth surface.**

Therefore, the active ingredient is: "THE EMULSION OF A POLYDIMETHYLSILOXANE IN OR BY A POLOXAMER" ....as defined in Section II of this filing.

c. Within the limitations of this protocol, concentration across a fourfold range did not measurably affect the extent of plaque reduction observed. This too is consistent with the proposed mode of action; ie, MICRODENT™ is non-invasive, does not work by anti-microbial action (which would be expected to be highly concentration dependant), and thus the controlling definition of the Quantities of the active ingredient is not to be found in classical mg/ml expressions as for the anti-microbial ingredients being simultaneously considered by the panel.

d. The only difference between the standard AF Emulsion (Test III and IV) and Q7-2587 is that Q7-2587 has a slightly higher average molecular weight due to a distillation process that "cuts off" the low end. The higher molecular weights are the same, although obviously in slightly greater proportion.

The implication is that within narrow ranges of molecular weights, there is no measurable difference in plaque reduction. Information too incomplete to submit to the Panel at this time (June 17, 1991) indicates that dramatically higher molecular weights of polydimethylsiloxane (1000 cs to 12,500 cs) may in some product formulations have a longer lasting (therefore more beneficial) effect on surface energy. This data will be submitted to the panel as it becomes available to Petitioner.

For this reason, Petitioner has specified a range of molecular weights (or viscosities resulting therefrom) within the Quantities of Actives Section II.

3. Statistical Significance

The Statistician's Report states, "Placebo has a significantly higher ($p < .05$) Plaque Index than any of the other formulations . The other formulations are not significantly different from each other."

This finding is exactly what the mode of action comments of this filing would predict. It is also consistent with the label claims made in the past and which the Petitioner is requesting the panel to approve.

It is particularly informative to note that when all the data was subjected to an ANOVA comparison, the statistical significance becomes very, very impressive, ($p=0.0001$). Petitioner believes there are few anti-plaque ingredients, including anti-microbials, that are sufficiently consistent as to achieve this level of statistical confidence

4. CONCLUSION

This clinical establishes unequivocally that Petitioner's MICRODENT™ is an effective plaque reducing ingredient.

Further, this clinical establishes unequivocally that only the ingredient as defined in Section II of this filing is the actual active.....surfactant alone is ineffective.

F. COMPLETE PROTOCOL

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PROTOCOL

COMPARISON OF CLINICAL EFFICACY OF MOUTHSPRAYS ON PLAQUE BUILDUP

I. OBJECTIVE

To compare the effect of five mouthsprays on plaque buildup:
(1) 20083-38 Placebo
(2) 20083-39 0.03% Simethicone AF Emulsion
(3) 20083-40 0.10% Simethicone AF Emulsion
(4) 20083-42 0.10% Q7-2587 Simethicone
(5) 20083-41 0.03% Q7-2587 Simethicone

II. STUDY DESIGN

Double-blind, cross-over clinical trial.

III. DURATION OF STUDY

The duration of the study will be five weeks.

IV. SUBJECTS

Fifty (50) adult male and female subjects will be entered into the study.

<u>Inclusion Characteristics</u>

1. Signed Informed Consent Form.
2. Good general health.
3. Subjects, ages 18 to 65 years, inclusive.
4. Minimum of 20 natural uncrowned teeth (excluding third molars).
5. Availability for the five week duration of the study.
6. Baseline plaque index of at least 2.0 as determined by use of the Quigley-Hein (Turesky Modification) Plaque Index Method (Appendix I).

<u>Exclusion Characteristics</u>

1. Presence of orthodontic appliances.
2. A soft or hard tissue tumor of the oral cavity.
3. Extensive or rampant dental caries.
4. Advanced periodontal disease (characterized by

the presence of purulent exudate, tooth mobility, and/or extensive alvelor bone loss).

5. Antibiotic therapy during the two weeks prior to entry into the study.

V. TEST PRODUCTS

1. FORMULA # 20083-38- Placebo
2. FORMULA # 20083-39- contains simethicone at 0.03%
3. FORMULA # 20083-40- contains simethicone at 0.10%
4. FORMULA # 20083-42- Contains simethicone Q7-2587 at 0.10%.
5. FORMULA # 20083-41- contains simethicone Q7-2587 at 0.03%.

VI. PROCEDURE

1. Screening and Selection of Subjects

Candidates will report to the clinical facility and will be screened by the examining dentist to identify those subjects who meet the Inclusion/Exclusion Characteristics. The findings of this initial screening procedure will be recorded on the Initial Screening Form (Appendix II). The first Fifty (50) candidates who meet the Inclusion/Exclusion Characteristics and sign an Informed Consent Form (Appendix III) will be entered into the study.

2. Stratification of Subjects

Subjects will be stratified into five balanced groups according to baseline plaque scores. Each group will be randomly assigned to one of the five test mouthsprays.

VII. PHASE I (WEEK 1)

1. Oral Prophylaxis

All subjects will receive a complete oral prophylaxis on Monday or Tuesday prior to their using their assigned mouthspray.

2. Supervised MouthSpraying at Clinical Facility

Immediately after their oral prophylaxis, subjects will spray their mouths with their

assigned mouthspray under supervision.

3. Instructions to Subjects

Subjects will be provided with their assigned mouthspray for home use. They will be instructed to spray their mouth with their asigned mouthspray five times daily for three days (Monday thru Wednesday or Tuesday thru Thursday).

Subjects will be instructed to refrain from all oral hygiene procedure, such as use of dentifrices, toothbrushes or other mouthrinses, dental floss, interdental stimulator, water irrigation devices, etc. during the study period. There will be no restrictions on eating, drinking and smoking habits.

4. Modified Quigley-Hein Plaque Examinations

On Thursday/Friday morning, all subjects will receive a plaque examination using the Turesky Method immediately after their supervised mouthspraying.

The examining dentist will dictate the findings of this examination to a trained recorder who will enter the data on a plaque examination form (Appendix IV) Each record will be signed by the examining dentist.

A Phase I Final Visit Form (Appendix V) will be completed for all subjects at this time.

VIII. **PHASE II**

Subjects will be instructed to resume their normal oral hygiene procedures immediately after their plaque examination on Thursday/Friday and to continue them until they report to the clinical facility again on the following Monday or Tuesday.

IX. **PHASE III (WEEK 2)**

1. Product Assignment

Each group will be assigned to the use of a different test mouthspray than was used in Phase I.

2. Oral Prophylaxis

All subjects will receive a complete oral prophylaxis on Monday/Tuesday prior to their using their assigned mouthspray.

3. Supervised Mouthspraying at Clinical Facility

Immediately after their oral prophylaxis, subjects will spray their mouths with their assigned mouthspray under supervision. A Phase III Initial Visit Form (Appendix VI) will be completed for each subject at this time.

Subjects will return to the clinical facility each morning (Tuesday through Thursday or Wednesday through Friday) to spray their mouths with their assigned mouthspray under supervision.

4. Instructions to Subjects

Subjects will be provided with their assigned mouthspray for home use. They will be instructed to spray their mouths with their assigned mouthspray five times daily for three days (Monday thru Wednesday or Tuesday thru Thursday).

Subjects will be instructed to refrain from all oral hygiene procedures, such as use of dentifrices, toothbrushes or other mouthrinses, dental floss, interdental stimulators, water irrigation devices, etc. during the study period. There will be no restrictions on eating, drinking and smoking habits.

5. Modified Quigley-Hein Plaque Examinations

On Thursday/Friday morning, all subjects will receive a plaque examination immediately after their supervised mouthspraying.

The same scoring procedure used in Phase I will be utilized at this time.

A Phase III Final Visit Form (Appendix VII) will be completed for all subjects at this time.

X. PHASE IV

Subjects will be instructed to resume their normal oral hygiene procedures immediately after their plaque examination on Thursday/Friday and to continue them until they report to the clinical facility again on the following Monday/Tuesday.

XI. PHASE V (WEEK 3)

1. Product Assignment

Each group will be assigned to the use of a different

test mouthspray than was used in Phase I or III.

2. <u>Oral Prophylaxis</u>

All subjects will receive a complete oral prophylaxis on Monday/Tuesday prior to their using their assigned mouthspray.

3. <u>Supervised Mouthspraying at Clinical Facility</u>

Immediately after their oral prophylaxis, subjects will spray their mouths with their assigned mouthspray under supervision. A Phase V Initial Visit Form (Appendix VIII) will be completed for each subject at this time.

Subjects will return to the clinical facility each morning (Tuesday through Thursday or Wednesday through Friday) to spray their mouths their assigned mouthspray under supervision.

4. <u>Instructions to Subjects</u>

Subjects will be provided with their assigned mouthspray for home use. They will be instructed to spray their mouths with their assigned mouthspray five times daily for three days (Monday thru Wednesday or Tuesday thru Thursday).

Subjects will be instructed to refrain from all oral hygiene procedures, such as use of dentifrices, toothbrushes or other mouthrinses, dental floss, interdental stimulators water irrigation devices, etc. during the study period. There will be no restrictions on eating, drinking and smoking habits.

5. <u>Modified Quigley-Hein Plaque Examinations</u>

On Thursday/Friday morning, all subjects will receive a plaque examination immediately after their supervised mouthspraying.

The same scoring procedure used in Phase I will be utilized at this time.

A Phase V Final Visit Form (Appendix IX) will be completed for all subjects at this time.

XII. PHASE VI

Subjects will be instructed to resume their normal oral hygiene procedures immediately after their plaque examination on Thursday/Friday and to continue them until they report to the clinical facility again the

following Monday/Tuesday.

XIII. PHASE VII (WEEK 4)

1. Product Assignment

Each group will be assigned to the use of a different test mouthspray than was used in Phase I , III or V.

2. Oral Prophylaxis

All subjects will receive a complete oral prophylaxis on Monday/Tuesday prior to their using their assigned mouthspray.

3. Supervised Mouthspraying at Clinical Facility

Immediately after their oral prophylaxis, subjects will spray their mouths with their assigned mouthspray under supervision. A Phase VII Initial Visit Form (Appendix X) will be completed for each subject at this time.

Subjects will return to the clinical facility each morning (Tuesday through Thursday or Wednesday through Friday) to rinse their mouths with their assigned mouthspray under supervision.

4. Instructions to Subjects

Subjects will be provided with their assigned mouthspray for home use. They will be instructed to spray their mouths with their assigned mouthspray five times daily for three days (Monday thru Wednesday or Tuesday thru Thursday).

Subjects will be instructed to refrain from all oral hygiene procedures, such as use of dentifrices, toothbrushes or other mouthrinses, dental floss, interdental stimulators, water irrigation devices, etc during the study period. There will be no restrictions on eating, drinking and smoking habits.

5. Modified Quigley-Hein Plaque Examinations

On Thursday/Friday morning, all subjects will receive a plaque examination immediately after their supervised mouthspraying.

The same scoring procedure used in Phase I will be utilized at this time.

A Phase VII Final Visit Form (Appendix XI) will be

completed for all subjects at this time.

XIV. **PHASE VIII.**

Subjects will be instructed to resume their normal oral hygiene procedures immediately after their plaque examination on Thursday/Friday and to continue them until they report to the clinical facility again on the following Monday/Tuesday.

XV. **PHASE IX (WEEK 5)**

1. Product Assignment

Each group will be assigned to the use of a different test mouthspray than was used in Phase I,III,V or VII.

2. Oral Prophylaxis

All subjects will receive a complete oral prophylaxis on Monday/Tuesday prior to their using their assigned mouthspray. A Phase IX Initial Visit Form (Appendix XII) will be completed for each subject at this time.

3. Supervised Mouthspraying at Clinical Facility

Immediately after their oral prophylaxis, subjects will spray their mouths with their assigned mouthspray under supervision.

Subjects will return to the clinical facility each morning (Tuesday through Thursday or Wednesday through Friday) to spray their mouths wtih their assigned mouthspray under supervision.

4. Instructions to Subjects

Subjects will be provided with their assigned mouthspray for home use. They will be instructed to spray their mouths with their assigned mouthspray five times daily for three days (Monday thru Wednesday or Tuesday thru Thursday).

Subjects will be instructed to refrain from all oral hygiene procedures, such as use of dentifrices, toothbrushes or other mouthrinses, dental floss, interdental stimulators, water irrigation devices, etc. during the study period. There will be no restrictions on eating, drinking and smoking habits.

5. Modified Quigley-Hein Plaque Examinations

On Thursday/Friday morning, all subjects will receive a plaque examination immediately after their

supervised mouthspraying.

The same scoring procedure used in Phase I will be utilized at this time.

A Phase IX Final Visit Form (Appendix XIII) will be completed for all subjects at this time.

XVI. DENTAL TREATMENT DURING STUDY

Subjects will be instructed to refrain from routine dental treatment (except emergency) during the course of the study.

XVII. STATISTICAL ANALYSIS AND POWER OF STUDY DESIGN

This study will be conducted at the Dental Research Center. The subjects in this study wil be utilized from

The chances of drop out subjects is negligible. The power in this study is based on fifty(50) subjects.

The plaque scores for the observations in each group will be compared using a mixed model analysis of variance. The power of the study design is such that of 0.15 difference between groups with regard to mean plaque scores can be detected at the alpha = .05 level and power = 0.8.

XVIII. ADVERSE REACTIONS

Subjects develop serious and/or unexpected adverse reactions they will be dropped from the study. These subjects will be kept under observation and monitored on a weekly basis until the symptoms subside.

XIX. DROPOUT FROM STUDY

A genuine effort will be made to determine the reason(s) why a subject fails to return for the necessary visit(s) or is dropped from the study. Subjects will be dropped from the study if any of the following occur:

0 Subject fails to report for any Plaque Examination

0 Subject fails to report for any supervised mouthspraying.

0 Subject receives emergency dental treatment which may interfere with the parameters under study.

0 Plaque scores of subjects who drop out of the

study at any time will be excluded from all statistical analysis.

A Final Visit Form (Appendix XIV) must be completed for all subjects entered into the study.

XX. SUBJECT RECORD FORMS

The following subject record forms will be completed by the investigator or obtained from the microcomputer printouts according to the following schedule.

VISIT	FORMS COMPLETED :
PHASE I	Initial screening Form Informed Consent Form Phase I Final Visit Form Plaque Exam Form
PHASE III	Phase III Initial Visit Form Phase III Final Visit Form Plaque Exam Form
PHASE V	Phase V Initial Visit Form Phase V Final Visit Form Plaque Exam Form
PHASE VII	Phase VII Initial Visit Form Phase VII Final Visit Form Plaque Exam Form
PHASE IX	Phase IX Initial Visit Form Phase IX Final Visit Form Plaque Exam Form

XXI. STUDY SUPPLIES

120 - 0.35 oz. bottles of Placebo
120 - 0.35 oz. bottles of 0.03% simethicone soln.

120 - 0.35 oz bottles of 0.10% simethicone soln.
120 - 0.35 oz. bottles of 0.10% Q7-2587 soln.
120 - 0.35 oz. bottles of 0.03% Q7-2587 soln.

XXII. **RESPONSIBILITIES OF INVESTIGATOR**

The investigator will have the responsibilities of insuring that the protocol is adhered to, completing the require forms, advising the sponsor of any side effects and returning any unused products to the sponsor.

XXIII. **STUDY SITE AND INVESTIGATOR**

This study will be conducted at

Reduced Plaque Formation by the Chloromethyl Analogue of Victamine C

by

SAMUEL TURESKY
NEVILLE D. GILMORE
IRVING GLICKMAN

THE CHLOROMETHYL ANALOGUE of Victamine C,* a cationic surface-active agent, reduces the formation of dental calculus in humans.[1,2] This chemical prevents crystallization of calcium phosphate in calculus smears in vitro,[3] but the mechanism whereby it inhibits calculus formation in vivo is not known. The following study was conducted to determine whether the chloromethyl analogue of Victamine C reduces the formation of dental plaque considered a precursor of calculus.[4-6]

EXPERIMENTAL METHOD

Plaque formation during a three-day experimental period was compared in six male dental students, ages 22-25, using a test aqueous mouthwash containing Victamine C analogue (0.1 percent-pH 6.0) and a control mouthwash of 0.26% aqueous solution of quinine sulfate (pH 6.0), which simulated the taste of the Victamine C analogue. For the purpose of this study, plaque was considered to be a soft concrescent deposit on the teeth which stains red following a 15-second rinse with 10 ml of 0.18 percent basic fuchsin solution (six drops of six percent alcoholic basic fuchsin in 10 ml of tap water) followed by a five second rinse with 10 ml of tap water.[7,8] A three-day experimental period was used because a measurable amount of plaque is formed during this time.[9,10]

The teeth of all subjects were cleaned free of plaque and calculus corroborated by disclosure with the standard basic fuchsin. This was followed by a three-day period without brushing or mouthwash at the end of which plaque was disclosed with fuchsin and scored. This provided a base line for plaque formation in each subject.

After the base-line period each subject was assigned another blank control period and two periods each on test and control mouthwashes in a sequence unknown to the examiners. Each trial period was preceded by an oral prophylaxis to remove plaque and calculus.

During each three-day trial period, with the exception of the two blank control periods, the subjects were instructed to use 20 ml of mouthwash as a rinse for one minute four times a day, after meals and before retiring, and follow it by two brief rinses with tap water. The subjects were given the mouthwash and a cup marked at the 20 ml level, but the use of the mouthwash was not supervised. They were also instructed to follow their normal diet but not to brush their teeth or use any other oral hygiene measures. The intervals between trials ranged from 4 to 18 days, during which usual oral hygiene measures were practiced.

Disclosed plaque was scored by the method of Quigley and Hein.[9] A score of 0 to five was assigned to each facial and lingual nonrestored surface of all the teeth except third molars, as follows:

- 0 = No plaque.
- 1 = Separate flecks of plaque at the cervical margin of the tooth.
- 2 = A thin continuous band of plaque (up to one mm) at the cervical margin of the tooth.
- 3 = A band of plaque wider than one mm but covering less than one-third of the crown of the tooth.
- 4 = Plaque covering at least one-third but less than two thirds of the crown of the tooth.
- 5 = Plaque covering two-thirds or more of the crown of the tooth.

An index for the entire mouth was determined by dividing the total score by the number surfaces examined.

At the end of each three-day period, prior to the use of disclosing solution, the mouth was examined for mucous membrane changes. The subjects were questioned regarding side effects after scoring for plaque.

Plaque was scored at random by either of two investigators according to their availability. Neither examiner was aware of the nature of the trial period at the time of scoring. A high degree of consistency within and between the examiners was established in trials involving 100 observations. Where a single examiner made both the observations for similar trial periods, the correlation coefficient (r) was 0.8723 (df = 27) for one examiner and 0.8935 (df = 17) for the other, and where each examiner made one of the two observations the correlation coefficient was 0.8071 (df = 23) with a difference between them of only 4.26 percent. Because of the consistency within and between examiners, and because the pattern of examiner-subject observations bore no relation to the experimental design, data are treated as coming from one examiner source.

From the Ziskin Memorial Research Laboratory, Department of Periodontology, Tufts University School of Dental Medicine, Boston, Massachusetts.

This report is from an investigation supported by the Research and Development Command, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-193-MD-2019.

*Victor Chemical Division of Stauffer Chemical Company.

TABLE 1
Whole Mouth Plaque Index Scores for Control and Test Trials

Subject	Plaque Index Blank Control 1	Blank Control 2	Active Control 1	Active Control 2	Test 1	Test 2
A	1.77	2.29	2.39	2.38	0.46	1.23
B	2.06	2.02	2.06	2.42	1.40	1.12
C	3.25	2.61	2.46	2.84	1.86	1.57
D	2.71	2.50	1.96	2.79	1.45	1.41
E	2.48	2.37	1.89	1.74	1.22	1.02
F	2.62	3.25	3.55	3.19	2.04	2.58
Mean	2.48	2.51	2.39	2.56	1.41	1.49
Standard error	.21	.15	.25	.20	.22	.23

RESULTS

There were no signs of mucous membrane change after trial periods. Most of the subjects reported a tingling of the mucous membrane for periods up to 45 minutes after using the test mouthwash. There were occasional comments of a "smoother feeling" of the teeth with the test mouthwash.

Analysis of variance (Table 1) indicated no statistical difference in the plaque indices among the four trial periods of the control series ($F = 0.11$) or between the two test periods ($F = 0.06$).

In all subjects the plaque index was less with the test than with the control. Differences between the mean indices of control and test trials ranged from 0.70 to 1.54, with an average reduction for the group of 1.03, which is highly significant ($t = 9.04$, $df = 5$) (Table 2).

Low plaque scores, representing little or no plaque, were more frequent after use of the test mouthwash than after the control (Table 3). If, for the purpose of statistical analysis, a score of 0 is given for each surface scored "low" (0 or 1) and a score of 1 for each surface scored "high" (2 or more), by totalling the scores and dividing by the surfaces so scored, a value results which represents the proportion of surfaces in each mouth scored as "high" plaque (2 or more). Comparison of mean values for test and control mouthwash trials scored in this way (Table 3-A) indicate that there was a reduction in "high" scores (2 or more) in test trials which ranged from .09 (9.0%) to .38 (73.1%) with a mean reduction of .32 (42.1%). This difference is highly significant ($t = 5.61$, $df = 5$).

Accumulation of plaque was not uniform throughout the mouth and a comparison was made of the distribution of high (2 or more) plaque scores in different areas. Comparisons were made between facial and lingual surfaces; between lingual surfaces of the mandible and maxilla; and between anterior (canine to canine inclusive) and posterior (first bicuspid to second molar inclusive) facial and lingual surfaces, shown in Table 4.

TABLE 2
Comparison of Mean Whole Mouth Plaque Indices for Control and Test Mouthwash Trials

Subject	Plaque Index Mean of Two Trials Active Control	Test	Difference
A	2.39	0.85	1.54
B	2.24	1.26	0.98
C	2.65	1.72	0.93
D	2.38	1.43	0.95
E	1.82	1.12	0.70
F	3.37	2.31	1.06
Mean	2.48	1.45	1.03*
Standard error	.49	.21	.11

*Highly significant ($t = 9.04$, $df = 5$).

TABLE 3
Distribution of Plaque Scores by Number of Surfaces Affec

Plaque Score	Number of Surfaces Blank Control Trial 1	Blank Control Trial 2	Active Control Trial 1	Active Control Trial 2	Tes. Tria 1
0	14	0	7	9	69
1	43	60	77	55	108
2 & 3	192	204	180	189	129
4 & 5	70	55	55	66	13
Total surfaces	319	319	319	319	319

TABLE 3A
"High" Plaque Scores
(Mean of Two Trials)

Subject	Active Control	Test	Difference	% Reduction
A	.77	.26	.51	66.2
B	.61	.29	.32	52.5
C	.82	.56	.26	31.7
D	.84	.49	.35	41.7
E	.52	.14	.38	73.1
F	1.00	.91	.09	9.0
Mean	.76	.44	.32*	42.1**

"High" plaque score = $\frac{\text{number of surfaces with score of 2 or more}}{\text{total number of surfaces}}$

*Highly significant ($t = 5.61$, $df = 5$).

**Percentage difference of the means

TABLE 4
Distribution of High Scores (2 or More) in Various Areas of the Mouth Given as Percentages of Total Surfaces

Area	Total Surfaces	Blank Control 1st Trial	Blank Control 2nd Trial	Active Control 1st Trial	Active Control 2nd Trial	Test 1st Trial	Test 2nd Trial
All Facial Surfaces	160	95.0	94.4	94.4	94.4	60.0	58.1
All Lingual Surfaces	159	69.2	67.9	52.8	65.4	29.9	30.8
Lingual Surfaces (Maxilla)	81	63.0	55.6	34.6	48.1	12.3	18.5
Lingual Surfaces (Mandible)	78	75.6	80.8	71.8	83.3	46.2	43.6
Anterior Surfaces (Facial and Lingual)	144	78.5	73.6	69.4	75.0	35.4	36.1
Posterior Surfaces (Facial and Lingual)	175	85.1	87.4	77.1	84.0	52.0	51.4

These data were analyzed as described above after assigning a score of 0 to the low plaque scores and 1 to the high plaque scores. In all test periods there were significantly more high plaque scores on facial than on lingual surfaces, and on the mandibular lingual than on the maxillary lingual surfaces. There was a tendency for higher plaque scores to occur on posterior rather than anterior surfaces (facial and lingual); this tendency was more pronounced in the test trials than in the controls.

DISCUSSION

A 0.1% aqueous mouthwash of chloromethyl Victamine C, a cationic surface-active chemical, when employed under the conditions of this study reduced the formation of plaque during a three-day experimental period. Chloromethyl Victamine C has also been shown to reduce calculus formation in vivo during eight-day and 21-day experimental periods.[1,2] The reduction in calculus formation demonstrated with Chloromethyl Victamine C may be related to its effect on plaque. The mechanism whereby it reduces plaque and the duration of its effectiveness have yet to be determined. Research into these questions is being pursued in our laboratory.

The findings regarding the distribution of plaque were interesting. Although the accumulation of plaque was reduced by the test mouthwash the distribution pattern remained the same as in the controls. Plaque tended to accumulate more on facial surfaces than on lingual surfaces, more on the lingual surfaces of the mandible than the maxilla, and more on posterior teeth than on anterior teeth. Subjects in this study did not brush their teeth during the experimental periods. It is therefore not feasible to compare the distribution pattern of plaque accumulation in this study with that reported in other studies[10,11] where subjects may have employed toothbrushing as a routine for oral hygiene.

CONCLUSION

A 0.1% aqueous mouthwash of the chloromethyl analogue of Victamine C produced a statistically signit cant reduction in the formation of disclosable dent: plaque during a three-day experimental period.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assis ance of Mrs. Miriam Smulow, Research Assistant in tl Department of Periodontology, Tufts University, School ‹ Dental Medicine.

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WHD-001

PRINCIPLE INVESTIGATOR:

BIOSTATISTICIAN:

SPONSOR:

WHD-001

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I. SUMMARY AND CONCLUSIONS

I. Summary and Conclusions

A. ABSTRACT

The Petitioner's active ingredient, MICRODENT™, was tested using a double blind, parallel treatment design protocol wherein subjects continued using their normal oral hygiene regimen, including brushing with an abrasive toothpaste.

Active and Placebo test groups of over 30 subjects per group used their assigned product delivered by a spray device six times per day. Plaque and gingivitis were scored at Baseline. The subjects received a dental prophylaxis and were scored again after three weeks.

The general trend line over all teeth surfaces showed that the mean plaque scores of the active group decreased from baseline while the placebo group increased.

The maxillary lingual surfaces demonstrated the greatest active ingredient effect. For subjects who complied with the protocol, the active ingredient produced a statistically significant reduction in mean plaque scores ($p < 0.01$) for a contiguous majority of maxillary lingual surfaces.

There was no effect on gingivitis scores.

I. Summary and Conclusions

B. Protocol Brief

1. INFORMATION SOUGHT

This clinical protocol was designed to address two fundamental questions:

> (a) Does the active ingredient of this petition, MICRODENT™, demonstrate a plaque reduction effect when tested over a three week period.
>
> (b) Would an effect be seen when normal oral hygiene (ie, brushing daily with an abrasive toothpaste) was employed in addition to the instructed frequent use of the active ingredient MICRODENT™ delivered in an oral spray?

Data interpretation addressed a third issue:

> (c) Prior studies clearly indicated that in the absence of brushing, the active ingredient had a plaque reducing effect and that the effect could not be attributed to the frequent introduction of surfactant alone. The active agent was the combination of poloxamer and polydimethylsiloxane. We questioned whether the well known plaque reducing physical effect of brushing with an abrasive would uniformly eliminate any added benefit of an oral spray delivery of MICRODENT™. Stated slightly differently, would there be tooth surface areas where delivery of the active throughout the day provided additional plaque reduction over abrasive brushing?

I. Summary and Conclusions

B. Protocol Brief

2. FORMULATIONS TESTED: ACTIVE vs PLACEBO

The exact formulations of MICRODENT™ in an oral spray vehicle, the placebo and abrasive toothpaste (gel) which follow are taken from Appendix D of the full protocol. See III Complete Protocol, below.

The concentration of MICRODENT™ in this experiment was 0.43%, consistent with the "Quantities of Actives" in Section II of this filing.

The ratio of Poloxamer to Polydimethylsiloxane was 40:1 (Simethicone, Dow Corning AF-30 is 30% polydimethylsiloxane by weight). This is also consistent with Section II of this filing.

The Placebo differed primarily in the absence of MICRODENT™. However, to provide a pleasant tasting placebo without the "smoothing" effect of the active ingredient, it was necessary to lower the alcohol and flavor content while increasing the viscosifier slightly to make up for loss in mouthfeel.

The Toothbrushing Gel formula was taken from a standard formulary, It had a standard level of silica gel abrasive (15%) but the usual sodium lauryl sulfate surfactant was substituted with poloxamer to keep the surfactant consistent throughout the test.

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I. Summary and Conclusions

B. Protocol Brief

3. PROTOCOL DESIGN ELEMENTS

The full text of the protocol designed by follows under sub-section III. below. However, for the convenience of the panel, we summarize the key design elements before proceeding to the results.

ELEMENTS:

(1) Double blind, parallel treatment.
(2) At least 30 subjects per test group.
(3) Normal oral hygiene and brushing habits.
(4) Subjects provided with toothbrush and abrasive toothpaste.
(5) Subjects pre-screened for oral health and a plaque index score at baseline of at least 2.0 (Turesky modification, Quigley-Hein).
(6) Following baseline examination, subjects were given a dental prophylaxis to bring the Plaque Index to zero.
(7) Plaque Index scores read at baseline (T=0) and three weeks (T=3wk). Groups balanced primarily by plaque scores.
(8) Gingivitis scores will be taken at baseline and three weeks, even though no effect of the active ingredient on gingivitis is expected. This will serve as additional assurance that the active ingredient is safe under frequent use conditions.
(9) Instruction card and product spray bottles specify six occasions of use daily with three sprays of 0.1 ml per use. After initial use instruction, subjects not observed for use technique.
(10) Multiple bottles for home, work and pocket distributed. Compliance monitored by collecting used bottles and weighing.
(11) Informed consent, Institutional Review Board, and other usual clinical ethics procedures followed University standards.
(12) Statistical Analysis performed by a qualified biostatistician, independent of the clinical investigator or sponsor.

I. Summary and Conclusions

C. STATISTICIAN'S REPORT AND FINDINGS

The biostatistician chosen to analyze the data from Dr. Menaker was Dr. Jonathan Clive of the University of Conn. Health Sciences Center (Farmington, CT). His CV is included in the appropriate section and his report follows this page.

Dr. Clive concludes:

"the product has demonstrated a significant ($p < 0.01$) clinical effect on a contiguous majority of the maxillary lingual teeth. The test group demonstrated a significantly greater reduction in mean plaque score than the control group, when results were adjusted for subject compliance."

"No differences were noted for changes in mean gingiva scores."

STATISTICAL ANALYSIS OF EXPERIMENTAL RESULTS

This section summarizes the data in terms of statistical significance, outlines the main points of data management and analysis, and highlights the significant findings of the study.

CONCLUSION

Based on results of a t-test of independent group means, we conclude that the product has demonstrated a signficant ($p < 0.01$) clinical effect on a contiguous majority of the maxillary lingual teeth. The test group demonstrated a significantly greater reduction in mean plaque score than the control group, when results were adjusted for subject compliance.

DATA MANAGEMENT

Data Entry

Data were entered and verified by the Data Services Department of the Health Center. All data were subject to a complete edit check. Following machine entry, data bounds were tested and several out of range values were changed.

Exploratory Data Analysis

The exploratory data analysis phase of the study involved calculating group means and standard deviations at baseline and followup examination. Sample moments were evaluated for plaque and gingiva scores, and for subject compliance. Tables I and II present summary measures for plaque and gingiva scores, broken down by selected classification measures.

The results of the exploratory data analysis were examined to determine the presence of general trends in the data, and to suggest specific clinical hypotheses to be evaluated.

DATA ANALYSIS

Hypothesis Testing

Hypothesis testing refers to the statistical evaluation of experimental results, using standard statistical testing procedures. A number of hypotheses were suggested a priori. Cross sectional group comparisons were made using t-tests of independent group means; longitudinal differences were evaluated by comparing mean differences for the two groups, also using t-tests of independent group means. Significance levels were adjusted to account for the number of tests being performed.

Baseline comparisons failed to detect significant differences between initial group means for plaque or gingiva scores, thus indicating the homogeneity of the groups at baseline. No statistically significant mean differences were observed for the breakdowns shown in Tables I and II.

Compliance Analysis

Analysis of patient compliance scores, discussed elsewhere in this report, indicated that the control group was significantly more compliant than the test group, even though the clinicians reported that naive subjects found product and placebo almost indistinguishable. The observed group means were 1.23 and 1.29 respectively, with the difference significant at the 0.05 level (two-tailed t-test of independent group means).

SIGNIFICANT FINDINGS

Experimental Results Confirmed

The exploratory data analysis indicated that possible experimental effects could be detected in maxillary lingual teeth. This observation was consistent with clinical evaluation as noted earlier. Examination of mean plaque change scores for individual teeth across groups indicated that a true experimental effect was manifest in a contiguous subset of maxillary lingual teeth, especially when adjusted for compliance. Examination of mean differences serves to greatly reduce the magnitude of the observed standard deviations, since observations for individual patients are highly correlated over time. Summary results are given in Table III.

Plaque Score Changes

Maxillary lingual teeth 4 through 13 inclusive showed the most statistically significant ($p < 0.01$) difference in mean plaque score change. The restricted subset of maxillary lingual teeth 5-12 showed a slightly larger mean difference.

Table III presents results for compliant and noncompliant patients. The degree of compliance exerts a strong influence on analytic results. A oneway analysis of variance using compliance as a covariate confirmed the results noted above.

Gingiva Score Changes

No differences were noted for changes in mean gingiva scores.

I. Summary and Conclusions

D. QUANTITATIVE RESULTS

The statistician's report clearly states that the only statistically significant finding was that for a contiguous majority of the maxillary lingual teeth, "The test group demonstrated a significantly greater reduction in mean plaque score than the control group, when results were adjusted for subject compliance."

However, some observations about subject compliance and the quantitative results obtained on other tooth surfaces may be instructive.

Subject Compliance

In some respects, the simultaneous introduction of a new active ingredient and a different form of oral hygiene technique could be expected to present compliance problems. Without the constant barrage of professional advice and media advertising to "Brush your teeth!", for some less motivated individuals to miss some of the specified use times could be expected. Hence, the protocol specified that returned samples would be weighed to determine uniformity of compliance between groups.

Additionally, with unsupervised use, thorough and uniform distribution of the 0.3 ml of spray from the tongue across the various areas of tooth surface could be expected to vary. The clinician (Dr. Menaker) observed after the instruction session that most of the spray seemed to be distributed by the subjects primarily across the maxillary lingual surfaces. The ability to distribute the material to the remaining areas of the mouth was frequently reported by the subjects as reduced.

One flaw (retrospectively) in the instruction set was to have not specified a length of time after spraying(say 20-30 seconds) for rubbing the teeth and gums before swallowing. [Reflecting that oversight, and based on the data in hand, all product label use instructions on spray versions of MICRODENT™ now contain such language] See Section I of this filing.

Another flaw (retrospectively} was to not include at least one spray per day which was supervised. This would have insured greater compliance, both in use frequency and use technique.

Significant differences in compliance between test groups was not predicted since the taste, mouthfeel and refreshment properties of the active and placebo products were similarly perceived. It is recognized, however, that there was opportunity for such to occur since the groups were homogenized on the basis of plaque scores,

Examination of the Subject Compliance Table (Sub-Section V of this clinical report) shows that Group A (Active) had six non-compliers out of 33 subjects while Group B (Placebo) had only one non-complier out of 34 subjects.

As noted in the statistician's report the compliance difference between groups was significant at the 0.05 level. When the predetermined non-compliance cut-off (actual use < .66 of instructed use) was invoked, the clear quantitative direction became statistically more significant even though the actual size of the means changed only slightly.

General Trend Lines:

In general, the mean plaque scores of All Surfaces of Group A (Active) decreased from the baseline while the Group B (Placebo) increased from the baseline (see Figure 5.). For the most significant surfaces (maxillary lingual) the decrease with use of the active was about 10% of the overall means. On some surface areas, the placebo increased 5-10%.
This trend was evident in the combined means of all surfaces. (See Figures 1. and 5., and Table IV of this sub-section and all tables and graphs compiled in sub-section VI of this clinical).

The only exception to this trend was the mandibular lingual set. Neither the active nor placebo groups changed appreciably from the baseline at week 3. Here the total plaque means were about 3.0 compared to about 2.5 for the rest of the surfaces. We would interpret this as the expected effect of the location of saliva glands, subsequent distribution of the agent as well as the difficulty in distributing the active spray under the tongue.

Conversely, the greatest effect was seen in the maxillary lingual surfaces. This is precisely where the distribution of active would be the most expected and consistent.

In fact, the magnitude of the Delta Mean Difference for compliers (Placebo Mean Difference, Baseline vs. Week 3 (MINUS) Active Mean Difference, Baseline vs. Week 3) decreases exactly in the order one would predict, given distribution of active, oral cavity physiology and toothbrushing technique effectiveness. That is: Max ling (.2132) > Max facial (.1741) > Mand facial (.1232) > Mand ling (.0775).

I. Summary and Conclusions

F. DISCUSSION

Petitioner submits that the findings of this Clinical Protocol unequivocally establish the following:

1. The active ingredient MICRODENT™ reduces plaque, even under the seldom tested rigor of continued normal oral hygiene, including brushing with an abrasive toothpaste, to a statistically significant extent.

2. While the active ingredient MICRODENT™ demonstrates moderate reduction in plaque over all surfaces, even with continued normal oral hygiene, its most significant effect ($p < 0.01$) occurs precisely where it is most effectively delivered by the tongue from the spray formulation tested; that is, the maxillary lingual surfaces.

3. Delivery site specificity is a frequently applied criteria for evidence of ingredient activity by the medical research community as it serves to indicate both beneficial effect and mode of action. MICRODENT™ meets this criteria.

4. These results adequately confirm, in a lengthy test (3 weeks compared to the 2 or 3 day tests most frequently employed in plaque efficacy evaluation) the plaque reducing efficacy of MICRODENT™ previously demonstrated under less rigorous, but common, protocol designs and results presented elsewhere in this response to the FDA "call for data".

5. There are no demonstrable deleterious effects whatsoever attributable to the frequent (6 times) daily use of the active ingredient MICRODENT™.

[continued]

Petitioner further submits that the data strongly support the following:

(a) The beneficial effect of MICRODENT™ is dependant upon adequate tooth surface contact, consistent with the Mode of Action discussed elsewhere in this petition.

(b) Since MICRODENT™ is a non-invasive, non-irritating ingredient without anti-microbial activity to disrupt the beneficial oral ecology, frequency of use is both required and to be recommended in any product use labeling.

(c) Given the site specificity and the statistically significant effect produced with frequent use of small volumes of a relatively low concentration of the active ingredient, it follows that MICRODENT™ should be equally or even more effective in:

(1) gels for brushing or rubbing over teeth and gums,

(2) interproximal devices such as dental floss and interdental stimulators,

(3) mouthrinses and pre-rinses, especially those which deliver higher concentrations of MICRODENT™ to all teeth surfaces and oral soft tissue,

(4) sugarless chewing gums delivering MICRODENT™ for extended periods of time to the teeth and gingival surfaces, and

(5) sugarless mints and candies delivering the active ingredient slowly for good distribution across all contact surfaces immediately after eating as is culturally customary for refreshment.

(d) The "cleaning plus surface energy altering" mode of action proposed by Petitioner is consistent with the quantitative and statistical findings of this and other clinicals presented elsewhere in this document.

(e) Other variants of MICRODENT™ falling within the proposed Active Ingredient Definition and Quantities of Active Ingredients which are comprised of higher molecular weight (higher viscosities) of medically approved polydimethylsiloxane, having a longer residence time on teeth surfaces should show equal or greater beneficial effect with no decrease in product safety.

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V. Efficacy Data

C. Finished Drug Products

1. **CONTROLLED STUDIES**

[CONTINUED]

DENTAL FLOSS STUDY

47-01

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DENTAL FLOSS STUDY

47-01

I. Summary and Conclusions

A. ABSTRACT

The Petitioner's active ingredient, MICRODENT™, was tested in a dental floss wherein a solid emulsion of the active ingredient was incorporated in the fibers of common dental floss grade Nylon. The design was a double-blind, stratified, six week flossing protocol with normal oral hygiene procedures continued.

The 30± subjects per test group were scored for plaque and gingivitis at baseline, were stratified according to plaque scores, recieved a dental phophylaxis, supplied with assigned product and instructed in flossing technique. Subjects returned at weeks two, four, and six weeks for scoring for plaque and gingivitis and supplied with new dispensers of assigned product.

Reduction in plaque by MICRODENT™ DENTAL FLOSS when compared to market leader standard dental floss (J&J Waxed Mint) was statistically significant by both ANOVA and "t" test (significant at the 0.05 level). Plaque Index was reduced about 10% of the overall gross means. Since previously reported floss comparisons (Wei & Vidra, 1982, review art.) failed to develop statistically significant differences, this result has unusual clinical significance.

The general trend line showed the standard dental floss tending to return to base line as the test progressed through week six (consistent with many other studies) while the MICRODENT™ DENTAL FLOSS was still tending downward at week six.

In a cross-over design, where a hygienist flossed all subjects, fluorospectrophotometric analysis of spent floss confirmed that MICRODENT™ DENTAL FLOSS removed ≈ 10% more plaque.

Reduction in gingivitis was similar for both products.

Group Compliance (quantity of floss used) was identical.

No adverse reactions to the active ingredient were observed.

I. Summary and Conclusions

B. LITERATURE REVIEW: DENTAL FLOSS

EFFECTS ON PLAQUE, GINGIVAL INFLAMMATION AND CARIES OF

VARIOUS TYPES OF DENTAL FLOSS

GENERAL LITERATURE CONCLUSION:

In spite of numerous attempts to demonstrate superiority with various commercial dental flosses there is no significant difference reported between waxed and unwaxed dental floss on plaque or gingival inflammation scores. Stannous fluoride treated floss did reduce S.mutans population interproximally. Professional flossing of first graders for 20 months reduced caries by approximately 50%.

OVERVIEW OF BIBLIOGRAPHY:

The major advance in dental floss has been the shift to nylon after WW II. Unfortunately, the balance of Bass, 1948 (#1) recommendations for the optimum characteristics of dental floss have been generally ignored, including the need for "splaying" which is sacrificed when floss is waxed or bonded as in most commercial flosses.

There is no clinical advantage in plaque and/or gingival inflammation scores for waxed vs. unwaxed floss according to Finkelstein et.al. 1979, Hill et.al. 1975, and Lobene et.al. 1982 (#2, 3 & 5 respectively). This is further confirmed by Wei & Vidra (1982) in their extensive review article on floss (#6).

The interproximal effect on S.mutans population reported by Keene et.al. 1977 (#4) when flossing with stannous fluoride soaked dental floss is the first "difference" reported in the literature between various flosses.

Flossing children regularly by professionals for 20 months was found to reduce caries by approximately 50% (#7 & 8)

COMPLETE LITERATURE REVIEW AVAILABLE

Since the findings of this clinical represent the first report (to Petitioner's knowledge) of a statistically significant difference in interproximal plaque removal between any forms of dental floss, it seemed appropriate to aid the panel in its deliberations by including with the Dental Floss Study an Annotated Bibliography and Reprints of pertinent literature on previous floss performance and flossing effect studies.

These will be found immediately following the Final Report

I. Summary and Conclusions

C. Protocol Brief

1. INFORMATION SOUGHT

Clinical studies reported earlier in this filing clearly indicated the plaque reducing efficacy of the active ingredient MICRODENT™. One of the unique properties of the solid emulsion form (see Section II of this filing) of MICRODENT™ is that it is constructed from a liquid (melt) emulsion in which initially the continuous phase is the poloxamer raw material and the discontinuous phase is the polydimethylsiloxane.

This melt emulsion enables the active ingredient to be uniformly distributed in and around the fibers of the floss construction. When reduced to practice with a unique floss making machine (US Patents issued for the Floss with MICRODENT™, its Use in the mouth and the Machine itself), the resulting floss is very pleasant to use compared with standard commercial flosses.

The following questions were addressed by this clinical:

(a). Does the incorporation of MICRODENT™ into a Dental Floss of standard denier (fiber thickness and count) cause the resulting Dental Floss to reduce plaque to a greater extent than standard waxed mint floss (largest selling floss in USA) ?

(b). How does that same incorporation of MICRODENT™ affect the gingivitis reducing properties compared to the same standard commercial dental floss ?

(c). Can an evaluation of instant plaque removal (analyzed as protein retained on floss) provide any additional insight into the mechanism by which MICRODENT™ DENTAL FLOSS works ?

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DENTAL FLOSS STUDY
47-01

I. Summary and Conclusions

C. Protocol Brief

3. PROTOCOL DESIGN ELEMENTS

The full text of the protocol designed by follows under sub-section II below. However for the convenience of the panel, we summarize the key design elements before proceeding to the results.

ELEMENTS:

(1) Double blind, stratified, parallel treatment.
(2) Twenty-nine and thirty subjects (Group A, Active) and (Group B, Placebo), respectively, completed the six week flossing protocol.
(3) Subjects followed their normal oral hygiene procedures except assigned floss was only interproximal product used.
(4) Subjects pre-screened for oral health and a Plaque Index score at baseline of at least 2.0 (Turesky modification, Quigley-Hein).
(5) Groups stratified according to baseline plaque score. Random distribution otherwise.
(6) Gingivitis (Lobene, Modified Gingival Index, 1986) scores taken at baseline.
(7) After baseline scoring, but before prophylaxis, subjects flossed by hygienist. Process repeated at end of Week 6 and floss pieces entered in protein analysis procedure for "mechanism" study.
(8) Subjects were given a dental prophylaxis to bring Plaque Index to zero.
(9) Subjects instructed in proper flossing techniques, and to floss daily. Subjects provided with standard toothpaste and toothbrushes.
(10) Plaque (two Indices) and gingivitis scores taken at 2, 4, and 6 weeks.
(11) Oral tissue examined for any deleterious effect due to Test Product or Placebo at each evaluation time.
(12) Previously weighed product dispenser units collected at end of each two week period and weighed to determine compliance.
(13) Informed consent, Institutional Review Board and other usual ethical procedures followed.
(14) Statistical Analysis performed by a qualified statistician, independent of clinical investigators or sponsor's representative.

D. STATISTICIAN'S REPORT AND FINDINGS

DENTAL FLOSS STUDY

47-01

I. Summary and Conclusions

D. STATISTICIAN'S REPORT AND FINDINGS

SUMMARY

Statistical analysis of the three variables tested (quoted below) demonstrated that for all three clinical observations, but especially plaque scores, the MICRODENT™ DENTAL FLOSS [Product A] Indices were significantly lower at week six (final) than the Placebo or Control Floss (J & J Waxed Mint Floss) [Product B].

PLAQUE (Turesky modification, Quigley-Hein)

> "Product A was significantly better than Product B in reducing plaque scores, (alpha = .05). Significant differences were also found at six weeks using a "t" test."

MODIFIED PLAQUE (Modified Quigley-Hein, limited to mesio and disto aspects of gingival embrasure areas)

> "Product A was significantly better than Product B in reducing Mod Plaque scores, (alpha = .05). Significant differences were also found at the six week period using a "t" test."

GINGIVITIS

> "There was no significant difference in the product effect on gingivitis raw mean scores. However, when gingivitis deltas were used there was a significant product effect. Significant differences were also found at the six week test period using a "t" test."

STATISTICAL ANALYSIS OF DATA

STUDY 47-01

AUGUST 9, 1989

INTRODUCTION

The relative efficacy of two dental flosses were to be compared by measuring (3) response variables on the teeth of two groups of subjects. There were twenty-nine and thirty subjects, respectively, in the two groups after six weeks of observation. The response variables were, (1) Plaque, (2) Modification Plaque, and, (3) Gingivitis. These response variables are more clearly identified in Protocal on this Study Number 47-01.

CONCLUSIONS

The results of statistical analysis for the product comparisons as measured by the three response variables are as follows:

PLAQUE

Product A was significantly better than Product B in reducing plaque scores,(alpha = .05), see the ANOVA table A and Figure A. Significant differences were also found at the six week test period using a "t" test. Those results are not included herein.

It is to be noted that ANOVA of the plaque deltas was not significant for Products, see ANOVA Table D.

MOD PLAQUE

Product A was significantly better than Product B in reducing mod plaque scores, (alpha = .05), see the ANOVA table B and Figure B. Significant differences were also found at the six week test period using a "t" test. Those results are not included herein.

It is to be noted that ANOVA of the mod plaque deltas was also significant, (alpha = .05), see ANOVA Table E.

GINGIVITIS

There was no significant difference in the product effect on gingivitis raw mean scores, see ANOVA table C. However, when gingivitis deltas were used there was a significant product effect, see ANOVA table F. Significant differences were also found at the six week test period using a "t" test, see Table G. Here product A produced significantly larger gingivitis deltas from baseline than product B.

DISCUSSION

While the results reported herein are significant for a Product difference, the improved qualities of Product A over Product B, as measured by the three response variables, would probally have been clearer if the tests had gone to eight weeks and or there had been at least forty subjects in each product group. Time was found to be a highly significant contributor to Plaque and Mod Plaque scores and to Gingivitis deltas. There were no product-time interactions found.

As an added check on the significance of the Products on the three response variables an Analysis-of-CoVariance was calculated for each of them. The covariate was baseline means. The results were essentially the same as the ANOVAs of the mean scores reported in Tables A, B, & C. They were significant for products in the Plaque and Mod Plaque ANOVAs and not significant for products in the Gingivitis ANOVA. However, the calculated significant level, in the Gingivitis ANOVA was .145.

Time level scores, statistics and histograms of score means are enclosed for reader perusal. They give the detailed analysis of the mathematics used to make the Figure A and Figure B graphs for Plaque and Mod Plaque. The raw Gingivitis scores, Product A compared to Product B, show Product A scores larger at baseline and two weeks but progressively smaller at four and six weeks. These results are significant for Products only if Deltas are used in the ANOVAs. These results are enclosed but not tablized.

The average deltas, with the standard deviations, for Plaque and Mod Plaque are shown in Table H by Product and Time.

As for demographics it is sufficient to note that there were five (5) male subjects in the Product A group and six (6) male subjects in the Product B group. The average age of group A was 33 years with a range of 18 to 50 years. The average age of group B was 32.5 years with a range of 20 to 49 years.

The statistical tests used in this study were all parametric and are based on the assumption that the populations being analyzed had a frequency function that followed the normal, or Gaussian, law. While distributions encountered in these analyses may have departed somewhat from the normal, it is pointed out that Analysis-of-Variance is a very robust statistical tool and not markedly effected by small departures from the normal law. Thus, no non parametric statistical tests were used.

At the request of   a set of tables for measuring departures from normality is being sent to   under separate cover, to forward to him.

ANALYSIS-OF-VARIANCE FOR MEAN SCORES

TABLE A

Analysis of Variance for plaq

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	10.180228	4	2.5450569	20.018	.0000
prod	.463441	1	.4634412	3.645	.0575
time	9.716786	3	3.2389288	25.475	.0000
2-FACTOR INTERACTIONS	.4661717	3	.1553906	1.222	.3024
prod time	.4661717	3	.1553906	1.222	.3024
RESIDUAL	28.988031	228	.1271405		
TOTAL (CORR.)	39.634431	235			

0 missing values have been excluded.

TABLE B

Analysis of Variance for modp

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	20.738705	4	5.1846764	24.188	.0000
prod	.810277	1	.8102771	3.780	.0531
time	19.928428	3	6.6428095	30.991	.0000
2-FACTOR INTERACTIONS	.5331398	3	.1777133	.829	.4791
prod time	.5331398	3	.1777133	.829	.4791
RESIDUAL	48.871268	228	.2143477		
TOTAL (CORR.)	70.143113	235			

0 missing values have been excluded.

TABLE C

Analysis of Variance for ging

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	9.9701141	4	2.4925285	31.616	.0000
prod	.0001327	1	.0001327	.002	.9677
time	9.9699814	3	3.3233271	42.154	.0000
2-FACTOR INTERACTIONS	.2561507	3	.0853836	1.083	.3571
prod time	.2561507	3	.0853836	1.083	.3571
RESIDUAL	17.975198	228	.0788386		
TOTAL (CORR.)	28.201463	235			

0 missing values have been excluded.

ANALYSIS OF VARIANCE FOR DELTAS

Deltas are defined as the Subject's response variable reading at time t_i minus that subject's response variable reading at time baseline.
t_i = 2 week, 4 week, & 6 week

TABLE D

ANALYSIS OF VARIANCE - Plaque

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TIME	0.084	2	0.042	0.403	0.669
PROD1	0.230	1	0.230	2.216	0.138
PROD1* TIME	0.409	2	0.204	1.969	0.143
ERROR	17.747	171	0.104		

TABLE E

ANALYSIS OF VARIANCE - Mod Plaque

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TIME	0.700	2	0.350	1.940	0.147
PROD1	0.687	1	0.687	3.807	0.053
PROD1* TIME	0.361	2	0.181	1.001	0.370
ERROR	30.870	171	0.181		

TABLE F

Analysis of Variance for delta - Gingivitis

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
MAIN EFFECTS	1.6978580	3	.5659527	7.598	.0001
time	1.3899763	2	.6949881	9.330	.0001
prod	.3078817	1	.3078817	4.133	.0436
2-FACTOR INTERACTIONS	.1791803	2	.0895901	1.203	.3029
time prod	.1791803	2	.0895901	1.203	.3029
RESIDUAL	12.737941	171	.0744909		
TOTAL (CORR.)	14.614980	176			

TABLE G

Gingivitis Deltas

By Time & Product

Product	2 Weeks		4 Weeks		6 Weeks	
	avg	std dev	avg	std dev	avg	std dev
A	-.32	.29	-.52	.29	-.61	.28
B	-.32	.25	-.42	.31	-.46	.22

"t" test at 6 weeks

AVG(A) = -.610
AVG(B) = -.457

N(A) = 29
N(B) = 30

Std dev (A) = .276
Std dev (B) = .219

Std dev Pooled = .249

"t"(calculated) = 2.36
"t"(table .05) = 2.00

Therefore significant at alpha = .05
Product A had significantly larger deltas from baseline than Product B, at 6 weeks.

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"t"(table .05) = 2.00

Therefore significant at alpha = .05
Product A had significantly larger deltas from baseline than Product B, at 6 weeks.

TABLE H

Deltas, Departures From Baseline

PLAQUE

Product	2 Weeks Avg	2 Weeks Std Dev	4 Weeks Avg	4 Weeks Std Dev	6 Weeks Avg	6 Weeks Std Dev
A	-.46	.32	-.51	.28	-.54	.34
B	-.49	.32	-.46	.31	-.34	.35

MOD PLAQUE

Product	2 Weeks Avg	2 Weeks Std Dev	4 Weeks Avg	4 Weeks Std Dev	6 Weeks Avg	6 Weeks Std Dev
A	-.60	.36	-.77	.37	-.80	.41
B	-.56	.49	-.69	.44	-.55	.46

E. QUANTITATIVE RESULTS

I. Summary and Conclusions

E. QUANTITATIVE RESULTS

On an absolute scale, the difference in plaque scores, either the usual Turesky modification of Quigley-Hein (Plaque) or the subsequent modification reported herein (Mod Plaque) which emphasized the plaque on those portions of the tooth surface mechanically contacted by the floss, was about 10% of the overall mean plaque scores at the end of the six week study.

The consistency of this effect across the test groups was such that statistical significance was established by both "t" test and ANOVA (both at the 0.05 significance level) for comparisons of gross means and delta means.

Compliance analysis (weighing of returned dispensers) indicated almost identical use of either the MICRODENT™ or Placebo flosses, giving further confidence that the 10% improvement in plaque reduction was, in fact, a real and repeatable finding.

This finding is consistent with the general amount of plaque reduction observed in the clinicals based on an oral hygiene spray delivery of the active ingredient, MICRODENT™.

The general trend line is also informative. Figure I in F. "Selected Tables and Graphs" which follows, shows a pattern for the Placebo which is quite normal for most flossing studies, ie, there is a trend to return toward baseline after the first few weeks. It is clinically significant, Petitioner believes, that the opposite trend for the MICRODENT™ DENTAL FLOSS is observed.

MICRODENT™ is still tending downward at the six week period.

I

CHEMICAL ASSAY OF PLAQUE REMOVED

BY FLOSS USED IN CLINICAL PROTOCOL 47-01

INTRODUCTION

Protocol 47-01 was designed to evaluate the relative cleaning effectiveness of a recently developed dental floss (Microdent) versus the conventional market leader floss (J&J waxed mint). The standard oral research scoring procedure most directly correlatable to the physical efficiency of the floss was the Modified Plaque Index which demonstrated a statistically significant difference (10%) between the two flosses in the visualized interproximal plaque after six weeks use.

Direct chemical analysis of the plaque* picked up by the two test flosses would provide an interesting mechanistic correlation to the clinical findings. Thus, after baseline scores were taken, but before the initial cleaning by the hygienist, two selected interproximal spaces per subject were flossed in a consistent manner by the hygienist. Each space was flossed twice, first with one floss then the other. The order of floss used alternated between spaces and between subjects.

CONCLUSION

Over 200 floss samples were analysed for protein (plaque equivalent). When Microdent Dental Floss was used first, it picked up approximately 8% more plaque than its J&J waxed counterpart used first. When second flossings in a space were compared, Microdent picked up 15% more plaque than J&J counterpart used second.

Even though publications by Bass as early as 1948 suggested that floss construction should affect the cleaning efficiency, numerous clinical studies have failed to establish any significant difference between the various sized and waxed flosses available commercially.

It is interesting to note that the results of these chemical analyses (presumably less subject to bias than the visual scoring procedures for evaluating plaque remaining on the teeth) were of a similar magnitude to that seen in the clinical plaque scores; ie, 10% less plaque in Microdent users versus J&J users.

We conclude that the protein removal study (proportional to plaque) provides adequate evidence that the reason Clinical 47-01 shows a reduction in interproximal plaque scores is primarily because Microdent Dental Floss actually cleans the interproximal spaces more effectively.

* Plaque assay performed double-blind by PhD (an employee of Petitioner) in, and with the cooperation of 3M Corporation, Central Research Division

PROCEDURE

After baseline scoring, the research dentist specified corresponding (left side/right side) interproximal spaces for each subject. A trained hygienist flossed the spaces as follows: (1) Right Anterior surface, up/down--right/left (0.5-0.7 inch traverse) followed by Right Posterior surface, up/down--right/left; (2) Into the same space the other floss was inserted using the same procedure; (3) the Left Anterior and Posterior surfaces were repeated as in (1) and (2) except that the order of floss was reversed.

The disclosing solution stained portion was noted for excessive blood, then clipped and placed into 1N NaOH according to the fluorescent spectrophotometric procedure described by Altman, et al (J. Pros. Dent. vol 42-5, Nov 1979, pp 502-506). The remainder of the assay followed this procedure which provided linear correlation between the protein assay and plaque dry weight.

Several samples were not tested due to work-up errors and inadvertent spills. Samples with indication of excessive bleeding were eliminated from the tabulation also.

A total of 51 Microdent first and 56 J&J first (total floss sample size = 214) were included in the tabulation.

The results are expressed as Fluorescent Intensity. Under the dilution and spectrophotometer setting employed, the flourescence numbers coincedentally approximated the mg of plaque dry weight on the piece of floss under test. More relevant of course is the linear proportionality of flourescence to plaque weight.

DATA

	Micro First	J & J Second	J & J First	Micro Second
Avg of 56 samples	0.317	0.204		
Avg of 51 samples			0.294	0.235

Micro vs J & J FIRST

$$\frac{0.317 - 0.294}{0.294} = 7.8\%$$

Micro vs J & J SECOND

$$\frac{0.235 - 0.204}{0.204} = 15.2\ \%$$

Protocol

**RELATIVE EFFICACY OF TWO DENTAL FLOSSES:
MECHANISM AND MEASUREMENT OF INTERDENTAL CLEANING**

Protocol Number
Study Number 47-01

Protocol

RELATIVE EFFICACY OF TWO DENTAL FLOSSES: MECHANISM AND MEASUREMENT OF INTERDENTAL CLEANING

Protocol Number:

Study Number: 47-01

Study Location:

Test Materials:
A: Microdent Dental Floss
B: J & J Waxed Floss

Sponsor's Representative:

Study Investigators:

Project Consultant:

Study Dates:		
	Starting Date	June , 1989
	Completion Date	August , 1989
	Final Report Date	September , 1989

Protocol

RELATIVE EFFICACY OF TWO DENTAL FLOSSES: MECHANISM AND MEASUREMENT OF INTERDENTAL CLEANING

Protocol Number
Study Number 47-01

OBJECTIVE

The objective of this stratified, parallel, seven week, double-blind, normal use condition clinical study is i) to determine interdental cleaning efficacy of the test product and a control in removing interproximal plaque and debris in humans, (ii) to explore the mechanism by which observed differences may occur, and (iii) to estimate the likelihood of increased compliance (hedonic acceptance) by test subjects at the conclusion of six weeks by cross-over use of the product for the 7th week.

TEST MATERIALS

Identification/Description

A: Microdent Dental Floss; at least 1000 mg of Microdent/25 yds.

B: Johnson & Johnson Waxed Floss

These products will be supplied by the Sponsor in coded, sealed and weighed containers.

Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations.

Storage Conditions

Products will be stored at room temperature in a controlled access storage room.

Retention

All used and unused test materials will be returned to the Sponsor within 30 days after issuance of the final report unless otherwise directed by the Sponsor.

REGULATORY COMPLIANCE

All aspects of this study will conform with the code of Federal Regulations, Title 21 Sections 50 and 56. The Investigator will obtain a signed consent form from each subject prior to initiation. The approved protocol and it's Informed Consent Form will be reviewed and approved through
Institutional Review Board.

QUALITY ASSURANCE

This study will be conducted in compliance with the approved protocol and Standard Operating Procedures.

PROCEDURE

Selection of Test Subjects

(1) Number/Sex 60 Male/Female Subjects (30/group to complete)
(2) Age 18-55 Years of Age
(3) Criteria

Subjects will be generally healthy adult volunteers, with a minimum of twenty flossable natural teeth (excluding extensively restored teeth and third molars). Subjects with orthodontic bands, retainers or partial dentures will be excluded from the study. Volunteers must have a minimum plaque index score of 2.0 according to the Turesky modification of the Quigley-Hein index. The subjects should practice normal oral care but not use floss or other interdental devices on a regular basis (more than once-a-week).

Subjects with gross oral pathosis or grossly carious teeth will be excluded from the study. Use of antibiotics and/or steroidal or nonsteroidal anti-inflammatory agents prior to the two weeks of the study initiation will be cause for exclusion from the trial. Additionally, pregnant or nursing females, and individuals with a history of diabetes, alcohol abuse and rheumatic fever or cardiac abnormalities will be excluded from the study. Smoking habits and use of oral contraceptives will be recorded but will not be exclusion to entry into the study.

Any subject who begins antibiotic or anti-inflammatory therapy during the course of the study must report this fact to the Investigator who should record it appropriately. A dental prophylaxis or periodontal therapy during the course of the study will also disqualify the subject.

Subjects in the same household will be assigned to the same group. A sufficient number of qualified volunteers will be entered into the trial to ensure a minimum of 30 subjects per group on completion.

Baseline (Day minus 2 - Day 1)

Subjects meeting all inclusion criteria and for whom none of the exclusion criteria are present will sign an Informed Consent form. The following intra oral examinations will be carried out as follows:

i) A complete intraoral hard and soft tissue examination will be performed. The areas examined will include the lips, tongue, hard and soft palate, gingivae, all mucobuccal fold areas, inner surface of the cheeks and sublingual areas. The results of the oral cavity examination will be recorded on the case report form (CRF I).

ii) Gingivitis: Will be evaluated by the Modified Gingival Index as described in: Lobene, R.R., Weatherford, T., Ross, N.M. et al: A modified gingival index for use in clinical trials. Clin Prev Dent 8:3, 1986.

0 = Normal (absence of inflammation)
1 = Mild inflammation (slight change in color, little change in texture) of any portion of the gingival unit
2 = Mild inflammation of the entire gingival unit
3 = Moderate inflammation (moderate glazing, redness, edema, and/or hypertrophy) of the gingival unit
4 = Severe inflammation (marked redness and edema/hypertrophy, spontaneous bleeding, or ulceration) of the gingival unit

iii) Plaque will be evaluated by the Modified Quigley-Hein Index as described in item IV below. However, evaluation will be limited to mesio-buccal, disto-buccal, mesio-lingual and disto-lingual aspects of the gingival embrasure areas of each tooth.

iv) Plaque will also be evaluated by the Turesky modification of the Quigley-Hein Index as described in J.Perio. 41:41-43, 1970. Eythrocine dye will be used to disclose the plaque on the tooth surfaces.

0 = no plaque

1 = separate flecks or a discontinuous band of plaque at the gingival margin.

2 = thin (up to 1 mm) continuous band of plaque at the gingival margin.

3 = band of plaque wider than 1 mm but covering less than 1/3 of the gingival third of the tooth surface.

4 = plaque covering more than 1/3 but less than 2/3 of the tooth surface.

5 = plaque covering 2/3 or more of the gingival third of the tooth surface.

Following the evaluation for plaque, the subjects will be randomly assigned (except same household subjects) to either product group, based on the stratification of their entry level plaque scores from item iii above. They will also secondarily be stratified by age and sex to obtain two generally well balanced groups. The examiner will be blinded to this information.

Immediately after plaque score determinations, a qualified hygienist will demonstrate use of the dental floss for the "Mechanism" aspect of the study. As part of the demonstration two contralateral interproximal areas in the same arch with similar amount of high interproximal (item iii above) scores will be identified by the examiner. The hygienist will floss the designated interproximal site with Floss A followed by Floss B. Standard technique and length of floss will be employed (wrap around, up-down and lateral strokes will be employed). On the contralateral side, the order of floss will be reversed otherwise following the same procedure.

The four spent flosses (2 each of A and B) will be collected by the hygienist and photographed in a single frame to demonstrate the differences in plaque and food debris removed using each floss. (Photographic technique details, supplies and equipment will be provided by the Sponsor allowing sufficient time to practice on site). The length of the floss exposed to the teeth will then be cut and stored in individual one dram vials containing 5 ml of 0.1 N HCl. Vials will be coded to reflect subject number, side of the mouth and sequence of floss use. The data will also be reproduced on a separate sheet for use at the final examination. Determination of debris and plaque removed from each floss strand will be arrived at by total protein assay carried out by the Sponsor.

At this time, either a dental prophylaxis will be given or volunteer appointed within seventy-two (72) hours for the prophylaxis and flossing will be reemphasized at this time.

The subject will then be provided with written instructions and asked to view a demonstration of the technique of flossing on videotape (A.D.A. Tape on Flossers #X825). The first impressions of the subject on actually using the floss during demonstration will be recorded on audiotape.

The stratification will determine the group allocation for each subject based on stratification scores and the appropriate product will then be dispensed. Collectors for used floss will also be provided along with a daily diary for the subject to record use frequency and time. A Colgate soft toothbrush and Colgate Tartar Control Toothpaste will be provided at Day 1 and thereafter as required. At the conclusion of their baseline visit, subjects will be instructed to floss at least once a day and more often if desired.

<u>Day 14 and Day 28</u>

Subjects will be required to return all previously dispensed products and obtain fresh supplies. Compliance will <u>NOT</u> be reinforced at this stage. Participants will be reminded not to comment to the Examiner on any aspect of the study. All baseline gingivitis, plaque and oral tissue examinations will be repeated and appropriately recorded on individual case report forms to maintain Investigator blindings. Future appointments will be confirmed.

Day 42

All dispensed products and diary will be returned and logged. All Day 28 examinations and procedures will be repeated. The "Mechanism" test using both flosses on contralateral teeth will also be repeated by the hygienist. The spent floss will be photographed and set aside for assay as before. At this stage the product code will be broken to allow cross over use of the second product for the next one week. A hedonic scale provided by the Sponsor will be filled out by each participant at this time.

Day 42 - 49

The subjects will be provided the second floss for the 7th week of the study. They will be required to record their hedonic acceptance on the provided diary. (Further details to be provided by Sponsor). A hedonic scale will be repeated to determine subject acceptance of each product. (Hedonic scale as described in the book, "Sensory Evaluation of Food and Related Product" by Howard Moskowitz).

Case Report Forms

Individual Case Report Forms will be used for each evaluation and will become part of the permanent record. Separate pages will be used for each index to avoid introduction of Investigator bias. The examiner will not have access to the Case Report Forms until the completion of the examination but will call out his finding to an assistant or recorder. Case Report Forms will be signed and dated on each examination day by the Investigator. Signature stamps and per signatures are not acceptable.

Completed, original report forms and questionnaires will be mailed to the Sponsor within two weeks of each visit cycle for all subjects in each group.

Return of Products

All used floss collection containers, diaries and unused materials will be returned to the Investigator and will be weighed by the Sponsor to determine the extent of compliance. After complete post-study inventory, all test materials will be returned to the Sponsor.

Adverse Reactions

All patient reports of stinging, burning, irritation, etc. will be recorded on the Case Report Forms. All changes noted during the oral cavity examinations will be recorded in the Case Report Forms. The Investigator will record his opinion of the relationship of the study materials to each adverse experience. Suspected allergic responses will be photographed and reported.

Dropped Subjects

Subjects will be discontinued from the study if any of the exclusion criteria become present.

If, in the Investigator's opinion, the subject is no longer an appropriate study participant, the subject will be removed from the study and the reason recorded on the Case Report Form.

The subject may discontinue his/her participation from the study at any time. The Investigator will attempt to determine the reason and record it on the Case Report Form.

REPORT

At the termination of the study, a report which includes the following information will be prepared and submitted:

- A description of the test material
- A description of the test system
- Dates of study initiation and termination
- A tabulation of adverse complaints
- A discussion of study data

Statistical Analysis

Baseline data will be analyzed for homogeneity of means and variances. There will be a check for outliers, both by individuals and subject means. An investigation of the distribution of the pooled means will be made to determine the applicability of parametric analysis of the gingivitis and plaque scores. Non-parametric analysis will also be applied.

Sample means and variances will be reported at each time level for gingivitis and plaque. Final analysis of data will be based chiefly on Analysis-Of-Variance. Both main effects and possible interactions will be investigated. Effort will also be made to evaluate effect of frequency of use.

The final report will include the results of the aforementioned statistics with supporting tables and or graphs, in addition to data listings and computer printouts.

All statistical results will be based on an alpha of 0.05, however where statistics are significant with an alpha of 0.10 this will be noted.

MAINTENANCE OF RAW DATA AND RECORDS

Original data or copies thereof will be available at Hazleton Laboratories America, Inc. to facilitate auditing the study during its progress and prior to acceptance of the final report. When the final report is completed, all original paper data as well as the final report, will be retained in the archives of for a period of two (2) years.

PUBLICATIONS

Any publications resulting from this study will be at the behest of the Sponsor and must be approved by well in advance of submission of such publications. This review is necessary to prevent premature disclosure of trade secrets.

PROTOCOL APPROVAL

I have read and accept this protocol.

5-21-89
Date

May 18, 1989
Date

5-18-89
Date

RELATIVE EFFICACY OF TWO DENTAL FLOSSES: MECHANISM AND MEASUREMENT OF INTERDENTAL CLEANING

Final Report Number 47-01

RELATIVE EFFICACY OF TWO DENTAL FLOSSES: MECHANISM AND MEASUREMENT OF INTERDENTAL CLEANING

Final Report

Study Number: 47-01

Study Location:

Test Materials:
A: Microdent Dental Floss
B: J & J Waxed Mint Floss
C: J & J Waxed Floss (for photography only)

Sponsor's Representative:

Study Investigators:

Study Supervisor:

Project Consultant:

Study Dates: June 14, 1989 to August 4, 1989

**RELATIVE EFFICACY OF TWO DENTAL FLOSSES:
MECHANISM AND MEASUREMENT OF INTERDENTAL CLEANING**

Final Report Number 47-01

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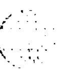

On Day 42 all dispensed products and diaries were returned. All Day 28 examinations and procedures were repeated. The crossover product was then dispensed. A six (6) week questionnaire, provided by the Sponsor, was completed by the subject.

On Day 49 all subjects that were provided with the crossover floss for the seventh week returned their product and diary and completed a seven week questionnaire.

Fifty-nine (59) subjects completed the study and their data was used for analysis (Table 1). Eight (8) subjects were dropped from the study due to subject non-compliance (Table 2).

Results, while not unequivocal, indicated that Floss A (Microdent) exhibited a slightly better reduction in plaque and gingivitis scores compared to Floss B (J & J). Statistically, this difference was borne out as follows by the Analysis of Variance:

	Mean Scores	Delta Scores
Gingivitis	*N.S. ($p=.9677$)	**S ($p=.0436$)
Plaque (Overall)	**S ($p=.0575$)	*N.S. ($p=0.128$)
Plaque (Interdental)	**S ($p=0.053$)	**S ($p=0.053$)

* NS = Not significant
**S = Significant at $p \leq 0.05$

TEST MATERIALS

Identification/Physical Description

A: Microdent Dental Floss; at least 1000 mg of Microdent/25 yds.

B: Johnson & Johnson Waxed Mint Floss

C: Johnson & Johnson Waxed Floss (for pre-prophylaxis photographic purposes only - product was not dispensed).

These products were supplied by the Sponsor in coded, sealed and weighed containers. The statistician was responsible for assigning the random code of each product to the individuals.

Purity and Stability

The Sponsor assumed responsibility for purity and stability determinations.

Storage Conditions

Products were stored at room temperature in a controlled access storage room.

Retention of the Product

All used and unused test materials were returned to the Sponsor on the following dates: 7/6/89, 7/20/89 and two (2) mailings on 8/7/89.

REGULATORY COMPLIANCE

All aspects of this study conformed with the code of Federal Regulations, Title 21 Sections 50 and 56. The Investigator obtained a signed consent form from each subject prior to initiation. The Approved Protocol (Appendix II) and the Informed Consent Form (Appendix IV) were reviewed and approved through Institutional Review Board (Appendix I).

METHODS

Selection of Subjects

(1) Number/Sex 67 Subjects

55 Females
12 Males

(2) Age 18-50 Years of Age

(3) Inclusion/Exclusion Criteria

Subjects meeting all inclusion criteria and for whom none of the exclusion criteria were present signed an Informed Consent Form and entered the study. Four (4) intraoral examinations were carried out as follows:

Procedure

Baseline (Day minus 2 - Day 1):

RELATIVE EFFICACY OF TWO DENTAL FLOSSES:
MECHANISM AND MEASUREMENT OF INTERDENTAL CLEANING

Final Report Number 47-01

OBJECTIVE

The objective of this stratified, parallel for six (6) weeks, double-blind, normal use condition clinical study was (i) to determine interdental cleaning efficacy of the test product and a control in removing interproximal plaque and debris in humans, (ii) to explore the mechanism by which observed differences may have occurred, and (iii) to estimate the likelihood of increased compliance (hedonic acceptance) by test subjects at the conclusion of six (6) weeks by cross-over use of the product for the seventh (7th) week.

SUMMARY

The purpose of this stratified, parallel for six (6) weeks, double-blind clinical study was to determine the relative efficacy of two (2) dental flosses by the mechanism and measurement of interdental cleaning. Sixty-seven (67) subjects were enrolled after satisfying admission criteria.

Oral soft and hard tissue examination and a gingival evaluation using the Modified Gingival Index was done. Plaque was evaluated using the Turesky modification of the Quigley-Hein Index and again using the above index but limiting the evaluation to the mesio-buccal, disto-buccal, mesio-lingual and disto-lingual aspects of the gingival embrasure areas of each tooth. Following stratification by plaque scores, age and sex, subjects were assigned to either product. A pre-designated posterior interproximal area was flossed by a hygienist sequentially by both flosses (A and C) and the order was reversed on the contralateral side. The spent floss were photographed to demonstrate degree of plaque removed. A standard length of each spent floss was cut and assayed for protein by the Sponsor, as a second measure of plaque removal.

After a prophylaxes, the subjects were dispensed their product (A or B) and shown a video demonstrating the flossing technique before dismissal.

On Day 14 and Day 28 subjects returned all previously dispensed products and obtained fresh supplies. Gingivitis, plaque, and oral tissue examinations were repeated and recorded on individual case report forms.

(i) A complete intraoral hard and soft tissue examination was performed. The areas examined included the lips, tongue, hard and soft palate, gingivae, all mucobuccal fold areas, inner surface of the cheeks and sublingual areas. The results of the oral cavity examination were recorded on the Oral Soft Tissue Forms.

(ii) Gingivitis was evaluated by the Modified Gingival Index as described in: Lobene, R.R., Weatherford, T., Ross, N.M. et al: A modified gingival index for use in clinical trials. Clin Prev Dent 8:3, 1986. (See Protocol, Appendix II)

(iii) Plaque was also evaluated by the Turesky modification of the Quigley-Hein Index as described in J.Perio. 41:41-43, 1970. Eythrocine dye was used to disclose the plaque on the tooth surfaces. (See protocol, Appendix II)

(iv) Plaque was also evaluated by the Modified Quigley-Hein Index as described in the protocol. However, evaluation was limited to mesio-buccal, disto-buccal, mesio-lingual and disto-lingual aspects of the gingival embrasure areas of each tooth.

Following the evaluation for plaque, the subjects were randomly assigned (except same household subjects) to either product group, based on the stratification of their mean plaque scores. They were also secondarily stratified by age and sex to obtain two (2) generally well balanced groups. The examiner was blinded to this information.

Immediately after plaque score determinations, a qualified hygienist demonstrated use of the dental floss for the "Mechanism" aspect of the study. As part of the demonstration, two contralateral interproximal areas in the same arch with similar amount of high interproximal plaque scores were identified by the examiner.

For all even numbered subjects, Floss A was used on the right interproximal side first, followed by Floss C. The order was reversed on the contralateral side. For all odd numbered subjects, Floss C was used on the right side followed by Floss A with the reverse order on the left side. Floss C (white colored) was used instead of Floss B (green colored) to maintain blinding of the hygienist with regard to Floss A (white colored). This floss (C) was used for pre-prophylactic photography as well as protein assay, where it was also necessary to maintain that the evaluator was blinded to the products. The hygienist flossed the site with the designated floss (Table 3) using a standard technique and length of floss (wrap around, up-down and lateral strokes) were employed.

The four (4) spent flosses (two each of A and C) were collected by the hygienist and photographed in a single frame to demonstrate the differences in plaque and food debris removed using each floss. The Sponsor demonstrated the photographic technique by stretching each floss across two (2) metal pins a few inches apart with the flossed area in the center. Floss types and sides were identified and labelled. The amount of erythrosine dye (indicating amount of floss removed) was photographed using appropriate lighting.

The length of the floss exposed to the teeth was cut and stored in individual one (1) dram vials containing 5 ml of 1.0N sodium hydroxide (NaOH). Vials were coded to reflect subject number, side of the mouth and sequence of floss use. Determination of debris and plaque removed from each floss strand was arrived at by total protein assay carried out by the Sponsor.

After the demonstration of flossing, a dental prophylaxis was given by a qualified dental hygienist.

The subject was provided with written instructions and asked to view a demonstration of the technique of flossing on videotape (A.D.A. Tape on Flossers number X825).

The group allocation for each subject was based on stratification scores and the appropriate product (A or B) was dispensed. A daily diary for the subject to record use frequency and time was provided. A Colgate soft toothbrush and Colgate Regular Toothpaste were provided at Day 1 and thereafter as required. At the conclusion of their baseline visit, subjects were instructed to floss at least once a day and more often if desired.

Day 14 and Day 28:

Subjects were required to return all previously dispensed products and obtain fresh supplies. Compliance was <u>NOT</u> reinforced at this stage. Participants were reminded not to comment to the Examiner on any aspect of the study. All baseline gingivitis, plaque and oral tissue examinations were repeated and appropriately recorded on individual case report forms to maintain Investigator blindings. Future appointments were confirmed.

Day 42:

All dispensed products and diaries were returned and logged. All Day 28 examinations and procedures were repeated. The "Mechanism" test using both flosses on contralateral teeth was repeated by the subject in the same order as before. At this stage the second (cross over) product was dispensed for the next one (1) week. A hedonic scale provided by the Sponsor (Appendix II, Attachment I) was filled out by each participant. Only subjects demonstrating uniform plaque areas were chosen by the Investigator and their appointment schedule dates are provided in Table 2.

Day 42-49:

The subjects were provided the second floss for the seventh (7th) week of the study. A hedonic scale was repeated at the seventh week (Appendix II, Attachment II) to determine subject acceptance of each product. (Hedonic scale as described in the book, "Sensory Evaluation of Food and Related Product" by Howard Moskowitz).

Case Report Forms:

Individual Case Report Forms (CRF) were used for each evaluation and became part of the permanent record. Separate pages were used for each index to avoid introduction of Investigator bias. The examiner did not have access to the CRF's until the completion of the examination but called out his findings to a recorder. CRF's were signed and dated on each examination day by the Investigator.

Data Collection and Recording

All CRF's and Examination Forms were retained in binders and audited for accuracy at each stage of the study. Original copies of all the CRF's were submitted to a representative of the client on the following dates: June 28, 1989, July 5, 1989, July 19, 1989 and August 1, 1989.

RESULTS

Sixty-seven (67) subjects were enrolled into the study and dispensed the product. Their demographics are listed in Tables 1 and 2. Fifty-nine (59) of the sixty-seven (67) subjects successfully completed the study. Eight (8) subjects were dropped due to non-compliance with the protocol (Table 2). Twenty-nine (29) subjects completed in Group A and thirty (30) subjects completed in Group B.

Mean gingivitis score at the end of the six (6) weeks were not significantly different between Floss A and Floss B by Analysis of Variance (ANOVA) [Table 4A]. However, an ANOVA of the delta value (difference between final and baseline score) for gingivitis demonstrated a greater reduction of gingivitis with Floss A (Table 4B). The distribution of scores for both products at each examination period are listed in Appendix IV.

Using the Turesky modification of the Quigley-Hein Plaque Index, significant differences were noted between the two products by raw mean scores (Table 5A) but not by delta values (Table 5B). Plaque reduction with Floss A was significantly greater ($p \leq 0.05$). The shift of these scores over the six (6) week period for both products are listed in Appendix V.

With the modification of the above index, scoring only the embrasure spaces ("Modified plaque score"), Floss A was significantly better than Floss B ($p \leq 0.05$) both by raw mean scores (Table 6A) and by delta values (Table 6B). The distribution of the scores for these products are listed in Appendix V.

The overall performance, therefore, of Product A seems better than Product B and the separation of scores would be more evident if the test period were extended to eight (8) or twelve (12) weeks, as noted in the statistician's report (Appendix V). Since the data was considered "normal" (Gaussian), only parametric analysis have been employed.

Adverse Reactions

No product related adverse reactions were noted.

Protocol Deviations

Five (5) subjects deviated from the protocol and they are listed below. Even though thirty (30) subjects were required in each group, Group A completed with only twenty-nine (29) subjects.

Subject Number	Date	Reason
20	7/13/89	The subject's dog ate floss and was dispensed number 81B.
35	7/14/89	The subject began taking ampicillin for one (1) week.

Subject Number	Date	Reason
41	6/29/89	The subject lost her floss and used husbands' product for three (3) days.
61	7/28/89	The subject reported pregnancy at five (5) weeks. Allowed to continue on Investigator's discretion.
78	8/4/89	Was out of town and did not return the product and diary - several attempt were made to contact this subject.

Subjects Dropped

Eight (8) subjects (numbers 16, 17, 18, 31, 47, 52, 67 and 74) were dropped from the study due to noncompliance with the protocol (See Table 2).

DISCUSSION

Two (2) objectives of this clinical trial were i) to identify the mechanism of action and ii) to determine likely effect of increased compliance (which will be explored independently by the Sponsors).

The third objective which related to inter-dental cleaning efficacy demonstrated Microdent Floss (A) to be statistically better compared to the J & J Mint Floss (B). However, the finding was not unequivocal.

The following approximate percent reductions of means from Baseline of the observed parameters are noteworthy.

	Baseline		Two Week		Four Week		Final
Gingivitis							
Microdent (A)	2.08	---16%---->	1.76	---25%--->	1.56	---30%--->	1.47
J & J Mint (B)	2.01	---16%---->	1.70	---20%--->	1.60	---22%--->	1.56

Delta Score = Significant (p = 0.0436)

	Baseline		Two Week		Four Week		Final
Plaque							
Microdent (A)	2.41	---17%---->	1.45	---22%--->	1.90	---23%--->	1.87
J & J Mint (B)	2.45	---21%---->	1.95	---20%--->	1.48	---14%--->	2.11

Mean Score = Significant (p = 0.0573)
Delta Score = Not Significant (p = 0.128)

	Baseline		Two Week		Four Week		Final
Mod. Plaque (A)	2.88	-- -21% ---->	2.28	-- -27% --->	2.11	-- -28% --->	2.08
	2.90	-- -20% ---->	2.35	-- -24% --->	2.21	-- -20% --->	2.35

Mean Score = Significant (p = 0.053)
Delta Score = Significant (p = 0.053)

In a comparable eight (8) week study, Lobene et. al[1] had noted a significant drop in gingivitis at four (4) weeks for all flossers, that then levelled off by eight (8) week exam. These authors also quote studies where no difference in plaque scores between flossers were noted at four (4) weeks and gingivitis scores had levelled off at three months.

However, in this study, after discounting the initial effects of prophylaxis, separation at eight (8) weeks is about 8% between the two (2) groups of flossers. This trend is consistent for all panelists, even though it is not uniformly statistically significant. This finding could be confirmed or negated with a larger population studied for an extended period of time.

REFERENCES

1. Lobene, Soparkar, Newman: Use of Dental Floss - Its Effect on Plaque and Gingivitis Clin. Prev. Dent. 4 (1) 5-8, 1982.

Feb. 1, 1990
Date

Feb 1, 1990
Date

TABLE 1
DEMOGRAPHIC INFORMATION

Entry Number/Initials		Product Number	Age	Sex	Product
1.	.	12	32	F	A
*2.	.	16	24	F	A
3.	.	11	30	F	B
4.	.	13	35	F	B
5.	.	20	44	F	A
*6.	.	17	27	F	A
7.	.	14	25	F	B
8.	.	15	27	F	B
9.	.	21	27	F	A
*10.	.	18	31	F	B
11.	.	22	18	M	A
12.	.	19	33	M	B
13.	.	24	22	F	B
14.	.	23	21	F	A
15.	.	26	31	F	A
16.	.	25	34	M	B
17.	.	27	32	F	A
18.	.	28	36	M	B
19.	.	29	37	F	B
20.	.	36	20	F	A
21.	.	30	28	F	A
*22.	.	31	28	F	A
23.	.	35	28	F	A
24.	.	32	37	F	B
25.	.	33	25	F	B
26.	.	34	26	F	B
27.	.	39	34	F	B
28.	.	37	32	M	A
29.	.	40	26	F	B
30.	.	38	50	F	A
31.	.	41	26	F	A
32.	.	44	40	F	A
33.	.	42	36	F	B
34.	.	45	50	M	A
35.	.	43	32	F	B
36.	.	50	38	F	B
*37.	.	52	31	F	B
38.	.	46	21	F	A
39.	.	53	26	F	B
40.	.	54	27	M	B
41.	.	48	39	F	A
*42.	.	47	40	M	A
43.	. .	57	41	F	B

TABLE 1
DEMOGRAPHIC INFORMATION
(Continued)

Entry Number/Initials	Product Number	Age	Sex	Product
44. .	49	35	F	A
45. .	59	20	F	B
46. .	51	35	F	A
47. .	61	30	F	B
48. .	55	32	F	A
49. .	62	24	M	B
50. .	56	30	F	A
51. .	58	38	F	A
52. .	63	38	F	B
53. .	65	35	F	B
54. .	60	42	F	A
55. .	64	32	F	A
56. .	68	46	M	A
57. .	66	28	F	B
*58. .	67	23	F	B
59. .	70	35	F	A
60. .	69	49	M	B
61. .	71	47	F	B
*62. .	74	29	F	A
63. .	72	30	F	B
64. .	78	39	F	A
65. .	76	34	F	A
66. .	73	46	F	B
67. .	79	21	M	A

* Drops

TABLE 2
SCHEDULE INFORMATION AND DROPPED SUBJECTS

Entry #/ Initials	Product #	Baseline Date	2 Week Exam	4 Week Exam	6 Week Exam	7 Week Exam
1.	12	06/14/89	06/28/89	07/13/89	07/26/89	08/02/89
2.	*16	06/14/89	(Dropped on 6/30/89 - missed exam)			
3.	11	06/14/89	06/29/89	07/12/89	07/26/89	08/02/89
4.	13	06/14/89	06/28/89	07/12/89	07/26/89	08/02/89
5.	20	06/14/89	06/28/89	07/12/89	07/28/89	08/02/89
6.	*17	06/14/89	(Dropped on 6/30/89 - missed exam)			
7.	14	06/14/89	06/28/89	07/13/89	07/26/89	08/02/89
8.	15	06/14/89	06/28/89	07/14/89	07/26/89	08/02/89
9.	21	06/14/89	06/28/89	07/12/89	07/26/89	08/02/89
10.	*18	06/14/89	06/28/89	(Dropped on 6/28/89 - missed exam)		
11.	22	06/14/89	06/28/89	07/13/89	07/27/89	08/02/89
12.	19	06/14/89	06/29/89	07/12/89	07/26/89	08/02/89
13.	24	06/14/89	06/28/89	07/12/89	07/26/89	08/02/89
14.	23	06/14/89	06/28/89	07/12/89	07/26/89	08/02/89
15.	26	06/14/89	06/28/89	07/12/89	07/26/89	08/02/89
16.	25	06/14/89	06/28/89	07/13/89	07/26/89	08/02/89
17.	27	06/14/89	06/28/89	07/12/89	07/26/89	08/02/89
18.	28	06/14/89	06/28/89	07/12/89	07/28/89	08/02/89
19.	29	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
20.	36	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
21.	30	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
22.	*31	06/15/89	(Dropped on 6/30/89 - missed exam)			
23.	35	06/15/89	06/29/89	07/19/89	07/27/89	08/03/89
24.	32	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
25.	33	06/15/89	06/29/89	07/14/89	07/27/89	08/03/89
26.	34	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
27.	39	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
28.	37	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
29.	40	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
30.	38	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
31.	41	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
32.	44	06/15/89	06/29/89	07/13/89	07/27/89	08/02/89
33.	42	06/15/89	06/29/89	07/13/89	07/27/89	08/03/89
34.	45	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
35.	43	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
36.	50	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
37.	*52	06/16/89	(Dropped 6/30/89 - pregnant)			
38.	46	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
39.	53	06/16/89	06/30/89	07/14/89	07/27/89	08/03/89

TABLE 2
SCHEDULE INFORMATION AND DROPPED SUBJECTS
(Continued)

Entry #/ Initials	Product #	Baseline Date	2 Week Exam	4 Week Exam	6 Week Exam	7 Week Exam
40.	54	06/16/89	06/30/89	07/14/89	07/27/89	08/03/89
41.	48	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
42.	*47	06/16/89	06/30/89	**(Dropped** 7/14/89 - missed exam)		
43.	57	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
44.	49	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
45.	59	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
46.	51	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
47.	61	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
48.	55	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
49.	62	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
50.	56	06/16/89	06/30/89	07/13/89	07/28/89	08/04/89
51.	58	06/16/89	06/30/89	07/14/89	07/26/89	08/04/89
52.	63	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
53.	65	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
54.	60	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
55.	64	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
56.	68	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
57.	66	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
58.	*67	06/16/89	**(Dropped** 6/30/89 - missed exam)			
59.	70	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
60.	69	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
61.	71	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
62.	*74	06/16/89	06/30/89	07/14/89	**(Dropped** 7/18/89 - non compliance)	
63.	72	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
64.	78	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
65.	76	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
66.	73	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89
67.	79	06/16/89	06/30/89	07/14/89	07/28/89	08/04/89

* **Dropped**

TABLE 3
SIX (6) WEEK MECHANISM FLOSS CODE

Subject Number	Right 1	Right 2	Left 1	Left 2
11.	A	B	B	A
21.	B	A	A	B
14.	A	B	B	A
24.	B	A	A	B
26.	A	B	B	A
30.	A	A	B	B
32.	A	B	B	A
54.	B	A	A	B
53.	A	B	B	A
34.	B	A	A	B
33.	A	B	B	A
42.	B	A	A	B
44.	A	B	B	A
38.	B	A	A	B
35.	A	B	B	A
41.	B	A	A	B
45.	A	B	B	A
66.	B	A	A	B
48.	A	B	B	A
57.	B	A	A	B
59.	A	B	B	A
62.	B	A	A	B
73.	A	B	B	A
79.	B	A	A	B
69.	A	B	B	A
20.	B	A	A	B

TABLE 5A
ANALYSIS OF VARIANCE FOR PLAQUE INDEX

Source of Variation	Sum of Squares	D.F.	Mean Square	F-Ratio	Sig. Level*
MAIN EFFECTS	10.180228	4	2.5450569	20.018	.0000
Product	.463441	1	.4634412	3.645	.0575*
Time	9.716786	3	3.2389288	25.474	.0000
2-FACTOR INTERACTIONS	.4661717	3	.1553906	1.222	.3024
Product Time	.4661717	3	.1553906	1.222	.3024
RESIDUAL	28.988031	228	.1271405		
TOTAL (CORR.)	39.634431	235			

TABLE 5B
ANALYSIS OF VARIANCE FOR DELTA VALUE PLAQUE INDEX

Source of Variation	Sum of Squares	D.F.	Mean Square	F-Ratio	Sig. Level*
Time	0.084	2	0.042	0.403	0.669
Product	0.230	1	0.230	2.216	0.138
Product* Time	0.409	2	0.204	1.969	0.143
Error	17.747	171	0.104		

* Significant at $p \leq 0.05$

TABLE 6A
ANALYSIS OF VARIANCE FOR MODIFIED PLAQUE INDEX

Source of Variation	Sum of Squares	D.F.	Mean Square	F-Ratio	Sig. Level*
MAIN EFFECTS	20.738705	4	5.1846764	24.188	.0000
Product	.810277	1	.8102771	3.780	.0531*
Time	19.928428	3	6.6428095	30.991	.0000
2-FACTOR INTERACTIONS	.5331398	3	.1777133	.829	.4791
Product Time	.5331398	3	.1777133	.829	.4791
RESIDUAL	48.871268	228	.2143477		
TOTAL (CORR.)	70.143113	235			

TABLE 6B
ANALYSIS OF VARIANCE FOR DELTA VALUE - MODIFIED PLAQUE INDEX

Source of Variation	Sum of Squares	D.F.	Mean Square	F-Ratio	Sig. Level*
Time	0.700	2	0.350	1.940	0.147
Product	0.687	1	0.687	3.807	0.053*
Product* Time	0.361	2	0.181	1.001	0.370
Error	30.870	171	0.181		

* Signficant at $p \leq 0.05$

TABLE 4A
ANALYSIS OF VARIANCE FOR GINGIVITIS INDEX

Source of Variation	Sum of Squares	D.F.	Mean Square	F-Ratio	Sig. Level*
MAIN EFFECT	9.9701141	4	2.4925285	31.616	.0000
Product	.0001327	1	.0001327	.002	.9677
Time	9.9699814	3	3.32332871	42.154	.0000
2-FACTOR INTERACTIONS	.2561507	3	.0853836	1.083	.3571
Product Time	.2561507	3	.0853836	1.083	.3571
RESIDUAL	17.975198	228	.0788386		
TOTAL (CORR.)	28.201463	235			

TABLE 4B
ANALYSIS OF VARIANCE FOR DELTA VALUE GINGIVITIS INDEX

Source of Variation	Sum of Squares	D.F.	Mean Square	F-Ratio	Sig. Level*
MAIN EFFECTS	1.6978580	3	.5659527	7.598	.0001
Time	1.3899763	2	.6949881	9.330	.0001
Product	.3078817	1	.3078817	4.133	.0436*
2-FACTOR INTERACTIONS	.1791803	2	.0895901	1.203	30.29
Time Product	.1791803	2	.0895901	1.203	30.29
RESIDUAL	12.797941	282	.0744909		
TOTAL (CORR.)	14.614980	176			

* Significant at $p \leq 0.05$

V. Efficacy Data

C. Finished Drug Products

2. PARTIALLY CONTROLLED STUDIES

V. Efficacy Data

C. Finished Drug Products

2. Partially Controlled Studies

(a) PRELIMINARY PROTOCOL AND DETERMINATION OF ORAL CLEANING BY FREQUENT USE OF AN ORAL HYGIENE SPRAY [TAKE-5 PLAQUE FIGHTER SPRAY™] CONTAINING MICRODENT™

PRELIIMINARY PROTOCOL AND DETERMINATION
OF ORAL CLEANING BY FREQUENT USE OF
AN ORAL HYGIENE SPRAY [TAKE-5 PLAQUE FIGHTER™]
CONTAINING MIICRODENT™

ABSTRACT

An analytical procedure and protocol are described by which debris accumulating in the oral cavity may be estimated. The procedures distinguished between debris removable by rinsing and that removed by brushing. Using these procedures, frequent use of an oral hygiene spray containing the active ingredient MICRODENT™, after meals and a controlled snacking protocol, throughout the day, resulted in a reduction of the accumulation of debris.

About 60% less removable debris, both rinsable and brushable, was found after use of the MICRODENT™ containing spray compared to use of a placebo spray without MICRODENT™.

March 1990

INTRODUCTION

Frequent cleaning of the surfaces of the oral cavity is generally recognized as the first step toward good oral health. Dental authorities recommend brushing with a suitably abrasive, surfactant containing, toothpaste after every meal. In actual practice, few Americans brush more than once a day. Some surveys suggest the national average for brushing to be about 1.2 times per day. Exacerbating oral desease due to their infrequent oral cavity cleaning habits is the increasing propensity of Americans to eat breakfast en route or at work, snack frequently, and not return home until after the evening meal; often with no interim cleaning of the mouth or teeth. Social perceptions about expectorating in public and concerns about the cleanliness of public restrooms effectively deter most of working America from even rinsing the mouth, much less brushing effectively between arising and retiring. Most choose to brush on only one of the latter two occasions.

It would seem, a priori, that introduction of an ingestable surfactant solution, formulated to provide good surface contact and residence time, pleasant taste and mouthfeel, into the oral cavity after meals and snacks would reduce the accumulation of food and cellular debris. One would not expect the effect of surfactant alone to equal that of brushing with similar frequency, although it possibly could improve oral cleanliness in the face of widespread individual resistance to frequent brushing.

An unpublished pilot clinical, measuring plaque build-up with/without such a surfactant introduction regimen surprisingly showed a reduction in plaque accumulation although the number of subjects was too few for statistical validation. The pilot clinical did not attempt to evaluate the more general question of cleanliness, with its social implications such as bad breath, intimate contact, etc.

The purpose of these experiments was to determine whether the surfactant effect of a commercial mouth cleaning spray, used frequently throughout the day, would produce a measurable reduction in oral cavity debris at the end of the day, with the debris subsequently removed for assay by rinsing and by brushing.

MATERIALS AND METHODS

Test Product: A commercially available oral spray, TAKE-5 TM, was used according to package directions. Directions included: "Pump 2 or 3 sprays onto tongue. Rub tongue over teeth, gums and surfaces of mouth. Swallow."

Each pump of the spray delivered 0.1 grams of solution. Label ingredients with possible cleaning effectiveness were Poloxamer 407 (0.4%), Ethanol (ca 40%), and Simethicone (0.03%). The latter would affect oral cleanliness only to the extent that its attachment to the oral surfaces retarded the redeposition of subsequent debris or increased surfactant contact due to its antifoam porperties. The remainder of the ingredients primarily serve to increase oral retention time and provide a pleasant experience.

Placebo: Although it was impossible to duplicate the total perception without the potential cleaning ingredients, a pleasant tasting, saliva flow inducing placebo was formulated with the remainder of the ingredients. The placebo was packaged in a spray bottle identical to the Test Product.

Debris Removal Solution: A 5.0% aqueous solution of Poloxamer 407 (Pluronic F-127, BASF Wyandott) effectively removed debris from the mouth and allowed a distinction to be made between the general debris in the oral cavity and that sufficiently attached to the tooth surfaces and gum crevices to require brushing.

Debris Removal Procedure: An oral approximation of the standard "three basin" handwashing test followed by a "scrubbing" analog was accomplished as follows:

(1) Fifteen ml. of the specified 5.0% surfactant solution was vigorously swished in the mouth for 60 seconds and expectorated into a beaker.

(2) An additional 15 ml. of the 5.0% surfactant solution was swished 60 sec. and expectorated.

(3) A third 15 ml portion was similarly swished to demonstrate that lesser quantities of debris was being removed with successive rinsings (rinse # 3 averaged about one-third the debris removed by rinse #1) and that most of the easily removed debris had been collected.

(4) Five ml. of the surfactant solution was introduced into the mouth and the teeth brushed vigorously for 60 sec. with a soft bristled brush. The foamy suspension was expectorated into a beaker, an additional 10 ml swished for 30 sec. and added to the brushed expectorate in order to transfer most of the brush-loosened debris to the same beaker.

Debris Assay: Each of the four expectorates were centrifuged for 5 min at 3500 RPM and the clear surfactant decanted. 15 ml. of 0.1M sodium acid phosphate (pH 4.5) buffer was used to resuspend the debris with the aid of a vortex mixer. After recentrifugation and decanting, the debris was again resuspended in 15 ml. fresh buffer. The debris was reduced to a uniform particle size with two passes through a standard ground glass tissue homogenizer having an OD of 19 mm (Uni-Form Homogenizer, Scientific Products Cat #T 3800-4). The Absorbance (Optical Density) was read in a Leitz Photrometer Model M fitted with a 550 mu filter. Previous experiments had demonstrated that the Absorbance was proportional to quantity of debris according to Beer's Law within the usual operative instrument range of 25-75 % Transmission.

Protocol: Participants were instructed to brush their teeth normally upon retiring, but refrain from brushing upon arising. Either the Test Product or Placebo was used following breakfast (two subjects used Placebo on Day One, two used Test Product on Day One and vice versa on Day Two).

A specified snack of two Ritz crackers was consumed at three equal intervals between breakfast and lunch and again after lunch. Participants used the Test Product and Placebo, according to package directions, 5 minutes after each snack and meal. Participants also consumed sucrose containing tea, coffee or water mid-way between each snack.

One hour after the last snack and spray procedure the participants followed rigorously the Debris Removal Procedure detailed above.

RESULTS

Table 1 details the debris removed at the end of the Test Product Day and the Placebo Day for each of the four subjects. Given the expected wide variability of an individual mouth to accumulate debris, each mouth was used as its own control in this cross-over design.

The quantity of debris removable by rinsing is expressed as the sum of the Absorbance for the three rinsings. The difference between Test Product and Placebo is expressed as percent of the debris removed after Test Product use divided by debris removed after Placebo use.

The debris removed by brushing was similarly compared.

The average rinsable debris (sum of determinations 1,2 and 3) after using Test Product was 59.4% of that removed by rinsing after using Placebo.

The average brushable debris in determination 4, after using Test Product, was 60.1% of that removed after Placebo use. The brushable debris was about 3 times the total rinsable debris removed, whether following Test Product use or Placebo.

As would be expected, greater differences between Placebo and Test Product were observed in those mouths which accumulated larger quantities of debris.

TABLE I

Person	Det. #	Test Product Absorbance X dil.	Placebo Absorbance X dil.	Debris Accumulation (Percent of Placebo) [A_t/ A_p x 100] Rinse	Brush
	1	.235	.297		
	2	.200	.180	84.6	
	3	.060	.108		
	4	.922	1.070		86.2
	1	.300	.860		
	2	.170	.400	37.8	
	3	.128	.320		
	4	1.620	2.592		62.5
	1	.172	.292		
	2	.127	.160	65.1	
	3	.095	.153		
	4	.760	.780		97.4
	1	.860	1.064		
	2	.186	.330	68.5	
	3	.155	.360		
	4	1.185	3.040		39.0
Sum of All Rinses		2.688	4.524	59.4	
Sum of All Brushes		4.487	7.482		60.0

DISCUSSION

Initially, it was concievable that a simple surfactant conditioning of the oral cavity would affect only very loose debris, such as might adhere to the soft tissue, while unable to remove appreciable quantities of debris attracted to the tooth surfaces. Thus the multi-step rinsing procedure followed by brushing was devised to partially separate the two adherence forms, if in fact they should be differentiated.

Since the one brushing resulted in a much greater debris removal than all three rinsings combined, it is once again evident that brushing must be an integral part of oral hygiene. What was surprising was that the frequent application of the surfactant spray following meals and snacks reduced both rinsable and brushable debris in about the same proportion. The protocol might be rendered more rigorous by the addition of a fourth rinsing step (approaching extinction of rinsable debris) and a second brushing step to demonstrate that effectively all the debris was accounted for.

Given the physical energy imparted to the teeth in brushing, this preliminary protocol does not distinguish between (1) a lesser amount of debris present after Test Product use and (2) a softer, more easily removed debris due to the frequent exposure to surfactant. However, given the lenghth of brushing time and the high level of surfactant to which the teeth were exposed for 3 minutes before brushing commenced, the first explanation seems more likely.

While the limited number of subjects render statistical validation improper, it would appear likely that a number or subjects per cell sufficient to achieve statistical power would demonstrate results of a similar magnitude, especially since there were no reversals (ie, Placebo effect greater than Test Product) in the 8 sets of determinations compared.

It would be appropriate to institute an expanded version of this test, under double blind conditions, as a mechanistic support of plaque accumulation clinicals or as substantiation of general oral hygiene utility.

The procedures and protocol are sufficiently convenient and reproducable to recommend themselves for evaluating other oral cleaning products, eg., pre-brush rinses, which have generated controversy over the "reduces plaque" claim whcn compared to "brushing alone" evaluated by subjective plaque staining and scoring techniques.

V. Efficacy Data

C. Finished Drug Products

3. CASE REPORTS

The "consumer-use", Mode of Action and generally accepted protocols for Efficacy Proof preclude the use of CASE REPORT methodology for the active ingredient, MICRODENT™

V. Efficacy Data

C. Finished Drug Products

4. PERTINENT MARKETING EXPERIENCES

V. Efficacy Data

C. Finished Drug Products

4. PERTINENT MARKETING EXPERIENCES

With over one million units of an oral hygiene spray with the active ingredient, MICRODENT™, [TAKE-5 PLAQUE FIGHTER SPRAY™] sold, there have been no reports of oral irritation or other deleterious effects which might be associated with frequent use of the active ingredient.

However, the type of marketing information most relevant to the issue of drug efficacy in the area of "plaque reduction" are those "home-use" consumer response studies which measure with considerable accuracy and statistical significance the way the finished product will be used and perceived by the consumer/patient.

A number of such studies, in which about 3400 consumers used either an oral hygiene spray containing MICRODENT™ or a dental floss with MICRODENT™, are summarized in this section. These studies establish:

(a) MICRODENT™ is perceived as dramatically more pleasant to use frequently than other oral care actives.

(b) MICRODENT™ containing products are perceived as "working", which leads to general maintenance of oral hygiene.

(c) MICRODENT™ DENTAL FLOSS has a higher "intent to use" and "intent to purchase" among non-flossers and regular flossers than standard waxed mint floss.

Summaries of these studies follow.

V. Efficacy
C. Finished Drug Products
4. Pertinent Marketing Experiences

(a) SALES SUMMARY OF MICRODENT™ CONTAINING PRODUCTS

As to Past Sales

MICRODENT™ was first introduced into the U. S. market in the fall of 1986 as the 0.43% active ingredient in TAKE-5 PLAQUE FIGHTER SPRAY™, the first plaque fighting oral hygiene spray marketed in the U.S. Four versions of TAKE-5 PLAQUE FIGHTER SPRAY™ have been sold since 1986, with over units sold nationally through major retailers such as: Walgreen Drug, Eckard Drug, Thrifty Drug, Long Drug, Wal-Mart, K-Mart, Pathmark, ShopRite, etc. These sales are detailed in Exhibit II of Volume 0.

TAKE-5 PLAQUE FIGHTER™ with MICRODENT™ is presently being marketed by one of Petitioner's licensee's, Synchronal, Inc., New York, NY. Note: Synchronal's marketing program includes national television advertising.

Professional spray versions of the active ingredient, MICRODENT™, are presently being marketed nationally under the trademark OMNiiDENT™ to over dentists by Petitioner's "professional licensee", OMNii International, Division of Dunhall Pharmaceuticals, Inc., Gravette, AR. These OMNiiDENT™ sprays are detailed in Exhibits I, II, and III of Volume 0. Over units are expected to be sold in the near term by dentists to their patients as a part of a professional "soft-tissue management program".

As to Present Sales

MICRODENT™ containing DENTAL FLOSS, and MICRODENT™ containing ALCOHOL-FREE MOUTHWASH are about to be introduced by OMNii International as well as by Synchronal, Inc. Both marketers have reviewed the clinical data submitted herewith and intend to make "plaque" claims for their versions of these products, consistent with Petitioner's proposed claims in Appendix I of Section VI. Facsimile Labels for these products are included in Volume I of this filing.

Additionally, a baby gum and tooth cleaner gel with MICRODENT™ for cleaning the "plaque-like film" that forms on babies gums prior to and during teething will shortly be marketed nationally by another licensee of Petitioner. Facsimile Labels for this product are included in Volume I of this filing.

Finally, MICRODENT™ treated, interproximal stimulators with "plaque" claims are scheduled for introduction to the "professional" market in early 1992 by OMNii International. Facsimile labels for this product are included in Volume I of this submission.

V. EFFICACY

C. FINISHED DRUG PRODUCT

4. PERTINENT MARKETING EXPERIENCES

(b). CONSUMER HOME USE AND RESPONSE DATA: SPRAY

TAKE-5™ SPRAY WITH MICRODENT™:
CONSUMER ACCEPTANCE
ABILENE TEST

The first consumer acceptance test on the oral hygiene spray marketed as TAKE-5™ was performed in Abilene, TX. in 1986. The demographics of this small, isolated city of 110,000 indicate it is representative of mid-sized mid American cities/towns.

INITIAL CONCEPT ACCEPTANCE

Using a cross-town grid which balanced income, racial demographics and alternated houses to prevent "back yard comparisons", adults answering the door were first qualified as moderately predisposed to extend oral care beyond brushing. The TAKE-5™ package front was shown while reading the back of the card whose text emphasized the mouth-cleaning, "out-of-the-bathroom" oral hygiene features. Of those so qualified, 89% indicated the top 3 boxes (6 box scale) on intent to purchase. After placement of 2 packages in each of 140 homes, the one week follow-up call revealed that 25% of the placements had not yet used the product.

We considered these to effectively be concept rejecters and subtracted these from the concept acceptor group in spite of their initial reply. Thus, the pre-advertising, pre-trial,no name brand, package acceptors were surprisingly greater than 60%.

CONSUMER RESPONSE AFTER ONE WEEK USE

Approximately 70% of these concept acceptor/users were reachable by phone at the pre-specified time and date. These 75 respondents were questioned for intent to purchase, product concept fit (i.e. promise/over promise and hedonic match to concept), positioning and quantity of TAKE-5 used.

INTENT-TO-PURCHASE

After one week of use, the top two boxes (6 box scale) were 64% of the concept acceptor/users. Inclusion of the "somewhat likely to buy" third box brought the positive intent to 83%.

PRODUCT PROMISE/OVER PROMISE

Perhaps the most significant finding was that after one week of use, the concept acceptors intent to purchase was inverted. That is, intent to purchase was greater after use than when based on concept alone. The promise of a clean mouth did not over-promise; rather, the product was better than expected. The consumer perceived the TAKE-5™ containing the active ingredient, MICRODENT™, was working.

		before use-concept	after use
(1)	definitely will buy	19%	36%
(2)	probably will buy	23%	28%
(3)	somewhat likely to buy	33%	19%

POSITIONING

Concept and execution of a cleaner mouth was very well accepted. As the original package concept did not emphasize pharmaceutical type claims (plaque, tartar, gingivitis, etc.) no probes for this position were made. In light of recent public reaction and controversy surrounding plaque control "over-promise" by a number of oral products, care was taken not to invoke the "magic" of a "plaque" claim, lest the product use frequency be biased on the basis of "I need to ".

The three questions directed to cleanliness, perception and believability were "just brushed feeling", "fresh mouth" and "clean mouth feeling". Those responses averaged 8.5 on a 10 point scale (where 0 = "not clean at all" and 10 = "as clean as I can imagine").

USE-UP RATE

Respondents were asked to bring their used sprays to the phone and estimate the quantity remaining (3/4, 1/2, 1/4, etc.). Product use up rate averaged 0.85 bottles/week among the 83% top 3 box users. This is equivalent to 4 uses per day, a figure reasonably consistent with the use frequency in the clinical protocols. The rate dropped to 0.45 for the 17% who did not particularly like the product (lower 3 box respondents).

TAKE-5 SPRAY WITH MICRODENT™
CONSUMER ACCEPTANCE
COMPOSITE OF MAJOR CORPORATION TESTS

Since the PP (predecessor company to Petitioner) sponsored test (Abilene, 1986), several major consumer marketing companies have evaluated TAKE-5 PLAQUE FIGHTER SPRAY™ for consumer response. PP and its successor company, Petitioner WhiteHill, provided over 1000 units of the product for these tests in return for summary descriptions of the results.

While methodologies varied slightly, the general approaches were similar to the Abilene study in enough areas to provide a composite view of the mutually similar questions/responses. The companies performing the tests were: Marion Merrell Dow, Colgate-Palmolive).

INITIAL CONCEPT ACCEPTANCE

The respondents were qualified for interest in oral care. Some studies focused on frequent brushers, regular flossers and mouth rinse users. In all cases, the concept of "doing something" for oral care that was convenient, out of the bathroom, and pleasant as well, scored high. Focus group approaches as well as more traditional "placements" all indicated that the pre-advertising, pre-trial, no name brand concept acceptance of greater than 60%, increased significantly when the product was "over labeled" or boxed with a major consumer product brand name.

CONSUMER RESPONSE AFTER USE

INTENT-TO-PURCHASE

The tests reported here used a four box scoring system, so "top two boxes" ("very likely" and "somewhat likely") are roughly equivalent to the top three box score of the Abilene Test. For all cases, the branded versions equalled or exceeded the 80% range on intent-to-purchase after one to three weeks use.

PRODUCT PROMISE/OVER PROMISE

Positive responses indicated that the concept's promises were well-met ("lauded" in the language of one contract test organization) in the areas of convenience and the perception of taste, freshening, and cleanliness. About the only negative in the concept/promise area concerned the believability of the "plaque fighter" claim. Some responses indicated that the highly perceived cleaning effect was "proof" of the plaque fighting, but the idea of "cleaning" clearly was more easy to believe, perhaps due to the current controversy which proceeded the "call-for-data".

POSITIONING

In addition to the "cleaner mouth", "convenient", and "pleasurable" positions elicited in the initial Abilene study, several interesting additional positions emerged:

(1). Multiple product locations - bath, kitchen, work desk, pocket, purse and car were frequently mentioned, with a surprising response from participants that they would like to have several to leave in various locations.

(2). Better than current alternatives to brushing (primarily gums and mints) between meals, "away-from-home", was a re-occurring theme. Most studies did not attempt to quantify this factor, but one indicated that over 50% found it better, about 40% rated it at mid-point ("about the same") and only 10% found it not as acceptable.

> NOTE: Informal interviews with long term TAKE-5 users had convinced PP that a positioning directed toward the market segment who use gum/mint as mouth cleaning/freshening products was "a natural" but this was the first quantitative evidence.

(3). Intense dislike of performing oral care in public restrooms (which are the only facilities a significant segment of the population have access to from the time they leave home until after dinner).

USE-UP RATE

None of these tests quantified the un-used portions, but all those evaluating their own tests expressed comfort with the magnitude of the 0.75 to 0.85 bottles/week number in the Abilene study. For example, in these tests 65% to 75% of the respondents used the product multiple times each day. "Four to five" was a frequent response. If from this we extrapolate to 3 sprays/use and 3 times per day, the use-up rate would be about 0.7 bottles per week. The multiple locations information re-enforces the reasonableness of this number.

V. EFFICACY

C. FINISHED DRUG PRODUCTS

4. PERTINENT MARKETING EXPERIENCE

(C). CONSUMER HOME USE AND RESPONSE DATA: FLOSS

MICRODENT™ DENTAL FLOSS
COMPOSITE CONSUMER ACCEPTANCE DATA

PP, the predecessor company to Petitioner, WhiteHill, provided 5000 units of packaged MICRODENT DENTAL FLOSS™ to a number of major consumer/oral care marketing companies for consumer and professional evaluations. To date, the results from the placement of over 2300 of these are in and made available to Petitioner under agreement. Respondents were balanced between flossers/non-flossers, and placed across more than 10 cities. Design variations ranged from small focus groups to mall intercept to telephone/mailed product, and included monadic, sequential monadic, and "use both at each flossing" exposures to MICRODENT™ DENTAL FLOSS and the control (most often J&J waxed mint, occasionally Oral-B unwaxed mint. Both branded and re-packaged dispensers were tested).

Direct comparisons (either sequential monadic or "both at each flossing" of MICRODENT™ DENTAL FLOSS and J&J were strongly positive toward MICRODENT™ in almost all categories, including intent-to-purchase or "switch brands". The more critical of these comparisons are detailed below.

Of the three "use presentations" only the strictly monadic left any doubt as to interpretation or statistical significance. This is not to surprising upon reflection, since in monadic tests both MICRODENT™ and Control were presented as "new" products.

In all tests, questions regarding like/dislike and specific positive attributes of "fresh", "clean taste" or negative attributes like "fraying", "hard to fit between teeth", were asked.

In a consumer product world where there has been almost universal dislike of the flossing process and has never been any appreciable difference between floss brands (or even private label), one should expect most monadic responses to be slightly above the mid-point and not appreciably different between relatively good product and an outstanding one. In a sequential or "both at once" design, the reverse becomes evident because it is obvious one has a choice, and one device is perceived as performing better.

The design factors varied so widely that providing an understandable composite picture requires the expressing of results in ranges. Specific numbers relate only to the design in question, hence some ranges are quite broad.

INTENT TO RECOMMEND

Professional (dentist or hygienist) recommendations clearly would impact intent to purchase for most consumers who are either concept acceptors or product preferrers. It is well established that approximately three out of four regular users of dental floss are introduced to flossing by their dentist and/or hygienist.

Accordingly, the Oral-B Company placed MICRODENT™ DENTAL FLOSS with a panel of practicing oral hygienists for evaluation. Oral-B uses this panel to assess oral care products that are commonly professionally recommended, eg., toothbrushes, dental floss, interproximal stimulators, etc.

This Oral-B Panel of Oral Hygienists responded very positively to MICRODENT™ DENTAL FLOSS. They preferred the floss with the active ingredient, MICRODENT™, over commercial flosses including J&J and Oral-B because:

(1) it was easier to work between the teeth (ie., the "splaying feature"), and

(2) patients responded positively to the taste and mouth feel (corresponding to the release of the active ingredient MICRODENT™).

Probes of the professional community have included dental researcher types, especially those involved with gum disease, who are essentially unanimous in their approval of both "increased compliance" and functional properties.

Among practitioners surveyed, most indicated either a willingness to switch recommendations to their patients or at least include MICRODENT™ DENTAL FLOSS samples with the J&J samples they give their patients. Given the advantage MICRODENT™ shows in head-to-head comparisons, this latter "dual sampling" may be the best strategy for establishing MICRODENT™ DENTAL FLOSS.

INTENT-TO-PURCHASE

When preference was asked as intent-to-purchase, the top two boxes ranged from about 60% for monadic use, up to 80% or 90% when direct comparisons are made. In all preference/purchase intent queries there was little difference between flossers and non-flossers.

Some of the test asked for overall preference as the bottom line questions. In these, MICRODENT™ DENTAL FLOSS was preferred over J&J about 2 to 1. Not an insignificant finding for an efficacious, plaque removing, product category where lack of compliance is a (the) major problem.

COMPLIANCE (FREQUENCY OF USE)

MICRODENT™ DENTAL FLOSS dramatically strengthened the consumer's stated intent to use floss with some degree of regularity. Of all the factors affecting plaque reduction potential for the category long term, this may be the most important.

Flossers and non-flossers were equally responsive to "will you floss more?" From 60% to 77% responded "very likely", or the top box on the question.

A surprising 80% of the MICRODENT™ preferrers indicate they will floss once a day or more. When regular floss users are segregated out, even about 1/3 of them say they will floss more often than now.

Underscore this response with the professional "most likely" to recommend or give away samples response and the impact of this compliance or frequency of use segment of the consumer tests becomes evident.

REASONS FOR PREFERENCE

The consumers were certainly not vague about why they preferred MICRODENT™. Product differentiation is unequivocal.

In the three major tests, "fresh mouth" responses ranged from (1) 70% top two boxes, to (2) 4 to 1 over J&J waxed mint and, to (3) 6 tot 1 over J&J waxed mint, when compared head-to-head.

"Taste" in general paralleled "fresh" with (1) 80% top two boxes, (2) 3 to 1, and (3) 4 to 1 over J&J waxed mint.

"Easy to use" or "fit between the teeth" questions led MICRODENT™ preferrers to mark 50% of the top two boxes, and rate this 1,5 to 1 over J&J unwaxed

"Clean: perception was 1.3 to 1 over J&J but when asked to rate "cleaning" on a non-comparative basis, some monadics showed little perceivable difference versus J&J. Other rating scales had preferrers indicating a strong 80% in "cleans excellent or very good".

Given the strong opinions held on positive attributes, it is no surprise that preferrers implied slight differences in "strength" or "fraying" for whichever product they preferred, although the inherent fibre strength is in fact equal.

COMPANIES PARTICIPATING IN TESTS

SUBJECT PERCEPTION OF FLOSS

IN

PROTOCOL 47-01

INTRODUCTION

Although not a formal part of the clinical evaluation of MICRODENT™ efficacy, it seemed of interest to probe the perception of those individuals participating in the clinical. Thus, after 6 weeks, a ten question questionnaire was administered which probed for hedonic factors of "difficult to use", "pain", "clean", "fresh", "feel", and "taste", plus physical parameters of handling, cutting and fraying and of course, intent-to-purchase. All questions were posed on a 0-10 point scale from "very bad" to "very good".

For the 7th week, participants previously using J&J were given MICRODENT™ and vice versa. At the end of week 7 a new questionnaire probed the same factors but on comparative terms ("compared to the floss you used the first 6 weeks, how did ....?").

INTERPRETATION

Care should be taken not to over interpret this consumer probe, especially compared to much larger, more standard consumer response surveys reported above. The reasons for this caution include: (1) the test panel was selected from non-flossers, most of whom could not even recall having used a waxed or flavored floss before, (2) the test groups (30) were too small for confidence in this type of survey and (3) motivation factors for participating in a clinical with oral exams, prophylaxis, etc. caused many participants to resent being asked to remain an extra 15 minutes for a questionnaire. This latter factor was very evident during the answering period.

Thus, the week 6 data tended to "bunch up" in the 9's and 10's due in part to not having a base of remembered perception. Week 7 was considerably more informative and centered around the mid-range of the scale as would be expected.

CONCLUSIONS

All but one subject perceived MICRODENT™ DENTAL FLOSS as more positive or equal to J&J waxed mint floss.

In the comparative week 7, MICRODENT™ DENTAL FLOSS averaged 2.8 points better than J&J waxed mint on the hedonic (pleasure/pain) factors of "pleasant to use", "remember to use",

"feeling in mouth", "fresh in mouth", and "taste (flavor)'.

As might be expected, the greatest difference of all was seen in "taste" with a delta of 4.6 (MICRODENT™ = 7.3: J&J = 2.7).

The intent-to-purchase comparative question was clearly confusing to the panel. It was also observed during week 6 interviews that about 50% were committed to continue flossing with any available floss once they had 6 weeks of "habit-forming" behind them. In spite of this, MICRODENT™ = 5.5 while J&J = 3.8 on intent-to-purchase.