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Research  
on a novel  
alcohol-free  
CPC mouthrinse  
for the control of  
plaque and gingivitis

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## Advances in therapeutic mouthrinses

Educating patients on the importance of preventive care remains a top priority for dental professionals. Despite the emphasis on proper brushing and flossing, the prevalence of periodontal diseases remains high. The incorporation of a chemotherapeutic rinse in patients' home care regimen is one approach to help improve their gingival health. Recently, Crest Pro-Health Rinse was introduced to offer patients the option of a pleasant, alcohol-free therapeutic mouthrinse.

This innovative, antibacterial formulation contains 0.07% high bioavailable cetylpyridinium chloride (CPC) in an alcohol-free base. CPC has a long heritage of use as a broad-spectrum antimicrobial against oral bacteria. It was one of only three antimicrobial systems to be deemed safe and efficacious by the FDA Plaque Subcommittee for the treatment of plaque-induced gingivitis when formulated within a concentration range of 0.045% to 0.10% CPC in a *high-bioavailable* mouthrinse matrix.

Clinical research shows the product provides protection against plaque, gingival inflammation and gingival bleeding. Perhaps even more importantly, the product was designed to encourage patient compliance. The absence of alcohol and its associated burn means patients who prefer to avoid alcohol-containing products for medical or personal reasons have another option available for home care.

This special issue of the *American Journal of Dentistry* presents clinical findings demonstrating the short and long-term plaque and gingivitis efficacy of Crest Pro-Health Rinse and high bioavailable CPC prototypes. Also included is a study presenting patient evaluations demonstrating product benefits when used under everyday *ad lib* conditions.

We hope you will find these papers interesting and educational. The *Journal* thanks Procter & Gamble, the manufacturer of Crest Pro-Health Rinse, for sponsoring this special issue.

Franklin García-Godoy, DDS, MS  
*Editor*

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## An alcohol-free therapeutic mouthrinse with cetylpyridinium chloride (CPC) - The latest advance in preventive care: Crest Pro-Health Rinse

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### Oral health means much more than healthy teeth

In 2000, the United States Surgeon General issued a comprehensive report on oral health in the United States.<sup>1</sup> The report, developed in conjunction with the National Institutes of Health and the National Institute of Dental and Craniofacial Research, outlined progress made over the last several decades in improving the oral health of the general public in the United States. The report also highlighted need gaps for specific population groups within our society and moreover suggested that less than optimal health of too many Americans results in “needless pain and suffering, [as well as] complications that devastate overall health and well being.” Importantly, the report highlighted contemporary research connecting systemic health of the population with their oral health, suggesting that “oral diseases and disorders in and of themselves affect health and well being throughout life”. As we strive for improved oral care of our patients, it is important to recognize that our efforts are directed not only for providing reduced edentulism, caries and periodontal diseases, but also include the treatment of oral conditions as a part of a holistic program of patient health care.

### Periodontal disease

Albandar<sup>2</sup> reviewed epidemiological data available on U.S. populations with respect to periodontal diseases, concentrating on major observations from four large size whole-population based surveys, NHANES I<sup>3</sup> (1971-1974), the Dental Health Outcomes Survey<sup>4</sup> (1981), the National Survey of Employed Adults and Seniors<sup>5</sup> (1985-1986), and NHANES III<sup>6</sup> (1988-1994) and several lower base size but specific “seniors focused” surveys.<sup>7-12</sup>

Primary observations related to periodontal health included findings that soft tissue oral disease remains a significant problem in the general population despite the best efforts of health care providers and oral care product manufacturers. The disease (Fig. 1) manifests itself in a number of ways, but in the U.S. can be generically differentiated between relatively healthy and extremely poor health patients. In generally healthy patients, the disease may still be present but usually not throughout the dentition. Over time, it can present localized problems, producing potentially catastrophic effects affecting parts of the dentition but not all. In a lesser proportion of the population, with poorer hygiene practices, limited resources, or with a high susceptibility for the disease, rampant disease throughout the dentition presents calamitous problems in both oral and general health. In addition, as the current population ages, the improved tooth retention being provided by modern hygiene aids will manifest in increased levels of less advanced disease which is only slowly progressing.<sup>14-16</sup>

### The first line of prevention – Mechanical oral hygiene

Perhaps the most surprising conclusion derived from epidemiologic surveys is that disease prevalence appears to remain

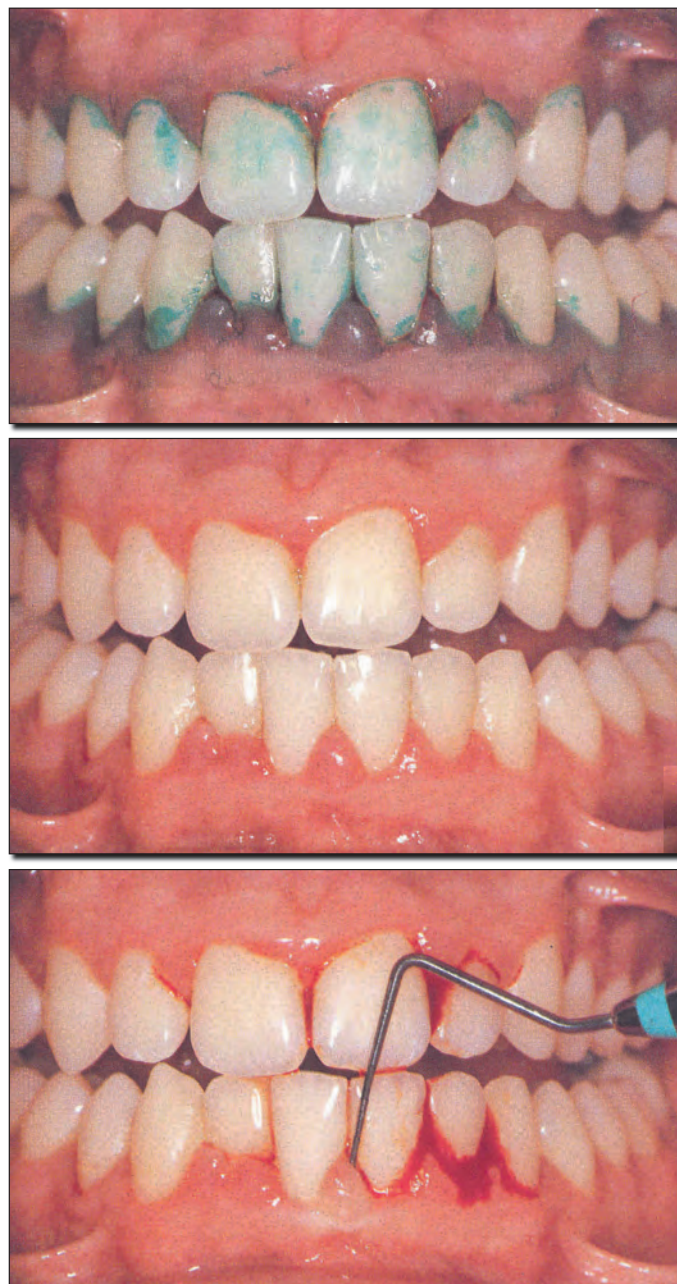


Fig. 1. Poor hygiene results in the accumulation of dental plaque (top) which in turn promotes the development of gingival inflammation (middle) and chronic gingivitis including swollen and bleeding gums (bottom). Untreated, gingivitis portends more destructive periodontal diseases. Reprinted with permission of Thieme Medical Publishers, Inc.<sup>13</sup>

high in spite of currently available dental therapy, hygiene and preventive procedures. Oral hygiene procedures that may contribute significantly to the prevention of soft tissue diseases include, as a first line of defense, mechanical plaque control

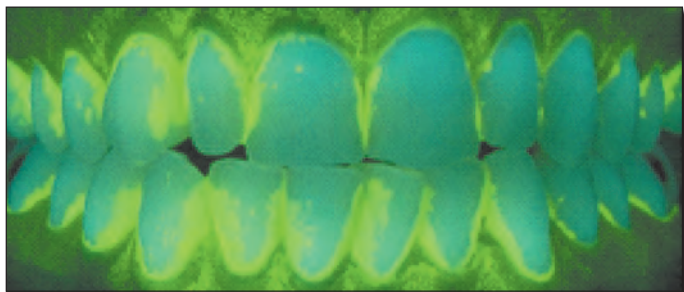


Fig. 2. Mechanical hygiene is less than optimal in most patients. This digital image of dental plaque disclosed by fluorescein reveals typical areas missed in regular toothbrushing and corresponding development of chronic supragingival plaque deposits in specific locations leading to localized gingivitis.

through oral hygiene (brushing and flossing).<sup>17-19</sup>

It is well known that deliberate and thorough toothbrushing and flossing are highly effective in arresting and reversing early gum disease. So why have these procedures not proven more effective? The answer lies in hygiene efficiency. Studies<sup>20-22</sup> show that the average toothbrushing time period is (optimistically) 30-60 seconds - not nearly enough for adequate cleaning of the dentition or harmful plaque deposits. As Fig. 2 shows, regardless of motivation, hygiene skill in average patients is limited. Patients require additional help to make the most out of their oral hygiene routines.

#### The second line of prevention: Chemotherapeutics

Careful consideration of information available from studies of epidemiologic and oral hygiene practices reveals the obvious: there is considerable room for improvement in the oral health of the general population. In this respect dental practitioners are at the mercy of their patients – without motivation, hygiene is inadequate. Moreover, the daily insult of developed plaque deposits is not amenable to effective professional intervention, which is primarily directed at thorough, but infrequent dental prophylaxes. The burden then falls to making more out of the hygiene that patients actually apply. This is where manufacturers of oral products, both over-the-counter (OTC) and prescribed can play a leading role. With respect to techniques that patients can apply, the second line of intervention thus includes the addition of chemotherapeutics to oral hygiene vehicles such as toothpastes and mouthrinses. The addition of fluoride to toothpastes in the mid-1950s heralded the modern era of applied chemotherapeutics to the prevention of oral diseases.

The incorporation in the 1950s of fluoride into toothpaste with high bioavailability and with clinically proven benefits provided patients with a health optimizing intervention that did not require a change in oral hygiene habits. Advertising focused on the end benefit of convenient, preventive care with fluoride – no cavities (Fig 3). This convenience has become the challenge for the oral products industry. Today, therapeutic options provided to dentifrices and mouthrinses include fluoride for the control of dental caries,<sup>23</sup> various ingredients for the control of dentin hypersensitivity,<sup>24</sup> and antimicrobials for the control of plaque and gingivitis.<sup>25</sup>

With respect to plaque and gingivitis, ingredients added to dentifrices and rinses with reported clinical benefits include antimicrobial ingredients such as triclosan, essential oil combinations, various metal ion antimicrobials (stannous, zinc, copper), chlorhexidine, and cetylpyridinium chloride.<sup>25-28</sup> These



Fig. 3. The introduction of fluoride into toothpastes – formulated in a form effective for the control of dental caries – heralded the modern era of preventive dentistry in the 1950s.

ingredients are thought to provide efficacy through the modulation of both the quantity of supragingival plaque on the teeth as well as the virulence of formed biofilms toward inducing the inflammatory response. Regardless of the form of application, the formulation of effective oral products is a complex undertaking. Antimicrobial ingredients need to show high solubility in the formulation, complete and rapid diffusivity into oral biofilms, rapid reactivity with microbial substrates and controlled retention to produce benefits following the actual application conditions.<sup>29,30</sup>

Mouthrinses produce a surprisingly difficult matrix in which to formulate effective antimicrobial therapies. Many antimicrobial ingredients derive their bactericidal and bacteriostatic effects from co-solubilization of the ingredients into the lipophilic (hydrophobic) portions of bacterial cell walls.<sup>31-32</sup>

Thus, the lipophilic portions of some of the molecules may have the highest reactivity with the bacteria. Fig. 4 shows the structure of the cetylpyridinium chloride (CPC) molecule, the ingredient formulated in Crest ProHealth Rinse<sup>a</sup> mouthrinse (Fig. 5), where the lipophilic side chain is observed. As will be discussed later, CPC was recently recommended as one of two active ingredients approvable for use in over-the-counter mouthrinses for the control of plaque and gingivitis.<sup>28</sup> In most instances, formulators of mouthrinses rely on alcohol to emulsify antimicrobial ingredients in bioavailable forms.<sup>33</sup> Some notable commercial examples include Listerine<sup>b</sup> antiseptic<sup>28</sup> (available

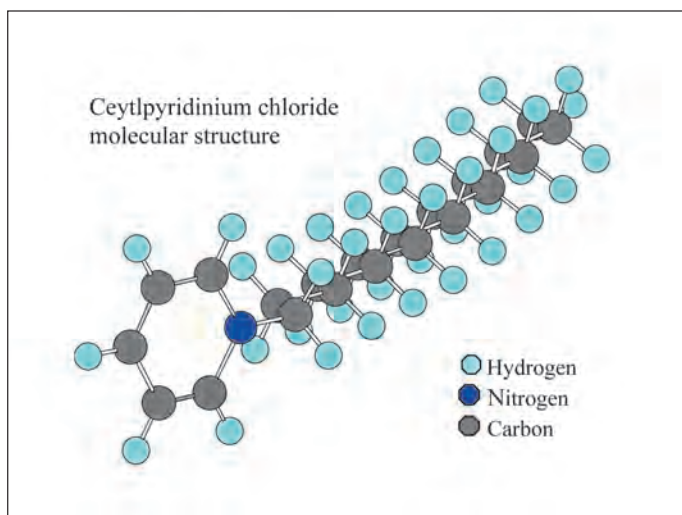


Fig. 4. The CPC molecular structure includes the cationic pyridinium ion which promotes substantivity through electrostatic binding *in vivo*. The long chain lipophilic alkyl portion can lyse bacterial membranes producing cidal activity.

as an OTC mouthrinse) which solubilizes essential oil combinations at therapeutic concentrations with the inclusion of between 21.6% to 26.9% alcohol, and Peridex<sup>c</sup> mouthrinse, which adds a slightly lower alcohol amount to solubilize flavor masking for the chlorhexidine bisguanide.<sup>34</sup> The delivery of soluble antimicrobials in mouthrinses is highly effective for producing chemotherapeutic antiplaque and antigingivitis efficacy, with both Listerine and Peridex demonstrating significant efficacy in clinical trials.<sup>35,36</sup>

#### Why an alcohol-free therapeutic mouthrinse ?

The use of alcohol as an emulsification ingredient in mouthrinses is well known.<sup>37</sup> From a toxicology point of view, alcohol has an extensive history of safe use in these product forms for the vast majority of the population.<sup>36</sup> On the other hand, alcohol-containing mouthrinses have properties which can, in practice, limit their utilization. Mouthrinses containing alcohol may be limited with respect to the populations that can tolerate the mouthrinse, local side effects and the time the rinse can be used *in vivo*, potentially affecting compliance and mouthrinse use in general.

To illustrate this latter point; in 1993, Bolanowski *et al*<sup>38</sup> reported on a study in which they observed increases in intraoral pain of subjects using mouthrinses containing in excess of 10% alcohol. These anecdotal observations, reported at the International Association for Dental Research meeting, were later followed by a much more detailed study, published in 1995.<sup>39</sup> In the study, the researchers tested a variety of commercial mouthrinses as well as control solutions containing a dose response of added alcohol, in a multi-sequence, blind, cross-over psychophysical study examining the effects of the rinses on induced pain. The population of 25 subjects were instructed on proper rinsing habits and used a subjective pain scale to evaluate rinse tolerability on a seven point scale ranging from no pain (score 0) to intolerable pain (score 6). In an interesting design feature, subjects carried out pain threshold evaluations at various time intervals during use. Thus, patients had the chance to evaluate overall tolerability of the rinses as well as the intensity of pain during usage. (One might think of overall tolerability as a measure of a patient's willingness to use



Fig. 5. Crest Pro-Health Rinse uses cetylpyridinium chloride as an active antimicrobial ingredient to kill bacteria, fight plaque and control gingivitis.

the product at all, while “pain threshold during use” tolerability may also be indicative of the potential to use products for the recommended rinsing times.). Overall, Bolanowski *et al*<sup>39</sup> observed a clear dose response and surprisingly linear relation on pain threshold for rinses and the alcohol content (Fig. 6).

Moreover, the researchers observed a clear time dependence on pain development during rinse use which was again dose dependent. Said differently, higher alcohol rinses induced more pain in subjects who also developed pain faster than lower alcohol rinses. Non-alcohol solutions had no promotional effects on patient discomfort.

Alcohol-free therapeutic rinses may be preferable for additional reasons. A second factor is related to potential side effects of alcohol itself. Ethanol has been shown to produce surface softening and increased wear rates of dental resins and composite materials.<sup>40,41</sup> In addition, it is well known that formulations containing significant quantities of alcohol are poorly tolerated in patients with mucositis, who are immunocompromised or who are undergoing head and neck radiation therapy for cancer.<sup>42,43</sup>

Along with the selected tolerability issues and special population issues cited above, alcohol-containing formulations may be undesirable for selected patients in situations in which ethanol exposure might be contraindicated. This might include patients being treated for alcoholism, pregnant and nursing women, and diabetics. There are also those patients who would choose to avoid alcohol ingestion based on social or religious reasons.

#### An alcohol-free cetylpyridinium chloride mouthrinse – Crest Pro-Health Rinse

As mentioned, mouthrinses containing therapeutic ingredients often use alcohol as a solubilization agent to deliver ingredient efficacy in a bioavailable form. This method has

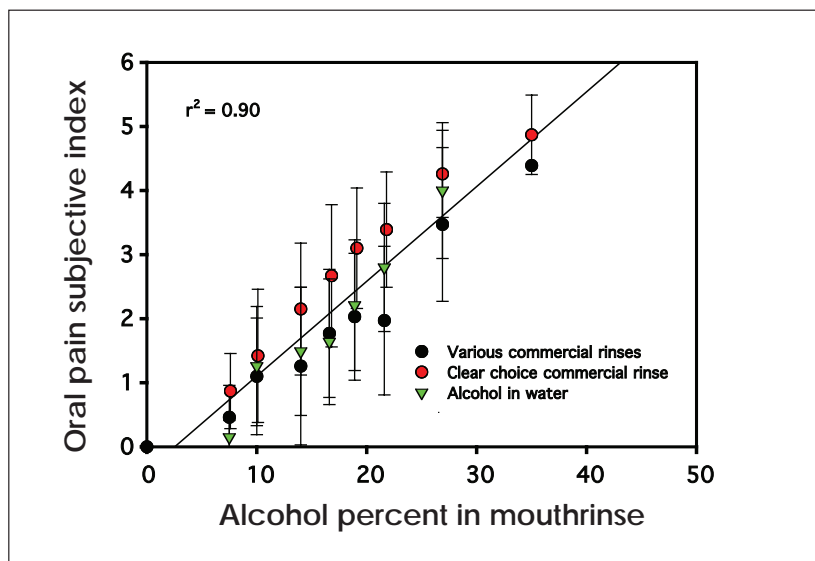


Fig. 6. The correlation between subjective oral discomfort and formulated alcohol levels in various mouthrinses including commercial products, water solutions (without flavor) and a commercial Clear Choice mouthrinse formulated also with various alcohol levels (Figure developed based on data in Bolanowski *et al*<sup>38</sup>). Note the variation in patient response for each product and alcohol level, and also the clear general association between alcohol concentration and subjective discomfort.

been successfully used in the development of two popular mouthrinses, Listerine and Peridex. On the other hand, there are circumstances where alcohol-free formulations may be preferred and these may include specific populations of consumers, who choose not to use available products due to esthetic preferences or rinse tolerability or who are unwilling to carry out prescribed dosage regimens.

The Crest Pro-Health Rinse discussed herein is formulated with 0.07% high bioavailable cetylpyridinium chloride (CPC) as the active ingredient for the prevention of plaque formation and gingivitis and gingival bleeding. CPC has a long history of use in oral care products, but is probably most well known as a portion of the antimicrobial combination used in Scope mouthrinse in the United States. In Scope, the CPC is formulated in a form that provides sufficient antibacterial activity to produce breath protection benefits, but is not formulated with bioavailability sufficient for plaque and gingivitis efficacy.<sup>44</sup> In Crest Pro-Health Rinse, the CPC is formulated in a high bioavailability base which does not require alcohol for solubilization. When formulated in a highly bioavailable form, CPC is a very effective antiplaque and antigingivitis ingredient evidenced by multiple clinical trials.<sup>45-53</sup> This efficacy prompted a subcommittee of the U.S. Food and Drug Administration in 2002 to recommend category 1 (safe and effective) monograph status for CPC mouthrinse.<sup>54</sup> This category 1 status is the same provision given to essential oils mouthrinse, namely Listerine.

### Summary and additional considerations

The addition of chemotherapeutic ingredients to toothpastes and mouthrinses is a convenient method to produce improved health benefits without requiring patients to change their daily hygiene regimens. The addition of new treatment options, with attributes that appeal to consumers, is an attractive means to increase hygiene focus of consumers and perhaps build the base of the population that may consider these means to improve their overall oral health. The foregoing discussion briefly

documented findings regarding health care status of average patients, approaches to improving oral hygiene, the role of mouthrinses in providing adjunctive health benefits to regular hygiene and the limitations of some marketed mouthrinses in selected population acceptability. There seems to be a clear place for the development of a therapeutic alcohol-free mouthrinse providing adjunctive plaque and gingivitis reduction benefits to regular hygiene procedures. This is the genesis of Crest Pro-Health Rinse introduced in this compilation of scientific articles. The clinical research summarized in this issue is focused on the therapeutic efficacy of Crest Pro-Health Rinse. Specifically, the papers show significant plaque and gingivitis benefits for Crest Pro-Health Rinse and high bioavailable CPC prototype rinses relative to both negative and positive controls (Listerine).<sup>50-53</sup> The issue also contains a summary of findings from a “real world” assessment that addresses factors related to compliance.<sup>55</sup>

Naturally, product selection is a complicated process and no one suggests that today’s many treatment forms, manual brushes, special flosses, electric brushes, antibacterial toothpastes and antibacterial mouthrinses described here provide a panacea. Many times, directions given to patients toward additional therapeutic actions require them to devote increased time for hygiene or to tolerate less than desirable esthetics or even side effects of treatments. Indeed, the use of CPC includes a variety of minor side effects that may affect a small portion of users including temporary effects on sensorial aspects of taste and the possibility in limited cases of reversible extrinsic tooth staining.<sup>56-58</sup> The potentiation of tooth staining in particular is observed to a greater degree in chlorhexidine rinses and is also routinely observed in the use of essential oil mouthrinses<sup>59-64</sup> and even with so-called bacterial antiadhesive rinses.<sup>65</sup> Importantly, these side effects can be easily managed as part of a hygiene strategy for patients.<sup>66-68</sup>

Alcohol-free therapeutic mouthrinses may provide significant benefits to a variety of patients with low tolerance or

preference for the alcohol-containing forms. The therapeutic index of Crest Pro-Health Rinse is comparable to available alcoholic rinse forms and may provide advantages in preference to consumers and patients requiring the added therapy provided by this treatment form. The statistics on mouthrinse utilization suggest that less than 50% of patients use mouthrinses; almost one half of these do not use therapeutic forms and most of the patients do not use these as directed for adequate complement to their oral hygiene. Crest Pro-Health Rinse provides a highly effective alcohol-free therapeutic mouthrinse alternative to consumers. It is hoped that this may drive increased compliance with overall health regimens as we continue to strive to produce a healthy dentition.

- a. The Procter & Gamble Co., Cincinnati, OH, USA.
- b. Pfizer Consumer Healthcare, Morris Plains, NJ, USA.
- c. Zila Pharmaceuticals, Phoenix, AZ, USA.

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# A 6-month clinical trial to study the effects of a cetylpyridinium chloride mouthrinse on gingivitis and plaque

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**ABSTRACT: Purpose:** To evaluate the effects of a novel mouthrinse containing 0.07% high bioavailable cetylpyridinium chloride (Crest Pro-Health Rinse) on the development of gingivitis and plaque *versus* a placebo control over a period of 6 months. **Methods:** This was a randomized, 6-month, placebo-controlled, parallel groups, double blind, single center clinical trial. One hundred thirty-nine generally healthy adults with mild-to-moderate gingivitis were enrolled in the study. Subjects were given Modified Gingival Index (MGI), Gingival Bleeding Index (GBI) and Modified Quigley-Hein Plaque Index (MQH) examinations followed by a dental prophylaxis. Subjects were then randomly assigned to either the cetylpyridinium chloride (CPC) rinse or placebo rinse and instructed to begin rinsing twice a day with 20 ml of their assigned mouthrinse for 30 seconds after brushing their teeth. Subjects were assessed for MGI, GBI and MQH scores after 3 and 6 months of product use. Oral hard and soft tissue examinations were also performed at all visits. **Results:** 124 subjects were evaluable at Month 3 and 119 at Month 6. After 6 months, subjects rinsing with the CPC rinse showed 15.4% less gingival inflammation, 33.3% less gingival bleeding, and 15.8% less plaque relative to the placebo group. All reductions were highly statistically significantly different ( $P < 0.01$ ). Results were similar at 3 months. Both treatments were well-tolerated. (*Am J Dent* 2005;18: 9A-14A).

**CLINICAL SIGNIFICANCE:** This study demonstrates that the Crest Pro-Health 0.07% CPC mouthrinse provided significant antiplaque and antigingivitis benefits when used twice daily for 6 months as an adjunct to toothbrushing.

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## Introduction

Plaque-induced gingivitis continues to be a major dental problem for adults, adolescents and children worldwide.<sup>1-5</sup> Studies have shown that dental plaque plays an important role in the development of gingivitis, which in turn can advance to periodontitis.<sup>6</sup> Furthermore, some studies suggest that individuals with certain systemic diseases may be at higher risk of developing periodontitis.<sup>7</sup>

The mechanical elimination of dental plaque is the basis of the prevention and the treatment of gingivitis and periodontitis. Prevention may be partially achieved by conscientious daily brushing and flossing to remove plaque that forms each day before inflammation occurs.<sup>8</sup> However, inefficient brushing and inadequate flossing by most people<sup>9</sup> can lead to an accumulation of plaque and ultimately gingivitis, particularly in areas that are difficult to reach. Using chemotherapeutic agents is one approach to help control plaque accumulation in these areas.

Antimicrobial toothpastes and mouthrinses have been investigated and marketed to provide additional anti-plaque/anti-gingivitis activity when used daily as an adjunct to a mechanical oral hygiene regimen.<sup>10-14</sup> Several clinical studies have demonstrated that the broad spectrum antimicrobial agent cetylpyridinium chloride (CPC) can help control supragingival plaque and gingivitis.<sup>15-17</sup> It has been reported, however, that the efficacy of CPC mouthrinses can be compromised by formulation excipients, such as emulsifiers, leading to situations where two CPC mouthwashes could contain the same level of CPC but differ significantly in their relative efficacy.<sup>18</sup> Taking this into account, the FDA Plaque Subcommittee reviewed extensive data on CPC and deemed it to be safe and efficacious for the treatment of plaque-induced gingivitis within a concentration range of 0.045% to 0.10% CPC when present in

a high-bioavailable matrix (as defined by prescribed performance assays).<sup>19</sup>

Recently, a new CPC rinse was introduced (Crest Pro-Health Rinse<sup>a</sup>) that meets these FDA guidelines. The product delivers 0.07% CPC in a high-bioavailable, alcohol-free formulation. The present study was conducted to investigate the long-term antiplaque and antigingivitis benefits of the CPC rinse relative to a placebo rinse.

## Materials and Methods

**Study design** - This was a randomized, 6-month, placebo-controlled, double-blind, parallel groups, single-center gingivitis clinical trial conducted at Dental Products Testing, Inc., West Palm Beach, Florida. Both the research protocol and written informed consent were reviewed and approved by an institutional review board prior to study initiation.

At the baseline visit, subjects who had not brushed nor flossed their teeth after 10 p.m. the previous night were given examinations to assess oral hard and soft tissue status and to measure gingival inflammation (Modified Gingival Index, MGI), gingival bleeding (Gingival Bleeding Index, GBI) and dental plaque (Modified Quigley-Hein Plaque Index, MQH). Subjects then received an oral prophylaxis and were randomly assigned in approximately equal numbers to one of the two treatment groups, balancing for gender and baseline smoking status:

- Experimental alcohol-free 0.07% CPC mouthrinse (Crest Pro-Health Rinse<sup>a</sup>);
- Alcohol-free placebo mouthrinse.<sup>a</sup>

Subjects were instructed to brush twice daily as they normally do, rinse thoroughly with water and then rinse with 20 mL of their assigned mouthrinse for 30 seconds. Subjects

Table 1. Baseline demographics characteristics.

Subjects included in the Month 3 analysis					
Treatment	N	Age		Gender % Female	Smoking status % Smokers
		Mean $\pm$ SD	Range		
Placebo rinse	64	39.3 $\pm$ 12.83	18 – 65	78%	19%
CPC rinse	60	36.5 $\pm$ 9.13	19 – 62	75%	15%

Ethnicity					
Treatment	N	% Black	% Caucasian	% Hispanic	% Other
Placebo rinse	64	19%	75%	6%	0%
CPC rinse	60	15%	63%	18%	4%

Subjects included in the Month 6 analysis

Treatment	N	Age		Gender % Female	Smoking status % Smokers
		Mean $\pm$ SD	Range		
Placebo rinse	62	39.5 $\pm$ 12.86	18 – 65	79%	19%
CPC Rinse	57	36.8 $\pm$ 9.09	19 – 62	74%	16%

Ethnicity					
Treatment	N	% Black	% Caucasian	% Hispanic	% Other
Placebo rinse	62	18%	76%	6%	0%
CPC rinse	57	16%	63%	18%	4%

were given a kit containing a commercial dentifrice with sodium monofluorophosphate (Colgate Cavity Protection<sup>b</sup>), two soft compact flat head toothbrushes (Oral-B<sup>c</sup>), dose cups, and their assigned mouthrinses at baseline and at 4-week intervals throughout the study. Subjects were given verbal and written instructions on product usage and instructed to use only the test products provided during the study. Subjects performed the first dosing in the presence of study personnel after they received their kits. All remaining product usages were unsupervised.

To preserve blinding, investigational products and kits were identical in their appearance. Subjects returned after 3 and 6 months for examinations to reevaluate all efficacy and safety parameters, including MGI, GBI, MQH and hard and soft tissue safety. Subjects and site personnel were blinded to treatment assignment.

**Study population** - One-hundred thirty-nine (139) subjects were enrolled in the study. Study subjects were generally healthy adult volunteers from 18-65 years of age. To participate in the study, subjects were required to have a minimum of 18 natural teeth, a baseline MGI score of at least 1.75 and not greater than 2.3, and a Turesky plaque score of at least 1.5. Prospective subjects with any of the following conditions were ineligible for participation: requirement for antibiotic pre-medication prior to dental procedures; use of antibiotic, anti-inflammatory or anti-coagulant therapy for 14 days prior to the baseline exam; diabetes; pregnancy; rampant caries; advanced periodontal disease; history of significant adverse events to oral hygiene products; or other medical conditions that the investigator deemed could compromise the evaluation of study results. All subjects provided written informed consent prior to participation.

At Month 6 the study population ranged in age from 18-65 years, with a mean (SD) age of 38.2 (11.25) years. Females accounted for 76% of participants. Seventy percent of subjects were Caucasian, 17% were Black, 12% were Hispanic, and

Table 2. Modified Gingival Index.

Score	Description
0	Absence of inflammation;
1	Mild inflammation; slight change in color, little change in texture of any portion of but not the entire marginal or papillary gingival unit;
2	Mild inflammation; criteria as above but involving the entire marginal or papillary gingival unit;
3	Moderate inflammation; glazing, redness, edema, and/or hypertrophy of the marginal or papillary gingival unit;
4	Severe inflammation; marked redness, edema and/or hypertrophy of the marginal or papillary gingival unit, spontaneous bleeding, congestion or ulceration.

Table 3. Gingival Bleeding Index as defined by Saxton & van der Ouderaa.<sup>22</sup>

Score	Description
0	Absence of bleeding after 30 seconds;
1	Bleeding observed after 30 seconds;
2	Immediate bleeding observed.

Table 4. Turesky Modification of Quigley-Hein Plaque Index.

Score	Description
0	No plaque;
1	Isolated areas of plaque at gingival margin;
2	Thin band of plaque at gingival margin ( $\leq$ 1mm);
3	Plaque covering up to 1/3 of tooth surface;
4	Plaque covering 1/3 to 2/3 of tooth surface;
5	Plaque covering $\geq$ 2/3 of tooth surface.

1% were of other ethnic origins. Eighteen percent of subjects at Month 6 were self-reported smokers (Table 1).

**Clinical assessment** - Gingivitis was scored at Baseline, Month 3, and Month 6 by the MGI on the buccal and lingual marginal gingivae and interdental papillae of all scorable teeth. (Table 2) MGI is slightly different from the Löe-Silness Gingival Index (GI) in that probing is not used to elicit bleeding and the scoring system for mild and moderate inflammation is redefined.<sup>20</sup> Previous studies comparing the two indices have demonstrated that MGI correlates significantly with GI.<sup>21</sup> Thus, MGI allows for noninvasive assessment of early and subtle visual changes in severity and extent of gingivitis.

Gingival bleeding was evaluated at Baseline, Month 3, and Month 6 according to the GBI as defined by Saxton & van der Ouderaa.<sup>22</sup> Each of three gingival areas (buccal, mesial, and lingual) of the teeth was probed, waiting approximately 30 seconds before recording the number of gingival units which bled using a 0-2 scale (Table 3). Measurement of plaque area was done at Baseline, Month 3, and Month 6 by the Turesky modification of the Quigley-Hein Plaque Index, which emphasizes plaque in contact with the gingiva, on six surfaces (distobuccal, midbuccal, mesiobuccal, distolingual, midlingual, and mesiolingual) of all scorable teeth after use of disclosing solution.<sup>23</sup> (Table 4).

Oral soft tissue assessments were conducted *via* a visual examination of the oral cavity and perioral area using a standard dental light, dental mirror, and gauze. Structures examined included the gingiva (free and attached), hard and soft palate, oropharynx/uvula, buccal mucosa, tongue, floor of the mouth, labial mucosa, mucobuccal/mucolabial folds, lips,

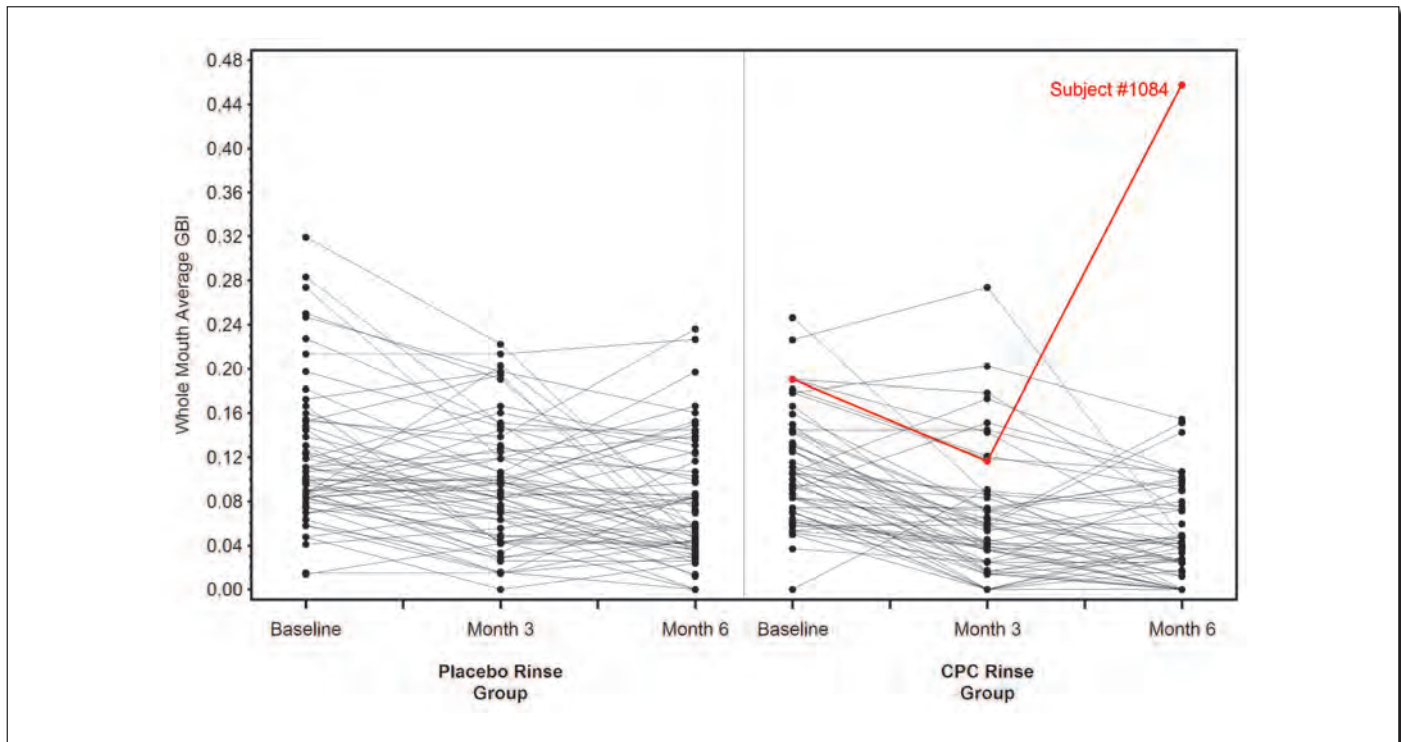


Figure. Individual GBI response over time (Note outlier at Month 6).

and perioral area. Oral hard tissues were assessed *via* a visual examination of the dentition and restorations utilizing a standard dental light, dental mirror, and air syringe. Abnormal oral soft/hard tissue findings noted after baseline or those that were present at baseline but worsened during investigational product usage were recorded as adverse events.

One examiner conducted MGI, GBI and safety examinations while a separate examiner assessed plaque. The same clinician performed the same measurements at all timepoints.

The whole-mouth average MGI, GBI and MQH scores were calculated for each subject at Baseline, Month 3 and Month 6 by summing the respective scores at each gradable site and dividing by the number of gradable sites. The proportion of sites bleeding was also calculated by summing the number of gradable sites with GBI scores of “1” or “2” and dividing by the number of gradable sites.

*Statistical analysis* - Descriptive summaries of the study population demographic data were prepared for subjects included in the Month 3 and Month 6 efficacy analyses. Evidence of imbalance across treatment groups was statistically assessed with two-sample *t*-tests and chi-squared tests.

Efficacy analyses were based on whole-mouth average MGI, GBI, and MQH scores, as well as the proportion of GBI sites bleeding. The 0.07% CPC rinse group was compared to the placebo rinse group with respect to each of these indices separately at Month 3 and Month 6. Analysis of covariance (ANCOVA) was to be used to model the post-baseline mean of each endpoint, using the respective baseline score as the covariate. The Month 3 and Month 6 data were to be modeled separately, with the Month 6 data of primary interest. The percent difference between treatments was to be calculated for each efficacy endpoint using the adjusted means from the ANCOVA models.

The ANCOVA efficacy analysis plan described above was executed, except for the Month 6 GBI data, where one subject (#1084) in the CPC rinse group was an extreme and influential outlier. As illustrated in the Figure, this subject’s GBI score improved from Baseline to Month 3 but then reversed at Month 6 to more than double the value at Baseline. The studentized residual for this subject at Month 6 was 6.56, confirming that this score was an extreme outlier. No explanation for this subject’s unusual Month 6 score could be found, either from a safety or compliance perspective. Given the fact that outliers can compromise the validity of traditional ANCOVA methods,<sup>24,25</sup> a rank ANCOVA that is robust to outliers was used to analyze the Month 6 GBI data.<sup>26-28</sup> Note that when the assumptions of ANCOVA are satisfied, *e.g.* no influential outliers, rank ANCOVA is less powerful (more conservative) than ANCOVA. However, when the assumptions of ANCOVA are not satisfied, *e.g.* influential outliers, rank ANCOVA results are more reliable than ANCOVA results. The percent benefit for the Month 6 GBI analysis was calculated using the median (robust to outliers) rather than the mean (not robust to outliers).

**Results**

Of the 139 subjects who were randomized to treatment, 124 were present and evaluable at the Month 3 visit and 119 at the Month 6 visit. (One patient was late for the Month 6 examination and missed the gingivitis assessment.) There was no evidence ( $P > 0.05$ ) of imbalance between groups with respect to age, gender, ethnicity or smoking habits at either Month 3 or Month 6 (Table 1).

*Modified Gingival Index* - The baseline mean MGI scores for subjects in the Month 3 analysis were 2.01 for the CPC rinse group and 2.02 for the placebo rinse group. At Month 3, the

Table 5. Modified Gingival Index results.

Month 3 Analysis				
Treatment	N	Baseline score (Mean ± SE)	Month 3 score (Adjusted Mean <sup>a</sup> ± SE)	% Reduction <sup>b</sup>
Placebo rinse	64	2.02 ± 0.013	1.92 ± 0.026	-----
CPC rinse	60	2.01 ± 0.014	1.68 ± 0.027	12.5%

The adjusted means were statistically significantly different (P<0.0001).

Month 6 Analysis				
Treatment	N	Baseline score (Mean ± SE)	Month 3 score (Adjusted Mean <sup>a</sup> ± SE)	% Reduction <sup>b</sup>
Placebo rinse	62	2.02 ± 0.014	1.88 ± 0.038	-----
CPC rinse	56	2.01 ± 0.013	1.59 ± 0.040	15.4%

The adjusted means were statistically significantly different (P<0.0001).

<sup>a</sup> Adjusted means and standard errors from analysis of covariance with baseline score as the covariate.

<sup>b</sup> % Reduction = 100% x (Placebo rinse mean – CPC rinse mean)/Placebo rinse mean.

Table 7. Proportion of bleeding sites results.

Month 3 Analysis				
Treatment	N	Baseline score (Mean ± SE)	Month 3 score (Adjusted Mean <sup>a</sup> ± SE)	% Reduction <sup>b</sup>
Placebo rinse	64	0.106 ± 0.0067	0.084 ± 0.0049	-----
CPC rinse	60	0.101 ± 0.0081	0.064 ± 0.0051	23.8%

The adjusted means were statistically significantly different (P=0.006).

Month 6 Analysis				
Treatment	N	Baseline score (Mean ± SE)	Month 3 score (Adjusted Mean <sup>a</sup> ± SE)	% Reduction <sup>b</sup>
Placebo rinse	62	0.105 ± 0.0068	0.074 ± 0.0060	-----
CPC rinse	56	0.096 ± 0.0055	0.050 ± 0.0063	32.4%

The adjusted means were statistically significantly different (P=0.007).

<sup>a</sup> Adjusted means and standard errors from analysis of covariance with baseline score as the covariate.

<sup>b</sup> % Reduction = 100% x (Placebo rinse mean – CPC rinse mean)/Placebo rinse mean.

adjusted mean score for the CPC rinse group was 12.5% lower than that of the placebo rinse group (1.68 vs. 1.92). The difference between groups was highly statistically significant (P<0.0001) (Table 5).

The baseline mean MGI scores for subjects in the Month 6 analysis were 2.01 for the CPC rinse group and 2.02 for the placebo rinse group. At Month 6 the adjusted mean for the CPC rinse group was 15.4% lower than that of the placebo rinse group (1.59 vs. 1.88). The difference between groups was highly statistically significant (P<0.0001) (Table 5).

**Gingival bleeding** - For subjects in the Month 3 analysis, the baseline mean GBI score for the CPC rinse group was 0.114 compared to 0.122 for the placebo rinse group. At Month 3 the adjusted mean GBI score was 23.4% lower for the CPC rinse group than for the placebo rinse group (0.072 vs. 0.094) and was highly statistically significant (P=0.006) (Table 6).

For subjects in the Month 6 analysis, the baseline median GBI score for the CPC rinse group was 0.106 compared to 0.102 for the placebo rinse group. At Month 6, the median

Table 6. Gingival Bleeding Index results.

Month 3 Analysis				
Treatment	N	Baseline score (Mean ± SE)	Month 3 score (Adjusted Mean <sup>a</sup> ± SE)	% Reduction <sup>b</sup>
Placebo rinse	64	0.122 ± 0.0080	0.094 ± 0.0056	-----
CPC rinse	60	0.114 ± 0.0087	0.072 ± 0.0058	23.4%

The adjusted means were statistically significantly different (P=0.006).

Month 6 Analysis				
Treatment	N	Baseline score (Median ± IQR)	Month 6 score (Adjusted Median ± IQR)	% Reduction <sup>c</sup>
Placebo rinse	62	0.102 ± 0.0648	0.060 ± 0.0796	-----
CPC rinse	56	0.106 ± 0.0726	0.040 ± 0.0644	33.3%

Based on a rank analysis of covariance, the treatments were statistically significantly different (P=0.002).

<sup>a</sup> Adjusted means and standard errors from analysis of covariance with baseline score as the covariate.

<sup>b</sup> % Reduction = 100% x (Placebo rinse mean – CPC rinse mean)/Placebo Rinse mean.

<sup>c</sup> % Reduction = 100% x (Placebo Rinse median – CPC Rinse median)/Placebo rinse median.

Table 8. Turesky Modified Quigley Hein Plaque Index results.

Month 3 Analysis				
Treatment	N	Baseline score (Mean ± SE)	Month 3 score (Adjusted Mean <sup>a</sup> ± SE)	% Reduction <sup>b</sup>
Placebo rinse	64	2.69 ± 0.050	2.41 ± 0.053	-----
CPC rinse	60	2.73 ± 0.056	1.93 ± 0.054	19.9%

The adjusted means were statistically significantly different (P<0.0001).

Month 6 Analysis				
Treatment	N	Baseline score (Mean ± SE)	Month 3 score (Adjusted Mean <sup>a</sup> ± SE)	% Reduction <sup>b</sup>
Placebo rinse	62	2.68 ± 0.051	2.34 ± 0.051	-----
CPC rinse	57	2.73 ± 0.058	1.97 ± 0.053	15.8%

The adjusted means were statistically significantly different (P<0.0001).

<sup>a</sup> Adjusted means and standard errors from analysis of covariance with baseline score as the covariate.

<sup>b</sup> % Reduction = 100% x (Placebo rinse mean – CPC rinse mean)/Placebo rinse mean.

GBI score was 33.3% lower for the CPC Rinse group than for the placebo rinse group (0.040 vs. 0.060) and was highly statistically significant (P=0.002) (Table 6).

Efficacy results for the proportion of sites bleeding were similar to the GBI results. Specifically, the baseline mean proportion of sites bleeding was 0.101 for the CPC rinse group and 0.106 for the placebo rinse group among subjects examined at Month 3. The adjusted mean proportion of sites bleeding at Month 3 was 23.8% lower for the CPC rinse group than for the placebo rinse group (0.064 vs. 0.084) and was highly statistically significant (P=0.006) (Table 7).

For subjects examined at Month 6, the baseline mean proportion of sites bleeding was 0.096 for the CPC rinse group and 0.105 for the placebo rinse group. At Month 6 the adjusted mean proportion of sites bleeding was 0.050 for the CPC rinse group compared to 0.074 for the placebo rinse group, or a 32.4% difference. The difference between groups

was highly statistically significant ( $P=0.007$ ) (Table 7).

**Plaque** - The baseline mean MQH score for subjects in the Month 3 analysis was 2.73 for the CPC rinse group and 2.69 for the placebo rinse group. At Month 3, the adjusted mean score for the CPC rinse group was 19.9% lower than that of the placebo rinse group (1.93 vs. 2.41). The difference between groups was highly statistically significant ( $P<0.0001$ ) (Table 8).

The baseline mean MQH scores for subjects in the Month 6 analysis were 2.73 for the CPC rinse group and 2.68 for the placebo rinse group. At Month 6 the adjusted mean for the CPC rinse group was 15.8% lower than that of the placebo rinse group (1.97 vs. 2.34). The difference between groups was highly statistically significant ( $P<0.0001$ ) (Table 8).

Neither treatment group had any significant adverse reactions or remarkable oral soft tissue findings related to mouthrinse use. One mild adverse event (angular cheilitis) was reported during the study in the CPC group and self-resolved.

### Discussion

Results of the study support the long-term antiplaque and antigingivitis benefits of a novel alcohol-free, high bioavailable<sup>18</sup> 0.07% CPC mouthrinse, further adding to the published evidence of efficacy of this therapeutic mouthrinse.<sup>29-31</sup> In this study, the CPC rinse reduced gingivitis and gingival bleeding by 15% and 33%, respectively, relative to placebo after 6 months usage. The proportion of bleeding sites was reduced by 32% relative to placebo. Statistically significant benefits were also observed for plaque.

Reports in the literature have consistently demonstrated that mouthwash rinsing is an important component of an oral care regimen. Many mouthwashes contain more than 21% alcohol, however, and may cause an unpleasant burning sensation. In addition, millions of patients prefer not to use alcohol-based products for reasons unrelated to product esthetics, including medical, religious, and age.

Patients with xerostomia, or dry mouth, are one group that can benefit from an alcohol-free therapeutic rinse. Xerostomia is the abnormal reduction of saliva. The condition can be a symptom of certain diseases or an adverse effect of certain medications. Over 400 medications are reported to cause a reduction in salivary gland production,<sup>32</sup> making xerostomia increasingly common among elderly patients who often take multiple medications.<sup>33</sup> The management of xerostomia principally consists of the avoidance of factors that might cause or aggravate dry mouth, the application of salivary substitutes, and the prevention of associated oral complications (e.g., caries).<sup>34</sup> There is general consensus among dental professionals that these patients should avoid alcohol-based mouthwashes since they may worsen the dry mouth effect.<sup>35-37</sup>

Crest Pro-Health Rinse offers therapeutic benefits to this group without the concern that alcohol will further exacerbate the symptoms. Other patient types may also prefer alcohol-free oral hygiene products, including diabetics, cancer patients, orthodontic patients, patients of certain religious faiths, and patients with a history of alcohol abuse.

In conclusion, this 6-month, randomized clinical trial shows Crest Pro-Health Rinse with high bioavailable CPC provides long-term gingival health benefits for the general

population. The rinse may be particularly appealing to certain patients who prefer to use alcohol-free products.

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# Comparative clinical trial of two antigingivitis mouthrinses

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**ABSTRACT:** *Purpose:* To compare the safety and the antiplaque and antigingivitis efficacy of two oral rinses. *Methods:* A randomized, double-blind, parallel groups, single-center study was conducted to evaluate the safety and efficacy of a high bioavailable, alcohol-free 0.07% cetylpyridinium chloride (CPC) rinse (Crest Pro-Health Rinse) and a positive control rinse containing essential oils (EO) and 21.6% ethyl alcohol (Cool Mint Listerine). Seventy-eight healthy adults were enrolled in a modified experimental gingivitis clinical trial. Four weeks before the baseline visit, subjects received a prophylaxis and were instructed to brush twice daily in a manner to approach optimum gingival health. At the end of the 4-week period, subjects were randomly assigned to treatment and instructed to use 20 ml of their assigned product for 30 seconds after brushing twice daily during a 21-day treatment phase. Plaque removal by brushing was prevented during the treatment phase for one mandibular quadrant (experimental gingivitis region) by means of a specially-manufactured tooth shield. Safety and efficacy measurements were obtained at baseline and at the end-of-treatment using the Modified Gingival Index (MGI), Gingival Bleeding Index (GBI), and Modified Quigley-Hein Plaque Index (MQH). At all visits, an oral soft tissue examination was performed for each subject. The efficacy data obtained in the experimental gingivitis region were analyzed with analysis of covariance. *Results:* Seventy-five subjects completed the study and were included in the data analyses. No statistically significant differences were detected between the two treatment groups for MGI, GBI or MQH measures. Results were similar for shielded interproximal sites. Both treatments were well-tolerated. (*Am J Dent* 2005;18:15A-17A).

**CLINICAL SIGNIFICANCE:** This randomized, controlled comparative clinical trial demonstrated that rinsing twice daily with the experimental alcohol-free 0.07% CPC rinse provides antiplaque and antigingivitis efficacy similar to that of the positive control EO rinse, a recognized antiplaque and antigingivitis mouthrinse that contains alcohol.

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## Introduction

Plaque-induced gingivitis is a common periodontal disease. The prevalence of gingivitis in adults exceeds 75% and in some populations approaches 100%.<sup>1</sup> One report involving a sample of approximately 15,000 subjects showed that gingivitis is present in about half the employed adult population in the United States.<sup>2</sup>

Many authors stress the importance of prevention, early diagnosis, and treatment of gingivitis in adults to prevent progression into advanced periodontal diseases.<sup>3</sup> Reports of an association between periodontal disease and some systemic diseases<sup>4-6</sup> further substantiate the need for prevention and treatment of early periodontal disease.

One of the primary causative factors in the development of gingivitis is inadequately controlled supragingival plaque.<sup>7</sup> While plaque can be controlled with proper daily hygiene, many patients find it difficult to comply. Inadequate flossing and inefficient brushing can lead to an accumulation of plaque and progress to gingivitis, particularly in areas that are difficult to access. A common strategy to supplement mechanical plaque removal is to incorporate a chemotherapeutic agent, such as an antibacterial mouthrinse, in the oral hygiene regimen.<sup>8</sup>

Conventional studies to establish the effects of antibacterial mouthrinses on gingival health following brushing are time-consuming and require large study populations. An alternative short-term clinical model was described by Loe *et al.*<sup>9</sup> In this experimental model, the development of gingival

inflammation is accelerated by eliminating the mechanical plaque removal caused by tooth brushing. This model was modified by Putt *et al.*<sup>10</sup> by incorporating a toothshield that is worn over one quadrant of the dentition, thereby avoiding the unpleasantness of abstention from toothbrushing for 3 weeks required by the original model. This experimental model was used to evaluate the safety and efficacy of a novel, 0.07% cetylpyridinium chloride rinse formulated in an alcohol-free delivery system (Crest Pro-Health Rinse<sup>a</sup>) *versus* a positive control rinse containing essential oils.

## Material and Methods

This was a randomized, double-blind, parallel groups, single-center study conducted at the University Park Research Center, Fort Wayne, Indiana. Both the research protocol and written informed consent were reviewed and approved by an institutional review board prior to study initiation. Four weeks before the baseline visit, 78 qualifying subjects received a prophylaxis and were instructed to brush twice daily in order to approach optimum gingival health. At the end of this 4-week period, subjects were randomized in equal numbers, balancing for baseline MGI and GBI, to one of the two treatment groups:

- Experimental cetylpyridinium chloride (CPC) rinse (Crest Pro-Health Rinse<sup>a</sup> - alcohol-free, high bioavailable 0.07% CPC)
- Essential oils (EO) rinse (Cool Mint Listerine<sup>b</sup> - 0.064% thymol, 0.092% eucalyptol, 0.060% methyl salicylate, 0.042% menthol, 21.6% alcohol).



Table 1. Modified Gingival Index.

Score	Description
0	Absence of inflammation.
1	Mild inflammation; slight change in color, little change in texture of any portion of but not the entire marginal or papillary gingival unit.
2	Mild inflammation; criteria as above but involving the entire marginal or papillary gingival unit.
3	Moderate inflammation; glazing, redness, edema, and/or hypertrophy of the marginal or papillary gingival unit.
4	Severe inflammation; marked redness, edema and/or hypertrophy of the marginal or papillary gingival unit, spontaneous bleeding, congestion or ulceration.

Efficacy and safety measurements were obtained at baseline and end-of-treatment (Day 21). Five contiguous teeth in either quadrant of the mandibular arch were shielded while brushing and served as the experimental gingivitis region. The following indices were measured.

- Modified Gingival Index (Table 1)
- Gingival Bleeding Index (Table 2)
- Modified Quigley-Hein Plaque Index (Table 3)

Safety was assessed by interview and clinical examination.

During the 3-week treatment phase, subjects wore the custom-made tooth shield over the experimental gingivitis region while brushing the remaining dentition twice daily for 60 seconds using a commercial dentifrice (Crest Cavity Protection<sup>®</sup>). Immediately following each brushing, subjects removed their toothshield, rinsed with water, and then rinsed with 20 ml of their randomly assigned test product for 30 seconds. Subjects were instructed to use their assigned products twice daily, allowing at least 4 hours between treatments. Subjects were also instructed to use only their assigned oral care product for the entire 3-week treatment period and were asked to abstain from all oral hygiene procedures, *e.g.* flossing, other than those performed as part of the study.

The average MGI, GBI and MQH scores of shielded teeth were calculated separately on a per-subject basis at Baseline and Day 21. The Day 21 means for each endpoint were modeled separately with analysis of covariance, using the respective baseline score as a covariate. All treatment comparisons were tested at the two-sided 5% significance level.

## Results

Of the 78 subjects enrolled in the trial, 67% were female, 96% were Caucasian and 4% were Black (Table 4). The mean (SD) age of the study population was 38.8 (9.95) years. Three subjects, all from the EO rinse group, were not evaluable for statistical analysis, none for safety-related reasons. The remaining 75 subjects completed the study and were included in all data analyses.

Treatments were well balanced with respect to all gingival health and plaque indices for the shielded sites at baseline (Table 4). With respect to MGI and GBI, the CPC rinse adjusted means at Day 21 were 3% (1.28 *vs.* 1.32) and 11% (0.39 *vs.* 0.44) smaller or in the direction of better gingival health than the EO rinse. With respect to plaque, the EO adjusted mean was 3% (3.02 *vs.* 3.12) different at Day 21 relative to the CPC rinse. None of these differences were statistically significant ( $P \geq 0.36$ ). Plaque and gingivitis results

Table 2. Gingival Bleeding Index as defined by Saxton & van der Ouderaa.<sup>11</sup>

Score	Description
0	Absence of bleeding after 30 seconds.
1	Bleeding observed after 30 seconds.
2	Immediate bleeding observed.

Scores recorded for buccal, mesial, and lingual sites, waiting approximately 30 seconds before scoring.

Table 3. Turesky Modification of Quigley-Hein Plaque Index.

Score	Description
0	No plaque.
1	Isolated areas of plaque at gingival margin.
2	Thin band of plaque at gingival margin ( $\leq 1$ mm).
3	Plaque covering up to 1/3 of tooth surface.
4	Plaque covering 1/3 to 2/3 of tooth surface.
5	Plaque covering $\geq 2/3$ of tooth surface.

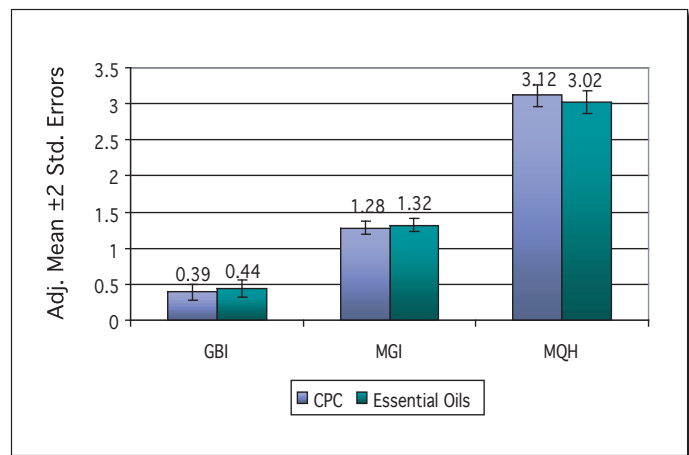


Fig. 1. Gingival health of shielded experimental sites at Day 21.

were similar when just interproximal sites from the experimental gingivitis region were analyzed (Table 5, Figure).

Both treatments were well-tolerated. No adverse, product related oral soft tissue changes were noted for either product at Day 21. Clinical examinations were generally unremarkable.

## Discussion

In this randomized, double-blind, experimental gingivitis study, an alcohol-free, 0.07% CPC rinse was not statistically different from the positive control EO rinse, an antiplaque and antigingivitis mouthrinse that has been granted the American Dental Association's Council on Scientific Affairs Seal of Acceptance as an adjunct for the prevention and reduction of gingivitis and plaque.<sup>12</sup> Results for interproximal sites were similar to overall results for all indices. No adverse events were observed with either product.

Subjects' gingival health and plaque levels in this study responded to the experimental gingivitis model generally consistent with that reported in the literature.<sup>13</sup> More specifically, both groups increased in mean MGI, GBI and MQH scores (gingival health worsened) during the 21-day treatment phase in which subjects did not brush the experimental gingivitis region. The relatively smaller increase in group mean plaque scores is understandable given that sub-

Table 4. Baseline demographics and gingival health characteristics for experimental sites.

Baseline characteristic	Demographic	CPC rinse (n=39)	Essential Oils rinse (n=39)	Overall (n=78)	Two-sided P-values
<b>Age (Years)</b>	Mean (SD)	37.6 (10.11)	39.9 (9.79)	38.8 (9.95)	0.30
	Min.–Max.	20–62	23–69	20–69	
<b>Modified Gingival Index</b>	Mean (SD)	1.00 (0.39)	1.00 (0.46)	1.00 (0.42)	1.00
<b>Gingival Bleeding Index</b>	Mean (SD)	0.22 (0.18)	0.19 (0.17)	0.21 (0.17)	0.37
<b>Modified Quigley-Hein Plaque Index</b>	Mean (SD)	2.81 (0.51)	2.86 (0.49)	2.84 (0.50)	0.65
<b>Sex</b>	Female	26 (67%)	26 (67%)	52 (67%)	1.00
	Male	13 (33%)	13 (33%)	26 (33%)	
<b>Ethnicity</b>	Black	1 (3%)	2 (5%)	3 (4%)	1.00
	Caucasian	38 (97%)	37 (95%)	75 (96%)	

Table 5. Efficacy analysis - Gingival and Plaque Indices for experimental sites at Day 21 (Analysis of Covariance).

Treatment group	N	MGI		GBI		MQH	
		Mean (SE)	P-value	Mean (SE)	P-value	Mean (SE)	P-value
<b>All experimental sites</b>							
CPC Rinse	39	1.28 (0.055)	0.65	0.39 (0.046)	0.41	3.12 (0.075)	0.36
EO Rinse	36	1.32 (0.057)		0.44 (0.048)		3.02 (0.078)	
<b>Interproximal experimental sites</b>							
CPC Rinse	39	1.38 (0.056)	0.38	0.38 (0.049)	0.84	3.20 (0.075)	0.21
EO Rinse	36	1.45 (0.058)		0.40 (0.051)		3.06 (0.078)	

jects arrived at the baseline visit without having brushed since the prior evening (Tables 4, 5).

Findings from this research demonstrate that the experimental CPC rinse has comparable antiplaque and antigingivitis activity to the positive control EO rinse when tested under the stringent conditions of no brushing. Results are consistent with other research showing that this particular CPC rinse provides a comparable antiplaque benefit to the EO mouthrinse when used as an adjunct to tooth brushing.<sup>14</sup>

Therapeutic rinses have become a common adjunct to oral hygiene regimens. The selection of an oral rinse is influenced by many factors, including product formulation and patient preference for esthetics. The majority of therapeutic rinses contain alcohol, which may not be desired for certain patient populations including children, patients of certain religious faiths, patients with xerostomia, recovering alcoholics, and others.<sup>15</sup> High levels of alcohol can also produce a burning sensation during use, a product attribute that may be unpleasant for some patients. The novel CPC rinse, designed for the broad population, may be particularly appealing to those patient groups seeking an antiplaque and antigingivitis rinse without alcohol or burn of alcohol.

In summary, this randomized, controlled, comparative clinical trial demonstrates that rinsing twice daily with an alcohol-free, 0.07% high bioavailable cetylpyridinium chloride rinse provides antiplaque and antigingivitis benefits comparable to a positive control mouthrinse.

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## Efficacy of a high bioavailable cetylpyridinium chloride mouthrinse over a 24-hour period: A plaque imaging study

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**ABSTRACT:** *Purpose:* To evaluate the antiplaque benefits of a 0.07% high bioavailable, alcohol-free cetylpyridinium chloride (CPC) rinse used after toothbrushing *versus* toothbrushing alone. *Methods:* A digital plaque image analysis technique was used to quantify *in situ* plaque formation in a subject population carrying out modified hygiene using standard fluoridated dentifrice or standard dentifrice augmented with 30 seconds mouthrinsing with an alcohol-free mouthrinse containing 700 ppm CPC. *Results:* Comparison of plaque formation 24 hours following “last hygiene” revealed that brushing followed by CPC mouthrinse use provided a statistically significant decrease in plaque coverage on teeth averaging 42% as compared with brushing only. Moreover, toothbrushing with a standard dentifrice in the morning resulted in 34% less plaque when subjects used the CPC mouthrinse 24 hours prior to examination. (*Am J Dent* 2005;18:18A-23A).

**CLINICAL SIGNIFICANCE:** These results support the strong retention and lasting antiplaque efficacy of high bioavailable CPC mouthrinse and suggest that the plaque biofilms formed during CPC use are susceptible to more efficient debridement.

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### Introduction

Gingivitis and periodontitis are the direct result of microbial dental plaque infection at the gingival tooth interface initiating a host response.<sup>1-3</sup> While specific microbial populations and pathogenicity have been correlated with disease processes, the generalized non-specific correlation between dental plaque and soft tissue disease is supported by the development of inflammation and bleeding in “experimental gingivitis” patients coupled with rapid resolution of disease upon reinstatement of hygiene measures.<sup>4-5</sup> Although frequent and effective hygiene presents a proven and effective route to the maintenance of soft tissue health, patients often do not exhibit the motivation, skill or discipline for adequate plaque control.<sup>6-7</sup> As a result, gingivitis prevalence remains high, even among educated patients who visit the dentist routinely.<sup>8-9</sup>

While we find that most patients find it very difficult to completely control plaque through hygiene alone, virtually all patients routinely use commercial dentifrices as part of their attempts toward a daily hygiene regimen and increasing numbers use mouthrinses as well. Due to their frequent application, dentifrices and mouthrinses therefore represent opportunistic vehicles in which to provide chemotherapeutic benefits of topical antimicrobials. The formulation of topical antimicrobial ingredients into dentifrice and rinse formulations has been the focus of significant academic and industry sponsored research. However, only a handful of antimicrobial ingredients have found their way into consumer products. Ingredients used in commercial forms today include chlorhexidine, triclosan, mixtures of essential oils and various metal salts including zinc compounds and stannous fluoride.<sup>9-14</sup> As a general rule, formulations containing these ingredients exhibit some modicum of efficacy in the control of dental plaque and plaque-induced gingival inflammation and bleeding. However, the effectiveness of these formulations is

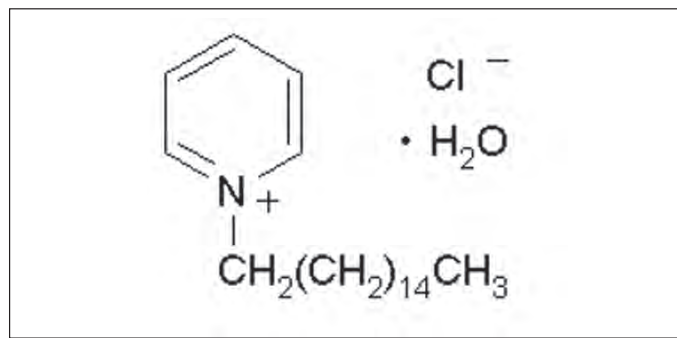


Fig. 1. Cetylpyridinium chloride structure

highly variable and would appear to be influenced by a variety of important factors including a combined spectrum of actions including:<sup>15</sup>

- Antimicrobial efficacy – preferably broad spectrum
- Adequate bioavailability – defined by plaque penetration and reactivity
- Demonstration of retained antimicrobial effects – that is lasting antiplaque antimicrobial actions following use.

Cetylpyridinium chloride (CPC) is a broad spectrum antibacterial ingredient with widespread use in oral care products.<sup>16,17</sup> The molecular structure of CPC, shown in Fig. 1, permits dual retention in the oral environment as both surfactant chains and cationic charges may adsorb to intraoral surfaces which are both lipophilic and anionic.

Like most antimicrobials, CPC may provide a spectrum of efficacy depending upon formulation excipients. For an ingredient like CPC it is vital to formulate with consideration for deactivation by anionic or hydrophobic dispersants, flavors or surfactants.<sup>18</sup>

Crest Pro-Health Rinse<sup>a</sup> is a therapeutic mouthrinse that provides antiplaque and antigingivitis efficacy when used as a

twice daily adjunct to regular toothbrushing. Crest Pro-Health Rinse utilizes CPC in an alcohol-free yet highly bioavailable formulation as its active antiplaque and antigingivitis ingredient. In this study, the chemotherapeutic antiplaque efficacy of Crest Pro-Health Rinse was evaluated in a modified brushing regimen model – with the specific intention to see if antiplaque efficacy of the mouthrinse extended to 24 hours post use.

### Methods and Materials

*Test formulations* - The dentifrice formulation used in the present study included commercial Crest Cavity Protection Dentifrice<sup>a</sup> (0.243% NaF in silica base) supplied in commercial packaging. The rinse formulation used in the present study included a 700 ppm CPC mouthrinse (Crest Pro-Health Rinse) mouthrinse. Toothbrushes used in the study included Oral-B 40<sup>b</sup> brushes for home use and disposable brushes for supervised brushing.

*Study design* - This study employed a sequential, non-randomized, treatment intervention design, where a baseline period of Crest Cavity Protection dentifrice utilization (Period A) was followed by 1 week of continued Crest Cavity Protection brushing with the addition of mouthrinsing (Period B). Study logistics are highlighted below. A key variation in this study design included modified hygiene logistics on three separate graded days, to permit a determination of antiplaque activity at a 24-hour time period following the last oral hygiene application.

*Washout – Pre-study A* - Rigorous hygiene day, Crest Cavity Protection use for 2 weeks to obtain equilibrium: *ie.* wash out brushing period.

*Period A* – 1-week Crest Cavity Protection use – Modified hygiene on selected days to produce 24-hour plaque regrowth.

*Period B* – 1-week Crest Pro-Health Rinse use with Crest Cavity Protection dentifrice – Modified hygiene on selected days to produce 24-hour plaque regrowth.

*Subject selection* - Subjects entering the protocol were part of a pre-qualified team panel at the Health Care Research Center in Mason, Ohio, who participated in part or wholly in previous plaque testing. These subjects were in excellent general health and oral health and did not take medications expected to influence the outcome of the study. Subjects agreed to use only their assigned oral products during the course of the study. Subjects in the panel characteristically were pre-screened to exhibit reproducible “off treatment” plaque levels on teeth both before and after hygiene applications and are trained/interested in new protocols and oral hygiene techniques. Subjects signed informed consents. The digital plaque imaging analysis protocol was reviewed and approved by the Institutional Ethics-Review Board.

*Pre-study rigorous hygiene and wash out regular brushing period* - All subjects projected for study participation entered the dental clinic at the start of the trial to participate in a “rigorous hygiene clean up”. The purpose of this clean up is

to provide a rigorous oral hygiene intervention of subjects participating in plaque growth and removal evaluations in the Digital Plaque Imaging Analysis Repeated Measures (DPIARM) protocol, rendering subjects essentially plaque free *via* visible grading of facial surfaces. Subjects so cleaned can be stratified into groups for study of antiplaque or cleaning formulations. Upon entry into DPIARM protocol, subjects were provided with a personal hygiene kit including disposable toothbrushes, dental picks, and dental floss. These were personally used in rigorous self oral hygiene procedures. Subjects presented to the dental clinic in our laboratory. They were provided with a fresh disposable toothbrush. Subjects were instructed to add a measured amount (1.5 gm) of high cleaning Ultrabrite<sup>c</sup> dentifrice and to brush their teeth for 2 full minutes – using a laboratory timer. Following brushing, subjects were instructed to rinse their mouths with tap water. Subjects were then asked to floss their entire dentition with dental floss available to each subject. They were also permitted the opportunity to use dental picks for any large spaces between teeth. Lastly subjects were asked to brush a final time with Ultrabrite dentifrice (a strong polisher/ cleaner) for 1 minute and rinse with water. Subjects used a dental mirror and disclosure tablets to verify a clean dentition which was also checked by the study monitor. Following this, subjects were provided with Crest Cavity Protection toothpaste (a standard dentifrice control) and an Oral-B 40 toothbrush. In this pre-conditioning phase, subjects were instructed to brush twice per day as they normally do for 2 weeks prior to initiation of the study (normal brushing sequence for these subjects includes typically morning and evening.) Plaque levels were not assessed in the pre-study A period.

*Period A* - Subjects from the pre-study A period reported to the dental clinic for product distribution but did not participate in another “rigorous hygiene clean up”. Subjects were provided with a soft Oral-B 40 toothbrush and new instructions. They were instructed to brush as they normally did twice per day through Saturday. During the remaining week, subjects were to be graded on three separate occasions (Monday, Wednesday and Friday). The Monday/Wednesday/Friday grading required modified hygiene behavior on Sunday, Tuesday and Thursday of the graded week. On Sunday, Tuesday and Thursday, subjects brushed normally in the morning, however they were asked to refrain from all hygiene the remainder of the day and evening – *e.g.* no pre-bed brushing or after dinner brushing. Subjects were then instructed to report to the imaging laboratory on Monday, Wednesday and Friday mornings prior to any food/beverage consumption and without oral hygiene. There, subjects disclosed dental plaque and carried out a “pre-brush a.m. plaque imaging”, after which subjects underwent a timed brushing for 40 seconds with assigned dentifrice provided in metered 1.5 gram doses using a disposable brush. Following brushing, subjects redisclosed dental plaque and subjected themselves to a second plaque imaging, a so-called “a.m. post-brushing plaque imaging”. Following rinsing of dentition of disclosing solution, subjects were free to have breakfast and lunch, as well as snacks, *etc.* throughout the grading day. All toothbrushing in this period included Crest Regular dentifrice only.

*Period B* - Following Period A, subjects reentered the dental panel clinic for a new, and additive product assignment. Subjects again did not participate in another “rigorous hygiene clean up”. Subjects were provided with a new tube of dentifrice and a blank labeled bottle of 0.07% CPC mouthrinse respectively. As in the Period A protocol, subjects were instructed to brush as they normally did, twice per day, with the evening brushing taking place right before they retired for the evening at the start. In all cases of mouthrinse use the following protocol was used. The mouthrinse took place directly following scheduled toothbrushing, following water rinses as directed in product labeling and previous clinical studies. Following brushing and water rinsing, subjects were asked to dispense roughly 20 ml of mouthrinse into their assigned dose cup, and rinse for 30 seconds (timer provided). Following evening rinsing, subjects were asked to expectorate mouthrinse, and not rinse with further water, eat or drink prior to retiring for the evening. In morning use, subjects were instructed not to eat or drink for 30 minutes following rinse applications.

Following the mouthrinse allocation, subjects were given these new hygiene instructions. Again, product use was affected by grading days for the plaque. During the week of rinse applications, subjects were graded on three separate occasions (Monday/Wednesday/Friday). The Monday/Wednesday/Friday grading required modified hygiene behavior on Sunday, Tuesday and Thursday of the graded week. On Sunday, Tuesday and Thursday subjects brushed and rinsed with assigned mouthrinse following use directions in the morning; however they were asked to refrain from all hygiene the remainder of the day and evening – *e.g.* no pre-bed brushing/rinsing or after dinner brushing/rinsing. Subjects reported to the imaging laboratory on Monday, Wednesday and Friday mornings prior to any food/beverage consumption and without oral hygiene. Subjects disclosed dental plaque and subjected themselves to a “pre-brush a.m. plaque imaging”, after which subjects underwent a timed brushing for 40 seconds with assigned dentifrice provided in metered 1.5 gram doses using a disposable brush. Following brushing, subjects redisclosed dental plaque and subjected themselves to a second plaque imaging, a so-called “a.m. post-brushing plaque imaging”. Following the post-brush plaque grading, subjects were asked to rinse 3 times more with plaque phosphate buffer rinse solution and 3 times more with water to attempt to wash out any residual fluorescein. Subjects then rinsed with their assigned rinse solution. Subjects were asked to refrain from eating/drinking (no coffee, *etc.*) for 30 minutes further. Following this, subjects were free to have breakfast and lunch, as well as snacks *etc.* throughout the grading day. Period B treatments occurred over 1 week providing three repeat measures of plaque formation.

*Evaluation parameters* - Dental plaque coverage was analyzed by a standardized digital imaging protocol previously described in detail.<sup>19</sup> Digital imaging included the capture of UV images of disclosed plaque in subjects. Plaque was illuminated using two Balcar long wave UV flashes (model FX60<sup>d</sup>) equipped with cutoff filters at 265 nm. The flashes

were powered by two Balcar 2400 power packs<sup>d</sup> and controlled by the computer image capture system. The flashes were positioned at 45° angles to the subject to reduce reflection to a minimum. To protect the subject’s eyes, UV filtering glasses were worn on imaging or, for experienced subjects, instructions were given to close the eyes during image capture. UV images were captured with a Fuji 1000 CCD camera<sup>e</sup> controlled by a desktop computer. For plaque measures, subjects sat in front of the camera and positioned their facial dentition in a specialized chin rest, at an identical distance of 45.5 cm from the image capture camera. Lip retractors were used by the subjects, who, after training, could position themselves for imaging to allow uniform lighting of the teeth and to capture unobstructed images. To ensure good subject positioning, a live image was displayed on a positioning monitor. Once a good position was established, a UV image was captured and a reference position image was stored according to a subject identification number. At subsequent visits, the stored positioning images could be used to realign the subjects to the same position if necessary. Repositioning in this instance was accomplished by overlaying a live image on top of the stored image. This was accomplished with a video blending system available on custom software in the Health Care Research Center laboratories.

The imaging system was calibrated using standard Munsell color charts with RGB values corrected (< 5%) for system stability in standardized assessments. Captured images were analyzed and classified with Optimas R macros. Discriminant analysis was used to statistically classify pixels into different anatomical categories, *e.g.* teeth, gums, plaque on gums, clean teeth, clean gums, *etc.* Total image pixels were collected and assigned to respective designations. The most reproducible analysis typically includes the ratio of plaque on teeth pixels to total teeth pixels (clean teeth + plaque covered teeth) thereby representing an “area” coverage estimate for each image and this analysis was carried out herein. The computer designated plaque area is used to calculate a percentage dentition coverage estimate for the facial plaque image, which is then compared for treatment effects. It is noteworthy that operator/analyst contribution to study measures only takes place in standardization of custom analysis rules for pixel classifications of “plaque covered tooth” or “plaque free tooth” *etc.* Once quantitative decision rules are established, plaque assessment is quantitatively managed by the computer decision rule selection grid, and no subjective clinician grading is involved. In this context then, the digital plaque image analysis (DPIA) is operator independent and no subjective clinician effects are permitted or expected.

Plaque disclosure for imaging utilized fluorescein buffer solution containing 1800 ppm fluorescein. Prior to photographing, subject plaque is disclosed by fluorescein as follows:

- Rinse for 10 seconds with 25 ml of phosphate buffer;
- Rinse for 1 minute with 5.0 ml of 1800 ppm fluorescein in phosphate buffer; and
- Rinse 3 x 10 seconds with 25 ml of phosphate buffer.

Table 1. Pre-brushing 24-hour plaque evaluations.

Treatment	Plaque coverage % (SD)	% Decrease in plaque coverage	Two-sided P-value*
Dentifrice	18.83 (7.36)	--	--
Dentifrice + 0.07% CPC mouthrinse	10.89 (5.86)	42	<0.0001

\* Paired-difference *t*-test P-value.

Table 2. Post-brushing plaque evaluations.

Treatment	Plaque coverage % (SD)	% Decrease in plaque coverage	Two-sided P-value
Dentifrice	7.61 (5.22)	--	--
Dentifrice + 0.07% CPC Mouthrinse	5.00 (2.71)	34	0.0092

\* Paired-difference *t*-test P-value.

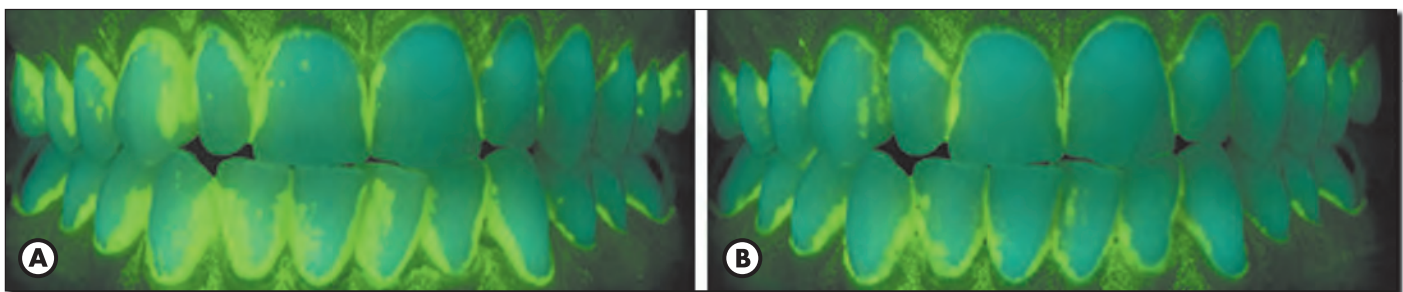


Fig. 2. Images of 24-hour plaque coverage in same subject using: **A.** Crest Regular Cavity Protection dentifrice only; and **B.** Crest Regular Cavity Protection dentifrice and adjunctive use of cetylpyridinium chloride mouthrinse.

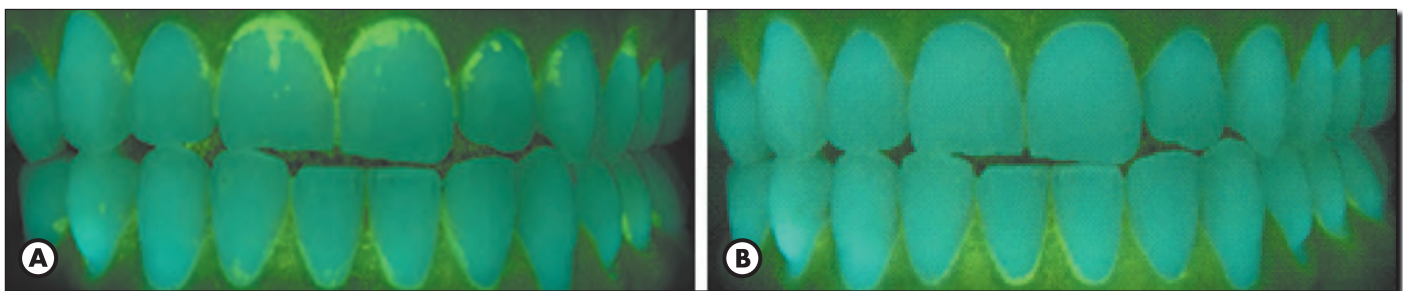


Fig. 3. Images of post-brushing plaque coverage in same subject using: **A.** Crest Regular Cavity Protection dentifrice; and **B.** Crest Regular Cavity Protection dentifrice and adjunctive use of cetylpyridinium chloride mouthrinse.

Phosphate buffer was comprised of 3.62 grams of monosodium phosphate and 0.349 grams of disodium phosphate diluted to 2 liters with ultrapure water. The final pH of this mixture is 5.5. The solution was prepared fresh each day.

*Statistical analysis* - The prebrushing percent plaque coverage scores for Monday, Wednesday and Friday mornings were averaged on a per-subject basis separately within Period A (Crest Cavity Protection dentifrice) and Period B (Crest Cavity Protection dentifrice + 0.07% CPC mouthrinse). Postbrushing scores were averaged similarly. These average plaque scores were then analyzed using a paired-difference *t*-test to compare treatments.

### Results

Sixteen subjects completed the full testing regimen. No complaints of oral discomfort or treatment related oral side effects were reported during the testing. Results of plaque

evaluations at the 24-hour plaque regrowth assessment point are shown in Table 1. Use of the 0.07% CPC mouthrinse provided a 42% statistically significant reduction in plaque coverage in mornings following a 24-hour plaque regrowth.

Results of plaque evaluations post-brushing (on the evaluation morning) are shown in Table 2. During use of the 0.07% CPC mouthrinse, subjects in the morning exhibited a 34% statistically significant reduction in plaque post-brushing. The plaque removed by tooth brushing averaged over 40% during mouthrinse use and 70% for subjects just using Crest Cavity Protection dentifrice. Naturally, the subjects had less plaque to remove when using the combined Crest Cavity Protection dentifrice with the additional mouthrinse use.

Visualization of images from both pre- and post-brushing measures are shown in Fig. 2 for the same panelists. As illustrated, the adjunctive use of 0.07% CPC mouthrinse produced clear reductions in dental plaque coverage at 24-hour plaque regrowth and post-brushing endpoints (Fig. 3).

## Discussion

Dental plaque formation is associated with the development of caries, gingivitis and periodontitis.<sup>3</sup> With respect to gingival disease, the literature reveals that scrupulous hygiene measures are necessary to effect full reductions in gingival disease. Antimicrobial therapies represent a useful adjunct to hygiene measures to produce clinically meaningful reductions in plaque and gingivitis. Antibacterials may reduce gingivitis through generalized reductions in plaque coverage; thinner plaques appear less virulent by suppressing the expression of specific bacterial metabolic products which may act as virulence factors or by suppressing growth of bacterial strains themselves that produce gingival immune response.<sup>20,21</sup>

This study used a digital imaging method to quantify plaque coverage on the teeth. With the advent of modern imaging processing techniques, computerized digital imaging is proving increasingly valuable in clinical applications including plaque assessments.<sup>19,22-28</sup> The technique applied in our laboratories is advanced in using a color determinant rule for plaque evaluation eliminating any operator or grader contribution to plaque estimation.<sup>19</sup> The technique is particularly valuable in the measurement of diurnal and time dependent actions of treatments/regimens on plaque formation since the imaging procedure does not disturb plaque growth or inhibition.

Cetylpyridinium chloride has been used for a number of years in cosmetic breath protection formulations, for example including the commercial Scope<sup>a</sup> mouthrinse. CPC was reviewed by a subcommittee of the Food and Drug Administration and recommended as safe and effective for the control of gingivitis and plaque in mouthrinses formulated in a high bioavailable matrix.<sup>29</sup> In the present study, a mouthrinse containing 700 ppm CPC demonstrated efficacy in the reduction of dental plaque formation for a period up to 24 hours following the last toothbrushing and rinsing in a modified hygiene protocol. The provision of plaque benefits for extended periods post-brushing is a valuable attribute for effective topical antimicrobials accounting for flexibility in habit variations in patients. The strength and duration of antiplaque benefits seen for the CPC mouthrinse in this study are consistent with soft tissue benefits observed with this ingredient and mouthrinse formulations in controlled clinical studies.<sup>30-32</sup>

- a. The Procter & Gamble Co., Cincinnati, OH, USA.
- b. Oral-B Consumer Services, South Boston, MA, USA.
- c. Colgate Palmolive, New York, NY, USA.
- d. Balcar, Torcy, France.
- e. Fuji Photo Film Co., Tokyo, Japan.

Ms. Kozak is a Principal Researcher, Dr. Gibb is a Statistician, Mr. Dunavent is a Statistical Analyst and Dr. White is Senior Research Fellow, at the Procter & Gamble Health Care Research Center, Mason, Ohio, USA.

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## A 6-month clinical study assessing the safety and efficacy of two cetylpyridinium chloride mouthrinses

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**ABSTRACT: Purpose:** To evaluate the effects of two experimental cetylpyridinium chloride (CPC) mouthrinses containing 0.075% and 0.10% CPC on the development of gingivitis and plaque *versus* a placebo control over a period of 6 months. **Methods:** This was a randomized, single center, parallel group, double blind, positive and placebo controlled clinical trial. A 0.12% chlorhexidine rinse served as the positive control for validation of the methodology. At the beginning of the trial, 366 subjects were balanced and randomly assigned to treatment groups. Subjects received a dental prophylaxis and began rinsing twice a day with 15 ml of their assigned mouthwash for 30 seconds after brushing their teeth. Subjects were assessed for gingivitis and gingival bleeding by the Löe-Silness Gingival Index method and plaque by the Turesky modification of Quigley Hein Plaque Index at baseline and after 3 and 6 months of product use. Oral soft tissue health was also assessed. **Results:** After 3 and 6 months, subjects rinsing with either 0.075% or 0.10% CPC had significantly ( $P < 0.0001$ ) less gingivitis, gingival bleeding, and plaque, on average, than those on placebo. The 6-month mean reductions in gingivitis, gingival bleeding, and plaque for the 0.075% and 0.10% CPC rinses *versus* placebo were 23%, 30% and 17%, and 20%, 27% and 19%, respectively. There was no statistically significant difference in efficacy between the two CPC mouthrinses. Reductions at 3 months were similar to those seen at 6 months. Significant benefits were observed with chlorhexidine, thereby validating the study. (*Am J Dent* 2005;18: 24A-28A).

**CLINICAL SIGNIFICANCE:** This study clearly demonstrates that CPC mouthrinses formulated to deliver therapeutic benefits when used twice daily can significantly prevent the development of gingivitis, gingival bleeding, and plaque over a 6-month period.

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### Introduction

Good oral hygiene is the most pressing oral health issue among patients, according to a recent survey of dentists.<sup>1</sup> Poor dental hygiene can lead to an accumulation of bacteria in the biofilm, which can lead to gingivitis and potentially progress to periodontitis.<sup>2</sup> Given the widespread occurrence of periodontal diseases among the general population,<sup>3</sup> incorporating antimicrobial products into patients' oral care regimen is a logical approach to enhance plaque removal achieved through brushing and flossing.

Various chemotherapeutic agents have been added to oral care products for years to augment mechanical plaque removal.<sup>4</sup> Only three agents, however, were classified as safe and efficacious for the treatment of plaque-induced gingivitis by the FDA Plaque Subcommittee following a 6-year review of over 40 active agents. These active ingredients include 0.45% stannous fluoride in a compatible dentifrice formulation, essential oils in a hydroalcoholic mouthrinse vehicle containing 21.6% to 26.9% alcohol, and cetylpyridinium chloride (CPC), when formulated in a mouthrinse within a concentration range of 0.045% to 0.10% high bioavailable CPC.<sup>5</sup>

The current study evaluated the antigingivitis and anti-plaque efficacy of two experimental mouthrinse formulations containing 0.075% and 0.10% cetylpyridinium chloride in a high bioavailable matrix *versus* a placebo CPC rinse after 3 and 6 months of use. A positive control chlorhexidine rinse was included in the study for validation purposes.

### Materials and Methods

This was a randomized, single center, parallel group,

double blind, 6-month clinical trial. Subjects who signed the informed consent and medical history forms and met preliminary entrance criteria were examined at baseline. Primary efficacy and safety measures included the Löe-Silness Gingival Index (GI) to measure gingival inflammation and bleeding, the Turesky modification of Quigley Hein Plaque Index (PI) and Oral Soft Tissue (OST) examinations. Subjects who did not meet the entrance exam for gingivitis and plaque were excluded. Approximately 1 week following the baseline examination, qualifying subjects received a thorough dental prophylaxis. Subjects were randomly assigned to the following four treatments, balancing for gender and baseline mean GI score: 0.075% CPC rinse, 0.10% CPC rinse, CPC placebo rinse, or 0.12% chlorhexidine rinse. The randomization was performed such that the sample size per group ratio was 2:2:2:1, with 1 representing the 0.12% chlorhexidine rinse group.

From Monday to Friday, subjects came to the clinical site each morning for supervised brushing and rinsing. Subjects were instructed to brush with a 0.243% sodium fluoride toothpaste (Crest Cavity Protection<sup>a</sup>) using a disposable Anchor<sup>b</sup> toothbrush, rinse with water, then rinse with 15 ml of product for 30 seconds. Subjects were instructed to follow the same instructions in the evening and on the weekends. Reexamination of subjects for the efficacy and safety parameters was conducted after 3 and 6 months of product usage (Fig. 1).

**Subject population** - A total of 366 subjects were enrolled in the study. To participate in the study, subjects had to give written informed consent, be at least 18 years of age, have a minimum of six gradable natural teeth with four molars, and

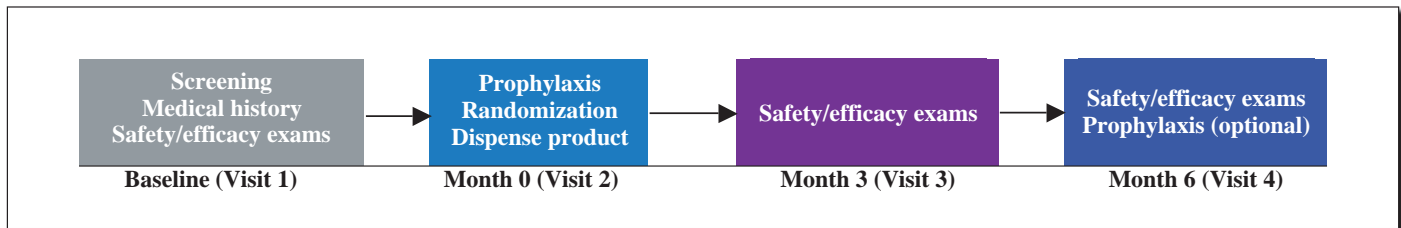


Fig. 1. Study design.

Table 1. Study population demographics of subjects completing examinations.

Visit/statistic	Placebo	0.075% CPC	0.10% CPC	Chlorhexidine
<b>Baseline*</b>				
Sample size	102	97	103	49
% Female	38%	37%	41%	40%
Mean Age (Range)	34.3 (18-66)	33.7 (18-59)	33.8 (18-57)	32.9 (18-53)
<b>Month 3</b>				
Sample size	87	79	86	36
% Female	43%	36%	46%	44%
Mean Age (Range)	34.7 (18-66)	34.2 (18-58)	33.8 (18-57)	34.3 (18-53)
<b>Month 6</b>				
Sample size	86	82	90	40
% Female	39%	41%	43%	38%
Mean Age (Range)	34.3 (18-66)	34.1 (18-59)	33.5 (18-57)	34.5 (18-53)

\* There was no significant difference (P> 0.10) between groups with respect to gender or age at baseline.

be in good general health. Subjects also had to have a baseline whole mouth average GI score  $\geq 0.50$ , at least 10 bleeding sites and a baseline plaque score  $\leq 3.0$ .

Patients were ineligible to continue in the study for the following reasons: participation in any other dental study; use of other oral care products; evidence of rampant caries, obvious periodontal disease, chronic neglect requiring urgent treatment, history of any medical diseases that may interfere with study (e.g., bleeding tendencies, infectious diseases); wearing removable or fixed orthodontic devices; use of antibiotics or immunosuppressives within 1 week prior to baseline, 3- or 6-month exams; use of anti-inflammatory drugs or analgesics within 48 hours of the baseline, 3- or 6-month exam; known hypersensitivity to chlorhexidine or tartrazine; oral prophylaxis outside of study; use of oral chlorhexidine products or participation in an oral rinse study within 3 months prior to baseline examination; pregnancy/nursing; noncompliance by missing more than five consecutive supervised rinses and/or more than 15% of all supervised rinses.

**Test materials** - The following test materials were used:

- 0.075% CPC rinse<sup>a</sup>
- 0.10% CPC rinse<sup>a</sup>
- CPC placebo rinse<sup>a</sup> (negative control)
- 0.12% chlorhexidine rinse (Peridex<sup>c</sup>, positive control).

All test formulations were supplied by Procter & Gamble.<sup>a</sup> The CPC mouthrinses were formulated to pass proposed performance assays by the FDA for over-the-counter (OTC) CPC mouthrinses. Subjects were supplied with two 16-ounce bottles of test product (all mouthrinses were packed in identical amber bottles), a 4.6 ounce tube of dentifrice in a

plain white tube, two toothbrushes and a 30-second timer. Subjects were resupplied with two additional bottles of mouthrinse, a new tube of toothpaste and two new toothbrushes each month. Unused mouthrinse was returned at the end of each month.

**Clinical assessment** - Subjects were instructed to not brush the morning of the Baseline, Month 3 or Month 6 examinations. Gingivitis, gingival bleeding, and plaque were the primary efficacy measures. Gingivitis and gingival bleeding were measured by the Löe-Silness Gingival Index<sup>6</sup> using the following criteria:

- 0 Normal gingiva.
- 1 Mild inflammation: Slight change in color, slight edema; no bleeding on probing.
- 2 Moderate inflammation: Redness, edema, and glazing; bleeding upon probing.
- 3 Severe inflammation: Marked redness and edema; ulceration; tendency to spontaneous bleeding.

Sites with GI scores of 2 or 3 were counted as bleeding. Plaque was measured using the Turesky modification of Quigley Hein Plaque Index with the following criteria:<sup>7,8</sup>

- 0 No plaque.
- 1 Isolated areas of plaque at gingival margin.
- 2 Thin band of plaque at gingival margin (< 1mm).
- 3 Plaque covering up to 1/3 of tooth surface.
- 4 Plaque covering 1/3 to 2/3 of tooth surface.
- 5 Plaque covering > 2/3 of tooth surface.

In addition to gingivitis and plaque examinations, assessment of the oral soft tissue (OST) was conducted at Baseline, Month 3 and Month 6 via a visual examination of the oral cavity and perioral area utilizing a standard dental light, dental mirror, and gauze.

**Statistical analysis methods** - A whole mouth average score for GI and PI was calculated separately for each subject at Baseline, Month 3 and Month 6 by taking the respective sum of scores at all sites and dividing by the total number of sites graded. A whole mouth total gingival bleeding score was also calculated for each subject at Baseline, Month 3 and Month 6 by summing the number of bleeding sites.

The gingivitis and plaque data were analyzed with analysis of covariance (ANCOVA). A separate ANCOVA model was fitted for each endpoint (GI, bleeding, PI) at Month 3 and Month 6 using the respective baseline score as the model covariate. In each case, an initial efficacy comparison was made between the positive control and CPC placebo groups. If this comparison achieved statistical significance (P $\leq$  0.05) the study methodology was considered validated and all pairwise comparisons between the CPC placebo, 0.075% CPC rinse and 0.10% CPC rinse groups were then made.

Table 2. Baseline gingivitis and plaque.

Endpoint/Statistic	Placebo	0.075% CPC	0.10% CPC	Chlorhexidine
<b>Gingival Index*</b>				
Number of subjects	102	97	103	49
Mean (SE)	0.814 (0.020)	0.792 (0.018)	0.800 (0.018)	0.794 (0.025)
<b>Number of bleeding sites*</b>				
Number of subjects	102	97	103	49
Mean (SE)	20.2 (1.27)	18.6 (0.90)	19.9 (0.97)	18.6 (1.04)
<b>Plaque*</b>				
Number of subjects	101	96	103	49
Mean (SE)	2.11 (0.044)	2.15 (0.039)	2.10 (0.039)	2.03 (0.056)

\* There was no statistically significant difference in the means between treatment groups.

Table 3. Month 3 Efficacy analysis results. Analysis of covariance.

Endpoint/Statistic	Placebo	0.075% CPC	0.10% CPC	Chlorhexidine
<b>Gingival Index*</b>				
Number of subjects	87	79	86	36
Adjusted mean (SE)	0.698 (0.015)	0.541 (0.015)	0.542 (0.016)	0.453 (0.024)
% Difference vs. placebo	N/A	23%	22%	35%
<b>Number of bleeding sites*</b>				
Number of subjects	87	79	86	36
Adjusted mean (SE)	15.5 (0.59)	11.0 (0.62)	9.6 (0.59)	7.5 (0.91)
% Difference vs. placebo	N/A	29%	38%	52%
<b>Plaque*</b>				
Number of subjects	86	78	86	36
Adjusted mean (SE)	1.95 (0.059)	1.53 (0.052)	1.47 (0.050)	1.29 (0.077)
% Difference vs. placebo	N/A	22%	25%	34%

\* In all cases the Chlorhexidine and CPC test groups means were highly significantly ( $P < 0.0001$ ) lower than the placebo group mean.

Table 4. Month 6 efficacy analysis results. Analysis of covariance.

Endpoint/Statistic	Placebo	0.075% CPC	0.10% CPC	Chlorhexidine
<b>Gingival Index*</b>				
Number of subjects	86	82	90	40
Adjusted mean (SE)	0.683 (0.016)	0.526 (0.017)	0.548 (0.016)	0.459 (0.024)
% Difference vs. placebo	N/A	23%	20%	33%
<b>Number of bleeding sites*</b>				
Number of subjects	86	82	90	40
Adjusted mean (SE)	15.9 (0.66)	11.1 (0.68)	11.6 (0.65)	8.8 (0.97)
% Difference vs. placebo	N/A	30%	27%	45%
<b>Plaque*</b>				
Number of subjects	85	82	90	40
Adjusted mean (SE)	1.97 (0.050)	1.63 (0.051)	1.60 (0.049)	1.35 (0.074)
% Difference vs. placebo	N/A	17%	19%	31%

\* In all cases the chlorhexidine and CPC test groups means were highly significantly ( $P < 0.0001$ ) lower than the placebo group mean.

The OST data from the Baseline, Month 3 and Month 6 examinations were also statistically analyzed. The analyses were performed pairwise for each test group as compared to the CPC placebo group. The adverse event data were analyzed using either Fisher's Exact Test, Mann-Whitney U Test or by chi-squared.

## Results

**Study population** - Three hundred and sixty-six subjects were enrolled in the study, all of whom were included in the analyses of safety data throughout the study. Subjects were generally well-balanced at Baseline with regard to gender, age, gingival health and plaque (Tables 1 and 2). Fifteen subjects who entered the study in protocol violation (primarily NSAID use) were excluded from all efficacy analyses throughout the study. Two hundred and eighty-eight and 298 subjects were included in the Month 3 and Month 6

efficacy analyses, respectively. Reasons for exclusion from the 3-month analyses included: medication use outside the study protocol (41), patient not available (10), noncompliance (5), adverse event (5), or non-study related medical reasons (2). The number of subjects excluded from the 6-month efficacy analyses for these reasons was 17, 17, 5, 8 and 6, respectively.

**Study design validation** - Results for Month 3 and Month 6 examinations showed that subjects in the chlorhexidine group showed significantly ( $P < 0.0001$ ) less gingivitis, gingival bleeding and plaque relative to the CPC placebo group, thereby validating the study methodology (Tables 3 and 4, Figs. 2-4). At Month 6 the chlorhexidine rinse showed benefits of 33%, 45%, and 31% relative to the placebo for these measures, respectively.

**Gingivitis results** - Both CPC treatment groups had signifi-

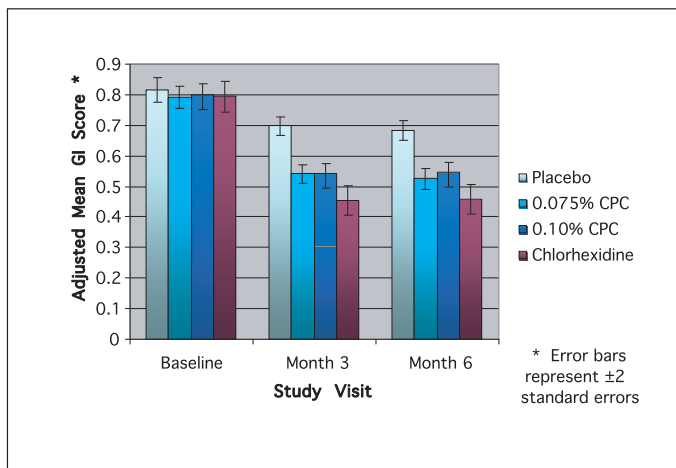


Fig. 2. Gingivitis results for evaluable subjects.

cantly ( $P < 0.0001$ ) lower mean gingivitis scores at Month 3 and Month 6 relative to the placebo group. At Month 6 the adjusted mean gingivitis scores for the 0.075% CPC and 0.10% CPC groups were 23% and 20% lower, respectively, than for the placebo group. Month 3 results showed similar CPC benefits with reductions of 23% and 22%, respectively. No significant differences were found between the two CPC treatment groups at Month 3 or Month 6 (Tables 3, 4; Fig. 2).

**Gingival bleeding** - Results for gingival bleeding were similar to those for gingivitis. The 0.075% CPC and 0.10% CPC treatment groups had statistically significantly ( $P < 0.0001$ ) fewer bleeding sites at Month 3 and Month 6 relative to the placebo. At Month 6 there were an average 11.1 and 11.6 GI bleeding sites in the 0.075% CPC and 0.10% CPC groups, respectively, compared to an average 15.9 GI bleeding sites in the placebo group. This represents an average bleeding site reduction of 30% and 27%, respectively. Bleeding reductions for the CPC groups at Month 3 ranged from 29% to 38% (Tables 3, 4; Fig.3).

**Plaque** - The mean plaque scores in both CPC treatment groups were highly significantly ( $P < 0.0001$ ) lower than the mean plaque score in the placebo group at Month 3 and Month 6. At Month 6 the magnitude of the plaque benefits were 17% (1.63 vs. 1.97) for the 0.075% CPC group and 19% (1.60 vs. 1.97) for the 0.10% CPC group. The Month 3 plaque benefits were 22% (1.53 vs. 1.95) for the 0.075% CPC group and 25% (1.47 vs. 1.95) for the 0.10% CPC group. There was no significant difference between the CPC groups with respect to mean plaque level at Month 3 or Month 6 (Tables 3, 4; Fig. 4).

**Safety** - There were no serious adverse events reported during the study that were deemed related to the test products. OST examinations showed that subjects rinsing with the chlorhexidine treatment had significantly more “tongue lesion” comments at Month 3 than those rinsing with either the CPC rinse or placebo rinse. There were no significant differences between either of the CPC rinse groups and the placebo rinse group in the number of subjects that had OST comments at 3 or 6 months.

**Discussion**

Results from this study show that two mouthrinses containing 0.075% and 0.10% high bioavailable CPC pro-

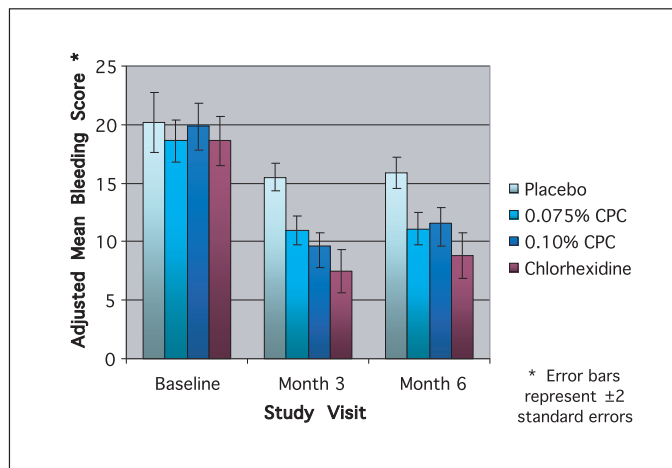


Fig. 3. Gingival bleeding scores for evaluable subjects.

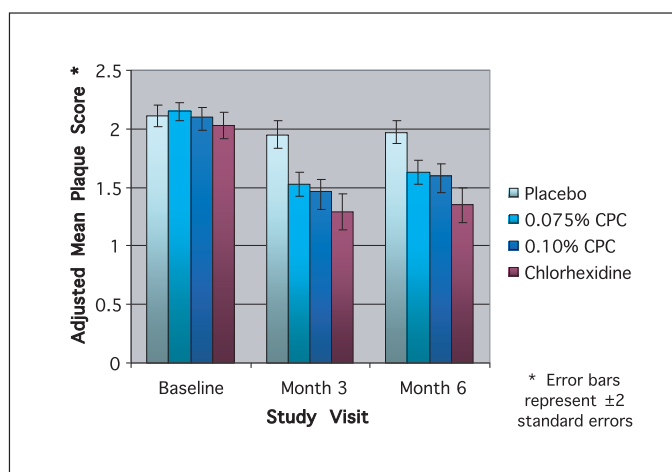


Fig. 4. Plaque scores for evaluable subjects.

vided statistically significant antiplaque and antigingivitis benefits over 6 months of use. Relative to the placebo rinse, the 0.075% CPC rinse showed a 23% reduction in gingivitis, 30% fewer bleeding sites and 17% less plaque after 6 months of use. The 0.10% CPC rinse demonstrated similar 6-month therapeutic benefits, showing 20% less gingivitis, 27% less bleeding and a 19% less plaque. The study design was validated, as the positive chlorhexidine control provided significant benefits for gingivitis, bleeding and plaque (33%, 45% and 31%, respectively) relative to the placebo control.

The therapeutic benefits of CPC are due to its broad-spectrum antibacterial action. CPC penetrates the cell membrane, allowing components in the cell to leak. This process disrupts bacterial metabolism, inhibits cell growth, and ultimately leads to cell death.<sup>9,10</sup>

It should be noted that certain excipients commonly used in marketed oral care formulations, such as surfactants, can diminish or neutralize the antimicrobial efficacy of CPC.<sup>11,12</sup> Published research<sup>13</sup> shows that formulations with high bioavailable CPC are associated with greater biological activity and also indicate that these formulations would have a higher probability of demonstrating clinical efficacy. The test products were formulated to have greater than 72% CPC bioavailability consistent with the current FDA requirements for a safe and efficacious CPC oral rinse for the treatment of plaque-induced gingivitis.

The benefits of CPC mouthrinses, when formulated to provide benefits against plaque and gingivitis, have been documented in other research.<sup>14-18</sup> In one 6-month trial,<sup>16</sup> a novel alcohol-free rinse (Crest Pro-Health Rinse) containing 0.07% high bioavailable CPC provided statistically significant reductions in plaque, gingivitis and gingival bleeding relative to placebo at 3 and 6 months. Separate studies show the new alcohol-free therapeutic CPC rinse provides antiplaque and antigingivitis benefits comparable to a recognized essential oils mouthrinse that contains alcohol.<sup>17,18</sup> In one study, the 0.07% CPC rinse and the essential oils rinse provided overall antiplaque benefits of 28% and 30%, respectively, relative to placebo.<sup>17</sup> A separate experimental gingivitis study demonstrated that rinsing twice daily with the experimental alcohol-free 0.07% CPC rinse delivered antiplaque and antigingivitis efficacy similar to that of the essential oils rinse.<sup>18</sup> Collectively, these trials show that twice daily use of a therapeutic CPC mouthrinse helps control plaque and gingivitis beyond mechanical plaque control.

- a. Procter & Gamble Company, Cincinnati, OH, USA.
- b. Anchor Brush Co, Morristown, TN, USA.
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## Practice implications with an alcohol-free, 0.07% cetylpyridinium chloride mouthrinse

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**ABSTRACT:** ***Purpose:*** Behavioral research was conducted to ascertain the relevance of an alcohol-free, 0.07% cetylpyridinium chloride (CPC) therapeutic mouthrinse to contemporary dental practice over a 6-month usage period. ***Methods:*** A randomized, single-blind study was conducted to assess practice-relevant compliance, acceptability and side effects associated with two mouthrinses. The target population was healthy adult mouthrinse users with a history of routine dental prophylaxis and maintenance care. Subjects were randomly assigned to a therapeutic mouthrinse with 0.07% CPC (Crest Pro-Health Rinse) or a cosmetic rinse control (Scope). Other oral hygiene was not standardized. Subjects completed a questionnaire and were examined by dental hygienists at baseline, and again after 3 and 6 months rinsing. At study completion, a dental prophylaxis was administered. ***Results:*** Compliance was generally favorable, with 273 subjects (89%) completing the 6-month rinsing study. Rinsing time generally stayed the same or increased relative to baseline. Groups differed among the subset who historically used an essential oils rinse (N=137), where those assigned to the alcohol-free therapeutic rinse exhibited significantly (P= 0.02) longer rinsing times compared to subjects using the alcohol-containing cosmetic rinse. Subject evaluations were generally positive with respect to both rinses. Side effects were minimal, with no between-group differences in hygienist-rated calculus or stain accumulation, or prophylaxis time. (*Am J Dent* 2005;18: 29A-34A).

**CLINICAL SIGNIFICANCE:** In a 6-month study, a high bioavailable 0.07% CPC therapeutic mouthrinse showed generally high compliance and favorable user acceptability, with similar side effects to those seen with a cosmetic mouthrinse. These findings suggest that the 0.07% CPC mouthrinse may be readily incorporated within the contemporary recall dental practice.

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### Introduction

The role of therapeutic rinses in the prevention and treatment of periodontal diseases has been long recognized.<sup>1</sup> Various antiseptics have been used in mouthrinse formulations, including bisbiguanides, essential oils, quarternary ammoniums and others.<sup>2</sup> Many of these agents are also found in other consumer goods, such as cleansers or deodorants. In rinses, these agents are commonly formulated with alcohol, not for any direct therapeutic reasons, but to help solubilize or disperse active ingredients or flavor oils.<sup>3,4</sup> Prominent clinical benefits include reductions in plaque or gingivitis attributable to immediate or sustained antimicrobial activity.

Usage may be short term (especially with chlorhexidine) or as a routine part of daily oral hygiene. There is considerable clinical trial evidence of the plaque and gingivitis benefits seen with therapeutic rinses relative to conventional toothbrushing.<sup>5</sup> Interestingly, delivery of therapeutic agents *via* rinse may offer some advantages over dentifrices. For example, use of an essential oils mouthrinse with routine toothbrushing is reported to yield better improvements in plaque, relative to a triclosan-containing anticavity dentifrice.<sup>6</sup> Ease of dispersion may contribute to some of the benefits, as evidenced by the malodor effects reported with some therapeutic mouthrinses.<sup>7,8</sup>

Relative to antibiotics, the common antiseptic rinses are recognized as having a generally lower incidence of adverse events, with little potential for resistance development, and demonstrated safety for routine use.<sup>2</sup> Except for isolated case reports involving mouthrinse abuse or accidental misuse,<sup>9</sup>

serious adverse events are virtually unknown. Clinical oral soft tissue effects are reported to not differ from normal controls.<sup>10</sup> Common side effects with therapeutic rinses include temporary taste alteration, superficial tooth staining, and calculus accumulation. Of these, taste alteration may be the most common. The phenomenon is reported for rinses containing chlorhexidine, essential oils, and cetylpyridinium chloride (CPC), among others.<sup>6,11-14</sup> Except for chlorhexidine,<sup>15</sup> taste perturbation is transient, with few-to-no reports of long term taste alteration. Other taste effects may be immediate, for example, the bitter taste or a burning sensation reported with some alcohol-containing formulations.<sup>11,12</sup>

Extrinsic tooth stain has been reported for chlorhexidine, delmopinol, CPC, and essential oils, among others.<sup>12,16-19</sup> Comparative trials usually show greatest stain accumulation with chlorhexidine compared to other antiseptics. Under certain conditions, this staining may be manifested after only a few days, with diet and other behaviors likely contributing to the extent and/or severity of staining.<sup>20,21</sup> In addition, supragingival calculus accumulation has been reported with some therapeutic rinses, most commonly for those containing chlorhexidine, and rarely, others.<sup>16,17,22,23</sup> Periodic treatment of stain and calculus accumulation is typically limited to routine dental prophylaxis. Along with taste, implications are primarily around rinsing compliance, especially with long term use.

Previous research has shown rinse formulation to impact on clinical effectiveness. CPC is a popular broad-spectrum antimicrobial that penetrates bacterial cell membranes, lead-

ing to cellular lysis, metabolic disruption, growth inhibition, and cell death.<sup>24</sup> Differences in rinse formulation may contribute nearly a two-fold increase in available concentration of CPC relative to Scope<sup>a</sup> or Cepacol<sup>b</sup> where CPC is less available.<sup>25</sup> Research has shown that formulations with high bioavailable CPC are associated with greater biological activity.<sup>25</sup> Based on these findings, an alcohol-free, CPC mouthrinse has been developed with a poloxamer emulsifier in order to increase bioavailability.<sup>4</sup> The formulation was designed to have a pleasing in-use experience, specifically no alcohol-burn, to enhance compliance. This high bioavailable, alcohol-free 0.07% CPC mouthrinse has been shown to yield significant antiplaque and antigingivitis benefits, similar in magnitude when compared to a positive control containing alcohol and essential oils.<sup>26</sup> Other controlled clinical trials report antiplaque or antigingivitis benefits with highly available CPC used for periods of up to 6 months.<sup>27,28</sup> These findings contrast with those previously reported for low available CPC when delivered using a highly flavored, cosmetic rinse.<sup>29</sup>

This new research was designed to ascertain the relevance of a novel, alcohol-free therapeutic mouthrinse to clinical practice. Subjects were randomly supplied with the new therapeutic mouthrinse or a cosmetic mouthrinse control. Dental hygienists were asked to evaluate response as part of routine prophylaxis maintenance care. The research investigated the following questions relative to an accepted rinse control:

- Would dental patients comply with therapeutic rinsing over an extended period?
- Is the rinse acceptable, or does taste or perception impact on usage?
- What are the common side effects, and impact on long term usage?

### Materials and Methods

A randomized, single-blind study was conducted to assess practice-relevant compliance, acceptability and side effects associated with two mouthrinses. The target population was healthy adult volunteers who routinely used mouthrinses. Study subjects were randomly assigned to a therapeutic mouthrinse (Crest Pro-Health Rinse<sup>a</sup>), or a cosmetic mouthrinse control (Scope). Treatment was unsupervised over a 6-month period.

A contract research organization recruited potential subjects from the general population in the greater Indianapolis, Indiana, USA area. The enrollment target was up to 300 subjects, to ensure at least 130 per group at study completion. Candidate subjects were screened by telephone to ascertain habits and oral hygiene practices. Eligibility was limited to generally healthy adults, 18-65 years of age, who routinely used a cosmetic or therapeutic mouthrinse (any type). Patients of record (having a history of routine dental care) were specifically targeted, with enrollment limited to individuals who had a routine dental prophylaxis within the previous 12 months. Subjects were excluded due to edentulism, presence of orthodontic devices, history of alcohol abuse, tobacco use exceeding 1 pack per day, or need for prophylactic antibiotic therapy prior to dental treatment.

At baseline, written and verbal informed consent was obtained, a baseline questionnaire was administered, oral health status was evaluated by a dental hygienist, and intraoral

photographs were obtained using standard methods. Study subjects were then randomly assigned to one of two mouthrinses, balancing for rinsing behavior, tobacco use, coffee/tea consumption, and dentifrice type (regular or anti-tartar). After 3 months, the questionnaire and oral health status evaluation were repeated. After 6 months, subjects returned for final clinical evaluations and intraoral photographs. A routine dental prophylaxis was administered, oral health status was assessed by a dental hygienist, and subjects were discharged from the study.

The test products were two rinses. The experimental group was a high bioavailable 0.07% CPC therapeutic mouthrinse in a blue mint-flavored, alcohol-free base. The control group was a low bioavailable 0.045% CPC mouthrinse in a green, mint-flavored, 15% alcohol base. In addition to the apparent color differences, these two rinses differed considerably in CPC availability<sup>30</sup> with approximately a two-fold increase in available CPC in the therapeutic rinse relative to the control. In clinical trials, the therapeutic rinse is reported to have significant antiplaque and antigingivitis benefits<sup>30</sup> while the control is recognized as having pleasant in-use characteristics. Test products were dispensed blind as to treatment assignment. Rinses were supplied in clear, 1.5 L stock mouthrinse bottles. Each bottle carried an investigational study label with warnings (*e.g.*: “do not swallow”) and contact numbers. Rinse bottles were packaged in a cardboard kit box, along with instructions for use. Supplemental mouthrinse was shipped directly to subjects upon request. In this “real world” study, subjects were not supplied with a standard dentifrice, toothbrush, or other oral hygiene products. Subjects were asked to suspend use of non-study mouthrinses for the duration of the research. Label instructions specified twice daily rinsing as part of routine oral hygiene practices following manufacturer’s instructions for use. All rinsing was unsupervised.

A subject questionnaire was administered at baseline, and again after 3 and 6 months. Subjects were specifically questioned on rinsing frequency, and open-ended questions were designed to elicit likes and dislikes, including side effects, during the 6-month treatment period. At study completion, rinses were assessed in five areas: taste, appearance, ease of use, burning and aftertaste, using a 5-point ordinal scale.

Oral health status was evaluated by one of 12 dental hygienists at baseline, 3 and 6 months. The baseline evaluation was conducted using a standard full mouth dental chart and instrumentation. Each hygienist was asked to evaluate calculus, stain/discoloration, and other oral conditions using a categorical 5-point scale. To model practice conditions, the baseline chart was available at the 6-month hygienist evaluation.

Demographic and behavioral parameters were summarized by group. Between-group comparisons in compliance, acceptability and clinical evaluations, all of which were ordinal in nature, were tested using the Wilcoxon Rank Sum test. Exact P-values were calculated. All testing was two-sided at a 0.05 level of significance.

### Results

A total of 308 subjects were assigned a test product (143 in the therapeutic rinse and 165 in the cosmetic rinse). Of these, 277 (90%) had a 3-month evaluation, and 273 (89%)

Table 1. Baseline reported oral hygiene behaviors.

All subjects - Baseline			
Frequency	Therapeutic rinse (N, %)	Cosmetic rinse (N, %)	Overall (N, %)
Brushing (N = 307)			
< 1/Day	4 (2.8)	2 (1.2)	6 (2.0)
1/Day	20 (14.1)	32 (19.4)	52 (16.9)
2/Day	94 (66.2)	116 (70.3)	210 (68.4)
>2/Day	24 (16.9)	15 (9.1)	39 (12.7)
Rinsing (N = 308)			
< 1/Day	27 (18.9)	23 (14.0)	50 (16.2)
1/Day	62 (43.3)	70 (42.4)	132 (42.9)
2/Day	47 (32.9)	65 (39.4)	112 (36.4)
>2/Day	7 (4.9)	7 (4.2)	14 (4.5)
Flossing (N = 306)			
< 1/Day	80 (56.3)	100 (61.0)	180 (58.8)
1/Day	43 (30.3)	41 (25.0)	84 (27.5)
2/Day	17 (12.0)	14 (8.5)	31 (10.1)
>2/Day	2 (1.4)	9 (5.5)	11 (3.6)

had a 6-month end-of-study evaluation. The sample was predominantly female (73%) and diverse with respect to ethnicity. The 35-44 age cohort was most commonly represented, with 32% of participants. Most (80%) reported consumption of coffee and/or tea. Tobacco users accounted for 11% of the study population.

At baseline, toothbrushing, rinsing and flossing were the most common daily oral hygiene behaviors (Table 1). Most subjects (81%) reported brushing at least twice daily, with manual toothbrushes outnumbering powered brushes by more than 4:1. Mouthrinsing was typically either once (43%) or twice (36%) daily, with Listerine (50%), Scope (21%) and store brands (16%) most prominently identified. Flossing was less common, with 59% reporting less than once per day usage.

User-reported rinsing times at Month 6 were generally comparable to or increased relative to pre-study (Table 2). Nearly 30% of subjects reported increased rinsing times with the assigned product, with increased rinsing more than three-fold more common than decreased rinsing. Overall, groups did not differ significantly (P= 0.141) in rinsing time at Month 6. Routine Listerine users accounted for approximately one-half of the overall sample. This subset exhibited differences in user-reported rinsing times (Table 3). In the therapeutic rinse group, 37% of subjects at Month 6 reported increased rinsing time *versus* baseline, compared to 20% in the cosmetic group. Groups differed significantly (P= 0.021) with respect to rinsing times in this subset.

At Month 6, subject evaluations were generally positive with respect to both rinses. In each of the five rinse assessment categories, "excellent" and "very good" were the first and second most common user-assigned ratings. Groups were generally similar with respect to rinse taste, rinse appearance and ease of use (Figs. 1-3). There were significant (P< 0.05) between-group differences in aftertaste, favoring the cosmetic rinse, and burning, favoring the alcohol-free therapeutic rinse (Figs. 4, 5). In addition, favorable flavor comments were the most common responses from both groups in the open-ended questions asked at months 3 and 6.

A total of 273 subjects completed the 6-month visit. The hygienist evaluation at that time period showed most subjects (73%) had some calculus accumulation (Table 4). The amount

Table 2. User-reported rinsing time at Month 6 *versus* baseline.

All subjects - Month 6 <i>versus</i> baseline				
Time	Therapeutic rinse		Cosmetic rinse	
	N	%	N	%
Rinse time decreased	8	7	13	9
Rinse time unchanged	74	60	99	66
Rinse time increased	41	33	38	25

Table 3. Listerine user-reported rinsing time.

Listerine user subset (N=137) - Month 6 <i>versus</i> baseline				
Time	Therapeutic rinse		Cosmetic rinse	
	N	%	N	%
Rinse time decreased	3	5	8	10
Rinse time unchanged	35	58	54	70
Rinse time increased	22	37	15	20

Table 4. Calculus accumulation at Month 6.

All subjects				
Calculus (Hygienist)	Therapeutic rinse		Cosmetic rinse	
	N	%	N	%
None	37	30	38	26
A little amount	60	49	83	56
Moderate amount	20	16	20	13
Large amount	5	4	5	3
Very large amount	1	1	3	2

was generally slight and by location, the lingual surfaces of the mandibular teeth were most commonly affected. Groups did not differ significantly (P= 0.73) with respect to hygienist-assessed calculus accumulation over the 6-month usage period.

There was less evidence of stain accumulation, with one-half of subjects ending treatment with no visible tooth stain (Table 5). Since there was no prophylaxis immediately prior to treatment, 20% started the rinse study with evident stain. Both groups showed accumulation of stain over time, with coffee/tea consumption (Table 6) and tobacco usage (Table 7) contributing to more stain development. Where present, staining was typically graded as slight in amount. Groups did not differ significantly (P= 0.75) with respect to hygienist-rated stain accumulation at Month 6.

Seventeen percent of subjects in each group did not receive an end-of-study prophylaxis, yielding 227 who received both a post-study dental prophylaxis and evaluation. For most patients (81%), hygienists rated the time needed for prophylaxis as routine or shorter (Table 8). Groups did not differ significantly (P= 0.616) with respect to prophylaxis time.

## Discussion

The randomized controlled trial is widely accepted as a "gold standard" for biomedical research. There has been considerable recent interest in expanding oral care studies into practice-based settings and beyond, to allow for more relevant data on patient response among the broadest possible populations.<sup>31</sup> In such research, clinician evaluation is believed essential to the identification of clinical problems. Accordingly, new research was conducted to assess the likely



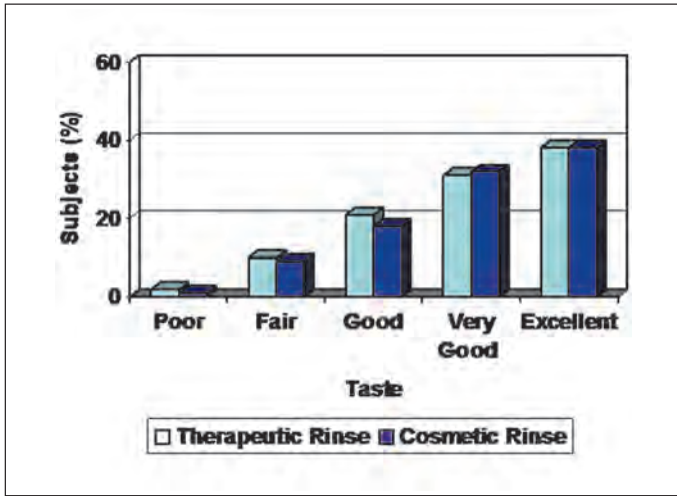


Fig. 1. Distribution of subject-rated rinse taste at Month 6.

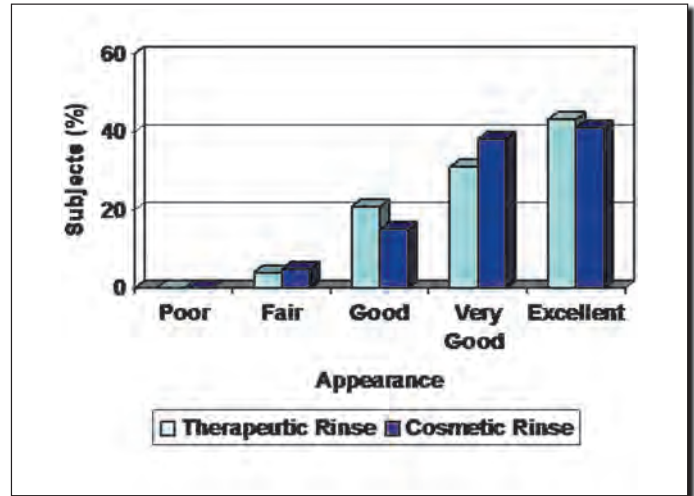


Fig. 2. Distribution of subject-rated rinse appearance at Month 6.

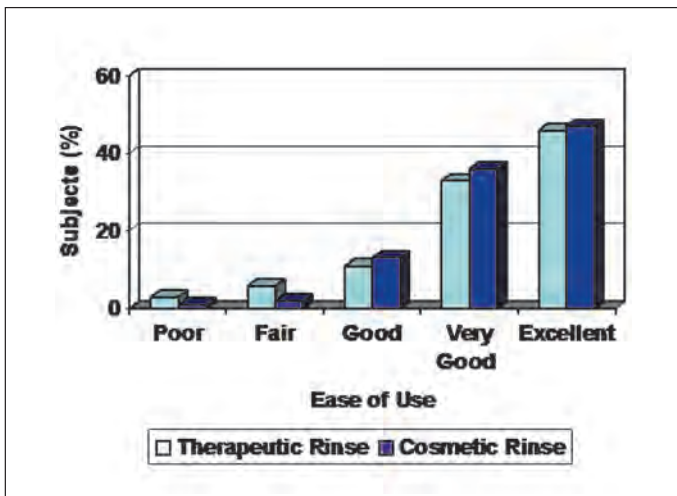


Fig. 3. Distribution of subject-rated rinse 30-second ease of use at Month 6.

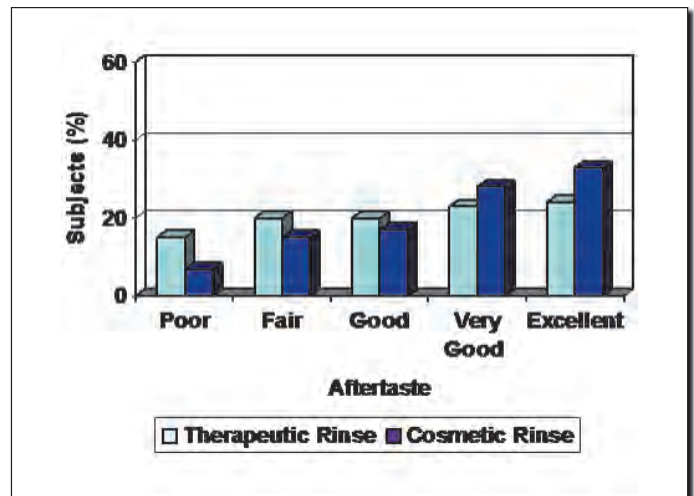


Fig. 4. Distribution of subject-rated rinse aftertaste at Month 6.

practice response seen with longer term use of an alcohol-free, high bioavailable 0.07% CPC mouthrinse. The research evaluated compliance, acceptability and side effects from both hygienists and patients who used either a therapeutic mouthrinse or cosmetic mouthrinse control unsupervised over a 6-month period as part of routine prophylaxis maintenance. The study population was considerable (~300), and evaluations were obtained from various hygienists, to obtain a diverse perspective on response.

Compliance was favorable, both on a daily basis and over the 6-month usage period. Most (89%) subjects reported routine daily rinsing with the assigned product throughout the 6-month period. While daily rinse times increased during the study compared to baseline, there were no between-group differences ( $P=0.14$ ). Of interest, one-half of study subjects used an essential oils mouthrinse at baseline. Among that subset, rinsing time increased significantly ( $P=0.02$ ) for the therapeutic rinse group compared to the cosmetic rinse. That is, routine Listerine users reported increased rinsing time with Pro-Health Rinse compared to those using Scope. Both the essential oils rinse and cosmetic rinse contain appreciable alcohol (21-27% and 15% respectively), while the therapeutic CPC rinse is alcohol-free. We speculate that differences relating to alcohol content (e.g., burning sensation) may have contributed to increased rinsing

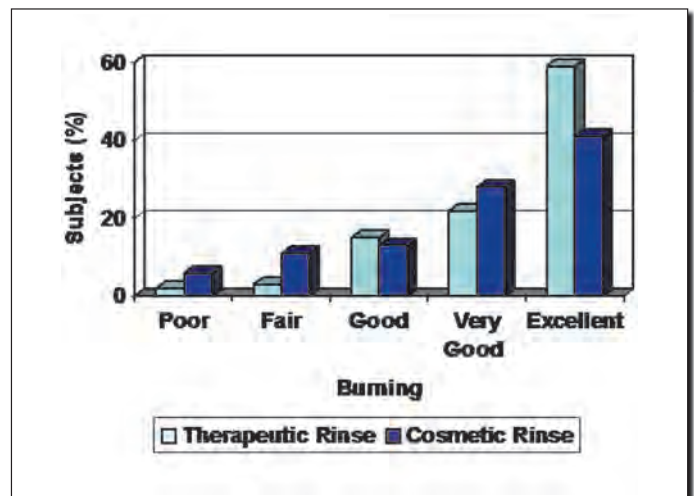


Fig. 5. Distribution of subject-rated rinse burning sensation at Month 6.

time among subjects who were switched from an alcohol-based to an alcohol-free rinse. Numerous other formulation differences between the essential oils and cosmetic rinses may have contributed to these rinse time differences. Further research would be needed to ascertain a causal relationship between alcohol-based rinse experience assessments and usage time.

Table 5. Tooth discoloration at Month 6.

All subjects	Therapeutic rinse		Cosmetic rinse	
	N	%	N	%
<b>Month 6</b>				
None	60	48	75	50
A little amount	47	38	55	37
Moderate amount	12	10	18	12
Large amount	3	2	-	-
Very large amount	2	2	1	1

Table 7. Distribution of stain-free subjects by tobacco usage and time.

All subjects	No tooth staining			
	Therapeutic rinse		Cosmetic rinse	
Behavior/time	N	%	N	%
<b>No tobacco usage</b>				
Baseline	98	78	116	80
Month 3	74	68	86	64
Month 6	53	48	69	53
<b>Daily tobacco usage</b>				
Baseline	13	77	11	61
Month 3	8	62	11	61
Month 6	6	46	6	33

Unlike compliance, taste, or even health, stain represented the single parameter in this research that could be evaluated by both clinicians and patients. Outcomes were evaluated in a general population, because behaviors such as tobacco use and tea consumption have been reported to affect the measured amount or severity of tooth staining in clinical trials using therapeutic mouthrinses.<sup>18</sup> In this new research, there were no significant between-group differences in staining. The response was likely not an artifact, as there was clear evidence of both temporal and behavioral effects. Hygienist evaluation showed more stain in both rinse groups over time, consistent with the length of time since last prophylaxis (irrespective of rinse). Moreover, coffee/tea and tobacco users were generally more likely to exhibit some staining. While the sample size for tobacco users was relatively small in this study, findings are consistent with the considerable body of research showing that these dietary and behavioral factors contribute to superficial stain development in the absolute.<sup>32</sup> Where present, most effects were slight, and not contributing to continuing participation. In summary, use of this therapeutic mouthrinse over a 6-month period yielded minimal staining, similar to that seen with a popular cosmetic rinse.

What are the implications for the recall practice? Use of therapeutic rinses such as chlorhexidine may, over the longer term, contribute to additional treatment need during dental prophylaxis due to calculus accumulation and/or staining. Such factors may limit professional use of these agents to only a few weeks.<sup>33</sup> In this new study, use of a 0.07% CPC therapeutic rinse over a 6-month period did not contribute to increased prophylaxis treatment. Both groups did have a minority (< 20%) of subjects with increased prophylaxis requirement, consistent with the length of time since previous treatment, as some subjects had not received a routine prophylaxis in over a year. However, after 6-months continuous use, there were no significant (P= 0.62) between-

Table 6. Distribution of stain-free subjects by coffee/tea consumption and time.

All subjects	No tooth staining			
	Therapeutic rinse		Cosmetic rinse	
Behavior/time	N	%	N	%
<b>No coffee and/or tea consumption</b>				
Baseline	27	84	25	86
Month 3	22	73	19	66
Month 6	17	57	14	50
<b>Coffee and/or tea consumption</b>				
Baseline	84	76	102	73
Month 3	60	65	78	63
Month 6	42	45	61	50

Table 8. Prophylaxis time at Month 6.

Subjects with a prophylaxis (N = 227)				
Prophylaxis time (Hygienist)	Therapeutic rinse		Cosmetic rinse	
	N	%	N	%
Less than usual	12	12	17	14
Usual	69	68	85	68
More Than Usual	21	21	23	19

group differences in hygienist-reported prophylaxis time for the therapeutic *versus* cosmetic rinse. These findings suggest that the 0.07% CPC mouthrinse may be readily incorporated within contemporary recall practices.

Behavioral research of this nature is not a substitute for randomized controlled clinical trials. Much of the information is impressionistic, subject to reporting bias and other effects. In addition, there were multiple clinical evaluators (dental hygienists) and specific health-related parameters were not measured. Like the randomized clinical trial, use of a recognized control and blinding of the subjects and evaluators aids in the interpretation and meaningfulness of the outcomes. When considered with evidence from randomized controlled trials, research like this study provides important perspective under in-use conditions, like those encountered daily in dental practices.

### Conclusions

In this research, a high bioavailable, alcohol-free 0.07% CPC therapeutic mouthrinse exhibited generally high compliance and good user acceptability over a 6-month usage period, with similar side effects to those seen with a cosmetic mouthrinse. These findings suggest that the 0.07% CPC mouthrinse may be readily incorporated within the contemporary recall practice.

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