

Appendix 1

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Responses To Agency Questions And Technical Summary

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RESPONSES TO AGENCY QUESTIONS AND TECHNICAL SUMMARY

Responses to Specific Issues Arising from the 3/20/00 Teleconference

In the March 20, 2000 teleconference between FDA and Warner Lambert, the Agency raised several questions regarding the Intraoral Caries Test (ICT) protocol we proposed to support a petition to amend the Anticaries Final Monograph. The petition would request permitting a variation of pH and dosing (relative to current monograph specifications) for a mouthrinse containing a combination of fluoride and the essential oils contained in Listerine® Antiseptic mouthrinse. These questions and the Company's responses are discussed below:

FDA Comment 1: "The company's choice of products for the test arms is not ideal. For a more valid comparison of the rinsing regimens, and to determine if the active antigingivitis ingredients in Listerine negatively impact on the anticaries activity of the fluoride, the same type of fluoride should be compared. If a USP reference standard is not available, an alternative is to use a marketed rinse containing 100 ppm acidulated phosphate fluoride (APF). If a marketed product is not available, one can be prepared in compliance with the monograph and used in the trial as the active control. It would also be more appropriate to use the currently marketed Listerine (without fluoride) as the negative control, since all ingredients other than the fluoride would be the same. This would be a better comparison to determine the effect of the fluoride and would rule out components of Listerine as contributing to the anticaries activity of the product."

Response: The Company acknowledges this concern, and agrees to compare the test product containing essential oils and sodium fluoride to a monograph-compliant formulation (positive control) containing the same fluoride source, administered twice daily, and to use currently marketed FreshBurst Listerine (without fluoride) as a negative control in the ICT study.

FDA Comment 2: "It is important that the proper statistical analysis is used. To comply with the anticaries final monograph, adequate demonstration of bioavailability in the biological testing models for fluoride dentifrices requires that the test product be significantly superior to placebo and equivalent to the reference standard. Therefore, we feel it is logical to request that W-L use appropriate statistical testing to demonstrate that the proposed combination oral rinse product is superior to placebo and not less effective than the active control. It appears as though W-L may be proposing to provide p values from a test designed to detect difference between groups, which does not allow extrapolation of conclusions about equivalence."

Response: The Company acknowledges this concern, and agrees that equivalence testing would be desirable, providing that it is technically and logistically feasible to perform a study with sufficient statistical power to permit the requisite statistical tests. We have pursued this possibility and have determined that *power calculations using data from multiple studies using the intraoral caries model (data provided by Drs. A. Dunipace and D. Zero, personal communication) require a subject population of at least 90 subjects to permit "at least as*

good as" statistical testing. Investigators experienced in the use of this model have indicated that there are practical limitations to the number of subjects in a given study, and that it would not be possible to run an ICT study with 90 subjects or more (D. Zero, G. Stookey, A. Dunipace, personal communication). This is discussed further in the ICT Study summary (below).

In view of this, we propose to conduct an ICT study in which statistical testing is used to establish that 1) the test product and dosing regimen is significantly superior to the negative control in providing enamel remineralization, and 2) the study is valid (i.e., the positive control is significantly superior to the negative control). We also propose that a statistical test be performed to determine whether the test regimen is significantly less effective than the positive control. While we acknowledge that this statistical testing will not establish equivalence of the test and active regimens according to a strict statistical definition, these tests would indicate that there is a reasonable expectation that the test product and dosing regimen provide meaningful anticaries efficacy.

Additionally, in order to support the effectiveness conclusions of the ICT study and provide "at least as good as" (i.e. one sided equivalency) testing between the experimental and positive control dosing regimens, we propose performing two additional studies in which statistical comparisons of test product and positive controls can be performed: 1) a human clinical fluoride clearance study to confirm that the test dosing regimen is "at least as good as" the reference dosing regimen in elevating salivary fluoride levels after use and 2) a rat caries test in which a comparison of anticaries efficacy can be made between the test product and positive controls and in which a determination can be made of whether Listerine essential oils or pH affect the anticaries activity of the test product.

Protocols for the proposed studies are provided in Appendices 2-4.

The agency also raised several questions regarding several of the subject selection criteria in the ICT protocol. These are addressed below.

FDA Comment 3: "Under inclusionary criterion #3, it is stated that adequate salivary flow is required. How will this be verified? If the subjects are excluded, will the labeling reflect this?"

Response: This criterion was included as a measure to reduce variance in the model, but is not vital for conduct of the test. To eliminate this issue, the current ICT protocol does not have salivary flow rate as an inclusion criterion.

FDA Comment 4: "Under exclusionary item #3, the statement "Any medical condition that could be expected to interfere with the subjects' safety during the study period" should be more specific if possible."

Response: This criterion was specified to protect subjects with medical conditions that might place them at risk subsequent to oral manipulation. The current ICT study protocol will contain language excluding subjects with "a condition requiring the use of pre-

medication for any dental procedures". This will be understandable to any clinician skilled in the art.

FDA Comment 5: "Since this product will be available OTC, individuals with severe gingivitis will most likely be using it, if so, they should not be excluded as stated in exclusionary item #4 "significant dental or oral soft tissue pathology such as severe gingivitis or active periodontal disease".

Response: As in the case of salivary flow rates, this criterion was intended to reduce variability in the study. The current ICT protocol will not include this exclusionary criterion.

FDA Comment 6: Regarding exclusionary item #5, "one or more carious lesions" the same comment applies here as to the above comments

Response: As in the case of salivary flow rates, this criterion was intended to reduce variability in the study. The current ICT protocol will not include this criterion.

FDA Comment 7: "Regarding information that should be submitted in the citizen's petition, W-L was told that the Agency would need to see data re: antiplaque/antigingivitis efficacy as well as anticaries efficacy, in order to determine whether either active ingredient(s) affected the performance of the other(s)."

Response: The Company had agreed to concurrently submit study data developed using a protocol design considered appropriate for Final Formulation Testing of antiplaque/antigingivitis mouthrinses. Protocols for the planned performance tests are submitted as Appendices 5 and 6.

Additional Information:

In addition to the issues discussed above, there are two specific areas where the studies presented herein differ from those previously discussed with FDA:

1. **Proposed ICT model:** The intraoral caries test protocol included herein is based on the ICT model of Zero *et al.* (1995). It differs from the previously discussed ICT model of Dunipace *et al.* principally in the design of the intraoral device (with buccal, rather than interproximal placement of enamel chips), the measure of remineralization (surface microhardness testing rather than microradiography) and the length of treatment legs (two rather than four weeks). The change in model is based on the following considerations:
 - Additional data provided by the principal investigator subsequent to the March 20 teleconference indicate that the variation of this model, using microhardness testing, is smaller than models using microradiographic assessment of lesions.
 - Additional data provided by the principal investigator subsequent to the March 20 teleconference indicated that a treatment effect of a fluoride mouthrinse can be demonstrated using microhardness test.

- The shorter treatment legs of the Zero model (2 weeks v. 4 weeks) impose less of a burden on the test subjects, and may provide for improved compliance and fewer dropouts.
- As with the Dunipace model, the present model has been previously shown to be a valid, workable model.
- Both microradiographic and microhardness testing have been widely used in the research community and for FDA submissions.

These considerations suggest that the currently proposed ICT model, which exhibits less apparent variability than the Dunipace model, will have comparable validity.

2. **Change in proposed formulation:** In prior discussions with the FDA, we focused attention on a proposed product in which Listerine essential oils would be incorporated into an acidulated phosphate fluoride vehicle (pH 4.2) containing 0.01% fluoride ion. This product was referenced to the APF rinse (pH 3.5, 0.01% fluoride ion) described in 21 CFR § 355.10(a)(3)(ii). **In the interim, we have obtained additional information which leads us to recommend that the proposed product be one in which 0.02% sodium fluoride is incorporated into a current Listerine mouthrinse product.** In contrast to the previously considered APF Listerine combination, the monograph-compliant reference product for a Listerine formulation with 0.02% sodium fluoride would be the 0.02% sodium fluoride rinse described in 21 CFR § 355.10(a)(3)(iii), which has a pH of about 7.

The issues of difference in pH and dosing for the currently proposed product and the monograph-compliant reference product remain the same as in the past. The proposed product and the monograph-compliant reference products differ in pH (4.2 versus 7, respectively). Additionally, the proposed product dosing regimen differs from that of the corresponding monograph formulation (20 ml for 30 seconds BID, versus 10 ml for 60 seconds BID, respectively). It is important to note that these differences are addressed in the three fluoride study protocols contained herein.

Rationale for formulation change: The change in formulation is based on two compelling considerations:

- **Better taste acceptability, leading to greater expectations of compliance.** Preliminary consumer feedback regarding the APF Listerine prototype indicated that the incorporation of the acidulated phosphate system into Listerine raised substantial issues of objectionable taste. These, in turn, raise significant concerns regarding expected compliance by consumers with the recommended dosing regimen. When 0.02% sodium fluoride was added to current Listerine products, consumers could detect no difference between the test product and the original product.

Greater similarity to the currently marketed product. The addition of sodium fluoride to the current FreshBurst Listerine formulation introduces far less variation from the clinically tested antiplaque/antigingivitis product than the incorporation of an acidulated phosphate fluoride system. Thus, there is an even greater expectation that the new product with fluoride would possess the same level of antiplaque and antigingivitis effectiveness as the currently marketed products. This will be confirmed with the appropriate performance testing.

SUMMARY OF PROPOSED STUDIES

Study 1: Intraoral Caries Test (ICT) (Clinical Protocol 936-9213)

Questions addressed:

1. Does FreshBurst Listerine with 0.02% sodium fluoride provide anticaries efficacy?
2. Does changing the dosing regimen from a 60 sec./10 ml rinse to a 30 sec./20 ml rinse affect efficacy?
3. Does the fixed combination of Listerine essential oils interfere with fluoride?

The study model will be the intraoral caries test described by Zero *et al.*, with the cells listed below. This crossover study will start with 42 subjects, with the intention that at least 36 subjects will complete all three legs.

Success Criteria: The test product and dosing regimen will be considered to provide anticaries effectiveness if it produces significantly greater remineralization than the negative control, and is not statistically lower in remineralizing efficacy than the positive control product regimen. Additionally, the experiment must be valid (i.e. the positive control produces significantly greater remineralization than the negative control). An additional statistical test will be performed to determine whether the test leg is significantly less effective than the positive control leg; however, this would not establish equivalence between the test and positive control legs. *A statistical test of equivalence (either one- or two-sided) between the test leg and the monograph-compliant positive control leg cannot be performed in this model, as it is not possible to run a study with enough subjects to provide adequate statistical power.* This is discussed in greater detail below.

Test cells for Intraoral Caries Test:

#	Function	Description of test solution	Dosing Regimens
1	Negative control	FreshBurst Listerine (no fluoride)	20 ml for 30 seconds
2	Test product	FreshBurst Listerine with 0.02% NaF	20 ml for 30 seconds
3	Positive control	FDA monograph compliant neutral 0.02% NaF rinse without Listerine essential oils	10 ml for 60 seconds

The primary endpoint will be enamel surface microhardness change (remineralization). Fluoride uptake will be a secondary efficacy measure.

Statistical testing will establish that the test product and positive control are both significantly better than the negative control. An additional statistical test will be performed to determine whether the test leg is significantly less effective than the positive control leg.

A separate salivary fluoride clearance study will be conducted to demonstrate the equivalence of fluoride availability in the oral environment after two dosing regimens. A separate animal caries test will be conducted to demonstrate the dose-independent equivalence of the anticaries effectiveness of the test product and the monograph-compliant neutral sodium fluoride positive control.

Discussion of statistical power and feasible population size considerations:

For the ICT model to be used here (Zero, 1995), the number of subjects required to provide 80% power for an "at least as good as" analysis between the proposed combination mouthrinse and positive control treatment is at least 90. This is based on an estimate for coefficient of variation (c.v.) of 40%, based on observed c.v.'s (range 33% - 50%) from previous studies using the Zero ICT model. Additionally, size calculations using c.v.'s from the Dunipace model [microradiographic endpoint] indicated that a population of 310 subjects would be required to permit "at least as good as" testing in a study using this model. Discussions with experienced investigators (D. Zero, A. Dunipace, G. Stookey) led to the conclusion that reliable ICT studies of this size cannot be conducted for a number of technical and logistical reasons including: a) a limitation of enough suitable subjects for the model at any given center; the steeply increasing difficulty of maintaining subject compliance over the duration of the study; and managing technical and analytical aspects of the study as the subject population markedly increases. To the knowledge of our consultants, the largest ICT study ever conducted by an investigator used 60 subjects; this is well below the current sample size estimate.

Study 2: Salivary Fluoride Clearance Test (Clinical Protocol 936-9201):

Question addressed:

Is a dosing regimen of 30 sec./20 ml rinse "at least as good as" a regimen of 60 sec./10 ml for delivery and clearance of fluoride from the mouth?

The study model will be the salivary clearance test described by Zero *et al.*, with the cells listed below. Success Criteria: The test dosing regimen (20ml for 30 seconds) will be considered "at least as good as" the reference regimen (10 ml for 60 seconds) if the lower limit of the one-sided 95% lower confidence interval for the ratio of the test dosing regimen to reference dosing regimen averages is at least 0.8 (i.e., 80%). *Since this model can be powered to provide for statistical comparison of the test and reference regimens, we will test the hypothesis that the test regimen provides fluoride delivery that is "at least as good as" the reference regimen.*

Test Cells for the Salivary Clearance Study

Function	Test Material	Dosing Regimens
Negative control	FreshBurst Listerine (no fluoride)	20 ml for 30 seconds
Test regimen	FreshBurst Listerine with 0.02% NaF	20 ml for 30 seconds
Reference Regimen	FreshBurst Listerine with 0.02% NaF	10 ml for 60 seconds

The endpoint will be salivary fluoride concentration over a 120-minute period after rinsing, expressed as the area under the curve of log-transformed salivary fluoride concentration over time.

The statistical test will establish that the test regimen is "at least as good as" the reference regimen control, and that both fluoride rinse regimens are significantly better than the negative control rinse.

Study 3: Rat Caries Test (Research Protocol 936-9212):

Questions addressed:

1. Does the fixed combination of Listerine essential oils interfere with fluoride effectiveness or in itself have an anticaries effect?
2. Does FreshBurst Listerine with 0.02% sodium fluoride provide anticaries efficacy in a rat caries model?
3. Does FreshBurst Listerine with 0.02% sodium fluoride have anticaries efficacy that is "at least as good as" a monograph-compliant neutral 0.02% sodium fluoride product?
4. Does pH have an effect upon the anticaries activity of 0.02% sodium fluoride?

The study model will be the rat caries test employed by the University of Rochester, with the cells listed below. ***This model can be powered to provide for "at least as good as" statistical comparison of the test and positive control products, although it is not suitable for evaluating different dosing regimens.*** **Success Criteria:** The test product will be considered to have anticaries efficacy if the caries score for the test group is statistically significantly lower than the mean for both of the fluoride-free groups, and the anticaries effectiveness of the test group is "at least as good as" that of the positive control group. While this model is neither a human clinical model nor a test suitable for evaluating dosing differences, it is a valid anticaries model recognized by FDA as necessary for supporting the comparability of anticaries efficacy of test and positive control dentifrices (independent of dosing).

Test Cells for the Rat Caries Test:

Cell #	Function	Product Name
1	Negative Control	Water
2	Test Mouthrinse	FreshBurst Listerine with 0.02% NaF
3	Fluoride-free Treatment Control	FreshBurst Listerine (no fluoride)
4	Sodium Fluoride Treatment Control	0.02% NaF mouthrinse (pH 4.2)
5	Positive Control	FDA monograph compliant neutral 0.02% NaF rinse without Listerine essential oils

The results will need to demonstrate:

- Listerine ingredients themselves do not affect anticaries effectiveness of sodium fluoride (cell 2 is equivalent to cell 4)
- A pH change will not negatively impact anticaries efficacy (cell 4 is equivalent to cell 5)
- The mouthrinse containing Listerine ingredients and sodium fluoride has anticaries efficacy (cell 2 is significantly better than cells 1 and 3)

- The mouthrinse containing Listerine ingredients and sodium fluoride is "at least as good as" the positive control (cell 2 is at least as good as cell 5)
- For study validation, the positive control must be significantly better than fluoride-free controls (cell 5 is significantly better than cells 1 and 3)

Study 4: Experimental Gingivitis Study (Research Protocol 931-1309):

Questions addressed:

Does FreshBurst Listerine with 0.02% sodium fluoride provide antiplaque and antigingivitis activity that is "at least as good as" current Listerine Antiseptic?

The study model will be the two-week experimental gingivitis reviewed by the FDA Plaque Products Subcommittee, with the cells listed below. Success Criteria: The test product will be considered to have antiplaque and antigingivitis efficacy if the plaque and gingivitis scores of the test and positive control groups are both significantly lower than the negative control group, and the antiplaque and antigingivitis effectiveness of the test group is "at least as good as" that of the positive control group.

Test Cells for the Experimental Gingivitis Study:

Cell #	Function of the Cell	Product Name
1	Negative Control	5% hydroalcoholic control (no essential oils)
2	Test Mouthrinse	FreshBurst Listerine with 0.02% NaF
3	Essential Oil Positive Control	Listerine Antiseptic

Study 5: Kill Kinetics Study (Research Protocol 936-9216):

Question addressed:

Does FreshBurst Listerine with 0.02% sodium fluoride provide rapid bactericidal activity that is "at least as good as" current Listerine Antiseptic?

The study model will be the kill kinetics test reviewed by the FDA Plaque Products Subcommittee, with the cells listed below. Success Criteria: The test product will be considered to have bactericidal activity comparable to the positive control if the mean log transformed colony forming unit (CFU) per ml count for the test product is no more than 0.25 log units higher than that of the positive control. In addition, the means for both the test product and positive control product must be least 3 logs lower than the mean for the negative control for each test organism.

Test Cells for the Kill Kinetics Study:

Cell #	Function of the Cell	Product Name
1	Negative Control	5% hydroalcoholic control
2	Test Mouthrinse	FreshBurst Listerine with 0.02% NaF
3	Essential Oil Positive Control	Listerine Antiseptic