



Pfizer Consumer Healthcare

February 20, 2004

Dockets Management Branch (HFA-305)
Food and Drug Administration
Room 1061, 5630 Fishers Lane
Rockville, MD 20852

Re: Reply Comments
Proposed Monograph for OTC Antigingivitis/Antiplaque Drug Products
Docket No. 81N-033P
68 Fed. Reg. 32232 (May 29, 2003)

In an August 25, 2003 *Federal Register* announcement, the Food and Drug Administration extended both the comment period and the reply comment period for an advance notice of proposed rulemaking (ANPR) for over-the-counter antigingivitis/antiplaque drug products, setting a February 23, 2004 deadline for submission of reply comments. Pfizer Inc has reviewed the submitted comments and is submitting reply comments on the following topics:

- I. Clinical Testing of New Dosage Forms of Category I Ingredients
- II. Final Formulation Test Methods
- III. Inclusion of Combination Products
- IV. Support Required for Plaque Reduction Label Claims
- V. Support Required for "Kills Germs" and Related Claims
- VI. Effectiveness Criteria for Moving Category III Ingredients to Category I

Since comments on these topics were submitted by a number of interested parties, our reply comments will address the general topic areas and not the specific comments of each of the

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respondents, with the exception of comments referring directly to claim support data submitted by the Warner-Lambert Co. (now Pfizer Inc) during the period of the Plaque Subcommittee's deliberations.

I. **Clinical Testing of New Dosage Forms of Category I Ingredients**

Pfizer agrees with the comments that support allowing dosage forms of Category I ingredients other than those forms reviewed by the Subcommittee and the proviso that these new dosage forms be suitable for oral topical administration. Pfizer also agrees with the requirement that effectiveness of a new dosage form be demonstrated by a single 6-month randomized, negative controlled, plaque/gingivitis clinical trial. However, Pfizer also notes the ambiguity with respect to effectiveness acceptance criteria that is presented by the requirement of only one trial and believes that these criteria should be specified. Pfizer agrees with the effectiveness standard established by the Subcommittee and supported in some of the comments, which is consistent with the revised ADA Guidelines¹; namely, a statistically significant mean 20% gingival index (GI) reduction across two studies, with at least a 15% reduction in any one study, and a statistically significant reduction in plaque, all compared to the negative control. Therefore, in order to demonstrate that the new dosage forms will have meaningful clinical effectiveness, Pfizer proposes that FDA require a single study with at least a statistically significant 20% reduction in gingival index or two studies with a

¹Imrey PB, Chilton NW, Pihlstrom BL et al. Recommended revisions to American Dental Association guidelines for acceptance of chemotherapeutic products for gingivitis control. J Periodont Res 1994; 29:299-304.

statistically significant mean 20% GI reduction across both, and neither study having a GI reduction of less than 15%. In both cases, a statistically significant reduction in plaque index would be required. Pfizer also believes that the percent reductions should be based on full mouth indices, and that indices using only a subset of scored sites are not appropriate for the primary demonstration of effectiveness since they can artificially inflate the percentages thereby precluding accurate comparisons with previous study outcomes.

II. Final Formulation Test Methods

Pfizer reiterates its belief that final formulation testing should include an in vitro test (such as a kill kinetics assay) to demonstrate that the antimicrobial spectrum of activity of the active ingredient has been maintained in the new formulation. Pfizer therefore disagrees with the comment claiming that such tests are not needed since they relate only to safety. Pfizer believes it is important to confirm that the new formulation has comparable activity to the clinically tested standard against representative Gram-positive and Gram-negative commensal and pathogenic bacteria, opportunistic organisms, and yeast, since any discrepancies in activity would likely not be revealed in a short-term antiplaque test or in a chemostat using an artificial plaque. Such confirmation is needed from both an effectiveness and a safety standpoint.

Pfizer also reiterates its belief that final formulation testing should include an in vivo test to demonstrate that the new formulation is effective against an in situ biofilm and gingivitis. In addition, test methods accepted for final formulation testing, whether *in vitro* or *in vivo*, should be methods that have been generally accepted by the clinical research community, shown to be appropriately predictive in peer reviewed

studies, and demonstrated to be sufficiently sensitive to differentiate between active formulations with differences in activity when compared in a single study.

Pfizer continues to support the guidance approach to final formulation testing, and includes in the Appendix additional information concerning the *in vitro* and *in vivo* test methods recommended for final formulation testing of mouthrinses containing the fixed combination of essential oils.

III. Inclusion of Combination Products

Pfizer reiterates its support for the inclusion of the rational combination products recommended by the Subcommittee in the proposed monograph, and agrees with those comments that support such inclusion. The three combinations recommended by the Plaque Subcommittee are rational from both a public health and a dental health standpoint, and there is no legal or regulatory constraint against inclusion in a monograph of new combination products that had not previously been marketed. In fact, as noted in some of the comments, several combination products were submitted and reviewed by the Subcommittee. Pfizer agrees that any new combinations should satisfy requirements for safety and effectiveness specified in the respective monographs governing each of the Category I ingredients.

IV. Support Required for Plaque Reduction Label Claims

Pfizer believes that claims for plaque reduction, control, prevention or removal should be based only on a reduction of plaque mass. Pfizer, therefore, disagrees with the comment from the Procter and Gamble Company (P&G) arguing that claims of “plaque reduction and/or plaque control” should be allowed for *any* statistically significant change in plaque. While other alterations in plaque, such as decreases in plaque

metabolic activity or plaque pathogenesis, may result in a gingivitis reduction, these changes should not be confused with plaque mass reductions. Based on data submitted, the Subcommittee clearly differentiated among agents on the basis of antiplaque mechanisms by categorizing stannous fluoride-containing products as antigingivitis and CPC- and essential oil-containing products as antigingivitis/antiplaque. For the former, clinical studies failed to demonstrate a significant reduction in plaque mass and the antigingivitis effect was explained by an inhibition of plaque metabolic activity. For the latter ingredients, the antigingivitis effect was explained primarily by a significant reduction in plaque mass. The respective plaque claims for these two categories were “helps interfere with harmful effects of plaque associated with gingivitis” and “helps control (or reduce, prevent, or remove) plaque that leads to gingivitis...” Clearly, the Subcommittee recognized that plaque reduction/control means something very different from a reduction in plaque virulence or metabolic activity. In addition, consumers and dental professionals have an expectation that a product claiming to reduce or control plaque will produce a decrease in plaque accumulation discernible by actual measurements, such as by the use of plaque indices or plaque weight, or by visual appearance.

In addition, the Subcommittee recognized that the activity of an active agent can be affected by the totality of the product, that is, the formulation in which the active agent is placed. This was reflected in data the Subcommittee reviewed for CPC-containing mouthrinses, some of which had only significant antiplaque activity and some of which were effective against both plaque and gingivitis. The fact that the way a product is formulated can affect its activity is also reflected in the Subcommittee’s requirement for

final formulation testing to confirm that the clinical effectiveness of a new formulation is comparable to that of the clinically tested standard whose data provided the basis for a Category I recommendation.

The influence of formulation on activity is also demonstrated in additional comments submitted by P&G in support of its request for a plaque reduction claim for the stannous fluoride dentifrice reviewed by the Subcommittee. As noted above, the Subcommittee concluded that the 6-month clinical study data submitted for this formulation failed to demonstrate a significant plaque reduction activity. An additional 6-month clinical trial conducted by P&G and included with its comments also failed to support substantial activity, with a plaque mass reduction of 6.9% compared to negative control. The only data submitted by P&G supporting a significant plaque reduction effect for its stannous fluoride dentifrice are included in two published 6-month clinical trials sponsored by the Colgate-Palmolive Company which were conducted on a Colgate stannous fluoride formulation *different from that submitted by P&G*. Pfizer believes that unless and until the FDA makes the determination that additional data submitted support a quantitative plaque reduction claim *for the stannous fluoride dentifrice formulation reviewed by the Subcommittee*, the use of a plaque reduction/control claim should be restricted to those products/ingredients that the Subcommittee concluded actually do produce significant quantitative reductions in plaque mass.

V. Support Required for "Kills Germs" and Related Claims

Since the Procter & Gamble Company (P&G) has in its comments cited data submitted by the Warner-Lambert Company (now Pfizer Inc.) to the Subcommittee,

Pfizer is responding directly to these comments. P&G is requesting that the monograph labeling include a provision enabling stannous fluoride to use an additional indication, "helps (select one) control, inhibit, or kill plaque bacteria that contribute to the development of gingivitis...", that is identical to the one included for the fixed combination of essential oils. P&G claims that they have conducted studies on the dentifrice which generated the same type of data that Warner-Lambert provided to the Subcommittee in support of this claim. This statement by P&G is only partly accurate. Prior to accepting this indication for the essential oils, the Subcommittee required Warner-Lambert to demonstrate that the killing of plaque bacteria was the primary mechanism by which the mouthrinse containing the fixed combination of essential oils produced its antiplaque/antigingivitis effects, and demanded a high standard of proof **including *in vivo* data**. Accordingly, Warner-Lambert presented data from *in vitro* kill kinetics assays to establish that the essential oils are, in fact, bactericidal, and then a series of *in vivo* studies **to demonstrate that bactericidal activity occurs in the mouth under actual use conditions**. These studies included: investigations into the level of viable salivary bacteria at various time points following a single rinse; bacterial reductions on the dorsum of the tongue (a reservoir for plaque bacteria) and at the gingival sulcus at various time points after a single rinse; demonstrations that preprocedural rinsing with the essential oil mouthrinse results in a significant reduction in viable aerosolized bacteria; bactericidal activity on representative bacteria in interproximal plaque; and *in situ* killing of plaque bacteria using a vital staining method, with an associated *in vitro* biofilm study to document the relationship between vital staining results and bacterial kill. A majority of the submitted studies had already been

published or have subsequently been published in peer reviewed journals²⁻¹². In contrast, all of the submitted P&G studies have been conducted using *in vitro* models which do not meet the same standard of clinical proof required by the Plaque Subcommittee for this claim to be granted to the fixed combination of essential oils.

² Ross NM, Charles CH, Dills SS. Long-term effects of Listerine Antiseptic on dental plaque and gingivitis. J Clin Dent 1: 92-95, 1989.

³ DePaola LG, Minah GE, Overholser CD et al. Effect on an antiseptic mouthrinse on salivary microbiota. Amer J Dent 9:93-95, 1996.

⁴ Jenkins S, Addy M, Wade W, Newcombe RG. The magnitude and duration of the effects of some mouthrinse products on salivary bacterial counts. J Clin Periodontol 21: 397-401, 1994.

⁵ Pianotti R, Pitts G. Effects of an antiseptic mouthwash on odorogenic microbes in the human gingival crevice. J Dent Res 57: 175-179, 1978.

⁶ Pitts G, Pianotti R, Feary TW et al. The in vivo effects of an antiseptic mouthwash on odor-producing microorganisms. J Dent Res 60: 1891-1896, 1981.

⁷ Pitts G, Brogdon C, Hu L et al. Mechanism of action of an antiseptic, anti-odor mouthwash. J Dent Res 62: 738-742, 1983.

⁸ Fine DH, Mendieta C, Barnett ML et al. Efficacy of preprocedural rinsing with an antiseptic in reducing viable bacteria in dental aerosols. J Periodontol 63: 821-824, 1992.

⁹ Fine DH, Yip J, Furgang D et al. Reducing bacteria in dental aerosols: Preprocedural use of an antiseptic mouthrinse. J Amer Dent Assoc 124: 56-58, 1993.

¹⁰ Fine DH, Furgang D, Korik I et al. Reduction of viable bacteria in dental aerosols by preprocedural rinsing with an antiseptic mouthrinse. Amer J Dent 6: 219-221, 1993.

¹¹ Fine DH, Furgang D, Barnett ML et al. Effect of an essential oil-containing antiseptic mouthrinse on plaque and salivary *Streptococcus mutans* levels. J Clin Periodontol 27: 157-161, 2000.

¹² Pan P, Barnett ML, Coelho J et al. Determination of the in situ bactericidal activity of an essential oil mouthrinse using a vital stain method. J Clin Periodontol 27: 256-261, 2000.

The same considerations apply to P&G's request for a comparable claim for cetylpyridinium chloride (CPC). In support of the claim for CPC, P&G has submitted, in addition to the *in vitro* studies, a study which looked at the *in vivo* kill of salivary bacteria. Whereas the studies on salivary organisms submitted by Warner-Lambert documented a significant reduction in levels of viable bacteria after only a *single rinse*, the P&G CPC study looked at salivary bacterial levels immediately prior to and 2 minutes and 2 hours after *13 rinses over a 5-day period*. Thus, even with this *in vivo* study, P&G has not produced a level of support for the claim anywhere nearly as rigorous as that required from Warner-Lambert by the Plaque Subcommittee.

In summary, Pfizer does not agree that the studies submitted by P&G constitute the same type of data submitted by Warner-Lambert in support of the requested additional claims for stannous fluoride and CPC. In fact, the studies contained in P&G's comments fall short of the standard established by the Subcommittee for allowance of these claims and are, therefore, insufficient to support the granting of these claims to the additional Category I ingredients.

VI. Effectiveness Criteria for Moving Category III Ingredients to Category I

Several of the comments dealt with the question of effectiveness criteria with which to assess the outcomes of 6-month plaque/gingivitis clinical trials including the question of what criteria should be used for moving Category III ingredients to Category I. Pfizer agrees with those comments supporting the continued application of the standards used by the Subcommittee in its

recommendation of Category I ingredients. These standards were based on criteria established by the American Dental Association with considerable input from clinical investigators and statisticians from the Task Force on Design and Analysis; namely, the requirement of at least two 6-month clinical trials, each of which demonstrates at least a 15% reduction in gingivitis with an average gingivitis reduction of 20% across both trials and a statistically significant reduction in plaque. ***Pfizer believes that it would not be in the best interest of either the public or the profession to lower the standards for future Category I ingredients when an expected level of effectiveness for such ingredients has already been established through the initial reviews.***

Thank you for your consideration of these reply comments. Please feel free to contact me if you have any questions or require any additional information or clarification.

Sincerely,



Don Jantzen
Director, Regulatory Affairs

Appendix – Final Formulation Effectiveness Testing of OTC Antigingivitis/Antiplaque Products Containing the Fixed Combination of Four Essential Oils

APPENDIX

Final Formulation Effectiveness Testing of OTC

Antigingivitis/Antiplaque Products Containing the Fixed Combination of

Four Essential Oils

The advance notice of proposed rulemaking (ANPR) for OTC antigingivitis/antiplaque drug products, published in the May 29, 2003 *Federal Register*, identifies the need for testing requirements to establish the effectiveness of final product formulations containing Category I active ingredients or combinations. In the ANPR, the agency has listed the test methods for each of the category I ingredients or fixed combinations that were presented to the Dental Plaque Subcommittee (the Subcommittee) by the respective sponsors, and has also requested "specific information from interested parties on testing protocols, effectiveness criteria, and statistical methods employed to analyze the data from these tests."

This document is further to the November 25, 2003 comments submitted by the Joint Oral Care Task Group (the Task Group) of the Consumer Healthcare Products Association (CHPA) and the Cosmetic, Toiletry, and Fragrance Association (CTFA). These comments specifically focus on final formulation effectiveness testing for antigingivitis/antiplaque drug products containing the fixed combination of essential oils that the Subcommittee recommended as Category I, as noted in proposed §356.26(p). These comments relate directly to the testing requirements listed in proposed §356.92 (b) of the ANPR, and supplement the April 27, 1998 submission on final formulation testing by Warner-Lambert to the Subcommittee

I. General comments on the ANPR presentation of tests for antigingivitis/antiplaque drug product containing essential oils:

A. Guidelines are more useful than fixed protocols over time: Since the state-of-the-science continues to evolve, thereby potentially rendering specific test procedures obsolete, we believe that, rather than codifying specific protocols, it would be preferable for the Agency to issue a Guidance document setting forth the design characteristics and success criteria of tests for establishing the effectiveness of final formulations relative to clinically tested standards. While such guidance could include examples of specific protocols, such as those contained in the April 27, 1998 Warner Lambert submission, it should also allow for the use of alternative tests, provided that their applicability to and validity for their intended purpose are well documented. Suggested guidelines for final formulation testing of products containing the fixed combination of essential oils are presented below.

B. Both *in vivo* and *in vitro* testing should be required for products containing the fixed ratio of essential oils. Proposed §356.92 (b) states that "One of the following tests should be conducted" and then lists an *in vitro* microbiological test and a clinical test to demonstrate *in vivo* activity. This is inconsistent with the basis of Warner-Lambert's April 27, 1998 submission, which specifically states, "A combination of *in vitro* and *in vivo* tests

should be required since *in vitro* tests alone, while able to confirm the antimicrobial activity of a given formulation, are not necessarily indicative of the *in vivo* antiplaque/antigingivitis activity of the formulation.” Both tests were listed as required in Section F. of the Subcommittee report (68 Fed. Reg. at 32240-32241). The scientific rationale for requiring both tests is discussed in greater detail in the following section.

C. Purpose and requirements of final formulation testing: The overall purpose of the test methods is to determine the comparable effectiveness of a final product formulation and a clinically tested standard; the test results should provide a reasonable expectation that the previously untested formulation will have clinical effectiveness comparable to that of the standard formulation containing the same level of Category I active ingredient. The Subcommittee recognized the need for such testing insofar as the way a product is formulated can have a significant impact on the effectiveness of active ingredients.

For antiplaque/antigingivitis products containing the fixed combination of essential oils, it should be demonstrated that

- i. The final formulation has the same *in vitro* antimicrobial spectrum of activity as the standard and
- ii. The final formulation has a level of clinically relevant *in vivo* effectiveness that is noninferior to that of the reference standard.

Tests for i. should include *in vitro* tests of antimicrobial activity, such as a 30-second kill time test, against a broad range of representative oral bacteria and yeasts, including potentially opportunistic microorganisms. It is important to demonstrate that the spectrum of activity shown for the standard has been retained in the test formulation, as this relates both to antiplaque/antigingivitis activity and safety with long-term use. The test should use product without dilution except for that caused by mixture with the challenge organism suspensions; this is how the product is used, and substantial dilution could theoretically alter the interaction of the active ingredients with the vehicle.

Tests for ii. should demonstrate *in vivo* activity using a human clinical model relevant to reduction and/or prevention of dental plaque and gingivitis. This *in vivo* testing is an important supplement to *in vitro* testing, since it is now well recognized that bacteria in a biofilm such as dental plaque can exhibit enhanced or differential resistance to antimicrobial agents compared to the planktonic form of organisms typically used for *in*

vitro determinations^{1,2,3,4,5}. In addition, intraoral conditions that are difficult to model *in vitro* can substantially influence the therapeutic effectiveness of an oral care composition. It is important that the *in vivo* test include measures of both plaque and gingivitis. While clinical studies of six months' duration or longer are the generally accepted model for establishing antigingivitis and antiplaque *effectiveness*, shorter term clinical models of 2-4 weeks' duration^{6,7,8,9,10,11} can be used to demonstrate *comparability* of the activity of a test product formulation and that of a clinically proven "gold standard" reference product.

¹ McLean, Robert J. C. 2002. An overview of biofilm molecular ecology, *Molecular Ecology of Biofilms*, pp.1-21. Editor(s): McLean, Robert J. C.; Decho, Alan W. Horizon Scientific Press, Wymondham, UK.

² Marsh, P.D. 2003. Plaque as a biofilm: pharmacological principles of drug delivery and action in the sub- and supragingival environment, *Oral Diseases*, 9 Suppl 1:16-22.

³ Fine DH, Furgang D, Barnett ML: Comparative antimicrobial activities of antiseptic mouthrinses against isogenic planktonic and biofilm forms of *Actinobacillus actinomycetemcomitans*. *J Clin Periodontol* 2001; 28:697-700.

⁴ Wilson, M., 1996. Susceptibility of oral bacterial biofilms to anti-microbial agents. *Journal of Medical Microbiology*. 44, 79-87.

⁵ Costerton, J.W. 1995. Overview of microbial biofilms, *Journal Of Industrial Microbiology*, 15:137-140.

⁶ Fornell J, Sundin Y, Lindhe. Effect of Listerine on dental plaque and gingivitis. *Scand J Dent Res* 1975; 83:18-25.

⁷ Mankodi S; Ross N M; Mostler K. 1987. Clinical efficacy of Listerine in inhibiting and reducing plaque and experimental gingivitis, *J. Clin. Periodontol*, 14:285-288.

⁸ Wennstroem, J.L. 1988. Mouthrinses in "experimental gingivitis" studies, *J. Clin. Periodontol.*, 15:511-516.

⁹ Lobene, R.A., Mankodi, S.M. Ciancio, S.G., Lamm, R.A., Charles, C.H. and Ross, N.M. 1989. Correlation among gingival indices. *J. Periodontol*. 60:159-162.

¹⁰ Ross NM, Mankodi SM, Mostler KL, Charles Ch, Bartels LL. Effect of rinsing time on antiplaque-antigingivitis efficacy of listerine. *J Clin Periodontol* 1993; 20: 279-281.

¹¹ Charles, C., Mankodi, S., Santos, S.L., Lynch M.C., Coelho J., and Wu M.M. 2003. Determination of the antiplaque/antigingivitis efficacy of essential oil containing mouthrinses with and without fluoride, using an experimental gingivitis model. *J. Dent. Res.* 82 (spec Is B) abstr 2758.

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D. Availability of Reference Formulations: To accomplish the goals of final formulation effectiveness testing, there is clearly a requirement for well characterized positive control formulations substantially identical to the clinically tested products used to secure Category I status. Pfizer Consumer Healthcare will work in conjunction with the Consumer Health Care Products Association and the U.S. Pharmacopeia to establish and make available reference products containing the fixed combination of essential oils for use as positive controls in final formulation testing.

Proposed Guidelines For Final Formulation Effectiveness Testing of Mouthrinses with the Fixed Combination of Essential Oils (§356.92 (b))

A. In vitro Antimicrobial Activity

1. Purpose and Principle: The purpose of *in vitro* final formulation testing is to confirm that the spectrum of antimicrobial activity of the test product is identical to that of the clinically tested reference standard. Activity against a broad range of representative oral bacteria and yeasts, including potentially opportunistic microorganisms, should be demonstrated using type strains of the test organisms as well as a fresh source of mixed oral microorganisms (e.g. saliva). Testing should use undiluted product, concentrations of test organisms approximating the total microbial load in saliva, and short exposure times representative of the duration of product use. The need to confirm that the spectrum of activity has been retained relates to both the antiplaque/antigingivitis effectiveness of the formulation and its safety with long-term use.

2. Test Method:

- (a) A kill time determination assay (also referred to as the kill kinetics assay or "Bahn test") should be used to assure that the test formulation has a spectrum of antiseptic activity comparable to that of the reference standard. This assay evaluates the extent to which an antimicrobial mouthrinse formulation kills standard cultures of microorganisms under defined conditions of time and temperature. This test is a recognized method for assessing the effectiveness of oral antimicrobial formulations¹², and is similar in concept to the kill time method described by NCCLS¹³ for evaluating bactericidal activity of antimicrobial agents.
- (b) The test should be carried out using the reference standard (positive control), test formulation, and a sterile water negative control.

3. Challenge microorganisms:

(a) The kill time determination should be conducted using a variety of standard laboratory strains of pathogenic and non-pathogenic Gram-positive and Gram-negative oral microorganisms as well as wild-type organisms obtained via saliva sampling. Organisms to be tested will include type strains of *Actinomyces viscosus*, *Streptococcus mutans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis* (formerly *Bacteroides forsythus*), *Candida albicans*, and Gram-negative enteric rods. In addition, the test should be conducted using a freshly collected pool of mixed-species oral microorganisms (i.e. from pooled human saliva) using the same experimental conditions.

¹² Oral Health Care Drug Products for Over-the-Counter Human Use; Establishment of a Monograph. 47 Federal Register 22898, May 25, 1982.

¹³ Methods For Determining Bactericidal Activity Of Antimicrobial Agents: Approved Guideline. NCCLS document M26-A Vol. 19, No. 18 (September, 1999)

4. Test conditions:

(a) The assay should use undiluted mouthrinse mixed directly with the test organism suspension and an exposure time of 30 seconds, in order to be representative of actual use conditions.

(b) Stock cultures should be grown under appropriate aerobic or anaerobic conditions and temperature to late log phase (typically 16-24 hours), gently centrifuged and resuspended to provide a concentration of approximately 10^{-8} to 10^{-9} colony forming units (CFU) per mL for bacteria (or 10^{-6} to 10^{-7} (CFU/mL for yeast). Typically, the concentration adjustment is performed turbidimetrically.

(c) The ratio of test rinse to microorganism should be such that the microbial suspension is diluted by a factor of 10 (i.e. 9 volumes of rinse to 1 volume of organism suspension).

(d) Tests conducted with *in vitro*-grown reference strains should be conducted in the presence of a biological fluid (such as sterile heat-inactivated serum) to account for possible interaction with proteins and other constituents of saliva that the products would be exposed to during use. Tests conducted with wild-type organisms in saliva would not require the addition of any other biological fluid.

(e) Tests should be conducted at 37° C.

(f) The experimental endpoint will be surviving organisms expressed as colony forming units (CFUs) per mL present in the reaction mixture after 30 seconds of incubation with test product (determined by appropriate dilution and plating on solid media).

5. Success Criteria:

(a) The kill kinetics determination for each microorganism will be considered valid if the average level of surviving organisms in the negative control (water) group is at least 10^6 to 10^7 CFU per mL, **and** the average level of surviving organisms in the reference product group is at least three logs lower than the negative control group.

(b) The test formulation will be considered effective for each microorganism if the average level of surviving organisms for each test strain (including saliva) is no more than 0.25 logs higher than the average level of surviving organisms in the reference product group.

B. *In vivo* Antiplaque/Antigingivitis Activity

1. **Purpose and Principle:** The purpose of *in vivo* final formulation testing is to assure that the test formulation has comparable effectiveness to that of the reference standard against bacteria in a biofilm, i.e., dental plaque, and gingivitis. A clinical test assessing direct effectiveness against a biofilm is required because the way a product is formulated can affect the ability of the active agent to penetrate the biofilm with sufficient rapidity during a 30 second rinse, thereby affording the active agent access to the plaque bacteria. Additionally, it is well documented that bacteria

contained in biofilms are considerably less susceptible to the effects of antimicrobial agents than the planktonic bacteria (organisms in suspension) used for in vitro kill kinetics determinations. Because the purpose of the study is to determine comparability of effectiveness with regard to the reference standard, and not to establish effectiveness of an antigingivitis ingredient *de novo*, a short-term clinical model is appropriate.

2. **Test Method:**

- (a) The *in vivo* activity of the test product should be demonstrated in a clinical trial using an experimental gingivitis model of at least 2 weeks' duration.
- (b) The study will include 3 cells: the reference standard (positive control); test formulation; and an appropriate negative control.
- (c) Subjects will be selected who are representative of the target population for the product, e.g., they will have baseline levels of supragingival plaque and gingivitis that indicate their usual mechanical oral hygiene procedures are not optimal. Mean baseline plaque and gingival indices will be scored for use as covariates in the statistical analysis.
- (d) After the baseline examinations, subjects will receive a complete dental prophylaxis.
- (e) Subjects will rinse twice daily for 30 sec with 20 mL of their assigned mouthrinse for the duration of the study, and will be required to refrain from any other mechanical or chemotherapeutic oral hygiene procedures during this period.
- (f) The oral examinations, including scoring of plaque and gingival indices, will be repeated at the conclusion of the study period.

3. **Success Criteria:**

- (a) The study will be considered valid if there are statistically significant differences in both plaque and gingivitis levels between the reference standard and negative control groups at the conclusion of the study, in the direction of greater efficacy for the reference standard.
- (b) Formulation comparability is established if the test formulation satisfies noninferiority statistical criteria for both plaque and gingivitis with respect to the clinically tested standard. Pfizer endorses the first noninferiority testing alternative presented in section 5.1.3 of the comments submitted on November 25, 2003 by the Joint Oral Care Task Group (the Task Group) of the Consumer Healthcare Products Association (CHPA) and the Cosmetic, Toiletry, and Fragrance Association (CTFA). Specifically:

This approach has three requirements in a single study that includes a test product, a reference product (positive control), and a negative control. Each of these requirements can be assessed using an appropriate analysis of variance or analysis of covariance model. Since all of the following criteria must be

simultaneously met, no multiple comparison adjustments are required.

1. The reference product mean must be statistically significantly superior to the negative control (two-sided 5% type I error rate)
2. The test product must be statistically significantly superior to the negative control (two-sided 5% type I error rate)
3. The test product must be demonstrated to be statistically significantly superior to the average of the negative control and the reference product (one-sided 5% type I error rate)

Requirements 1 and 2 are assessed via direct contrasts of the reference and test product means with the negative control mean. Requirement 3 can be assessed by calculating a 95% one-sided confidence interval for the following contrast of means (μ_s) in a statistical model and comparing that bound to 0. Noninferiority is concluded if the appropriate bound is less than 0.

$$\mu_{\text{Test}} - \frac{1}{2}(\mu_{\text{Neg}} + \mu_{\text{Reference}})$$

Requirement 1 ensures that the reference product is demonstrated to be superior to the negative control in the study. This helps ensure the validity of the study. Requirement 2 ensures that the test product is superior to negative control. Requirement 3 ensures that the test product is substantially more similar to the reference product than to the negative control.