Acceptance Program Guidelines

Fluoride-Containing Dentifrices

American Dental Association
Council on Scientific Affairs 2005

FLUORIDE-CONTAINING DENTIFRICES

Council on Scientific Affairs

Scope:

These guidelines apply to fluoride-containing dentifrice products used for the control of dental caries. Such products may further contain active agents to control other dental indications such as hypersensitivity, gingivitis, and calculus. However, these guidelines only address requirements necessary to determine the anti-caries efficacy of such products. Each additional clinical indication will require either further clinical and/or laboratory proof as defined in the appropriate guidelines. If no guidelines are available for a clinical indication, then the manufacturer should support the indication based on the preponderance of evidence.

I. SUBMISSION DIRECTIONS

1. General Information

A Submissions are to be sent to the Council Office:
Director, Acceptance Program
Council on Scientific Affairs
American Dental Association
211 East Chicago Avenue
Chicago, Illinois 60611 - 2678

B Submissions are to be sent in triplicate, along with one single-sided copy for duplicating purposes. Three copies of the submission should also be supplied in an indexed CD-ROM format with appropriate links to PDF files. For each product, three samples from three different lots, and three placebo samples shall be provided. Market samples are preferred. If possible the submission should be less than 200 pages exclusive of appendices.

C A manufacturer is advised that the review process is complex. Typically, notification of Council action may be expected 90 to 150 days from the receipt of a complete submission by the Council. More time may be required if additional information or clarification is needed from the manufacturer.

D When a product is classified as "Accepted" the classification is for 5 years. Renewal of the classification will be considered by the Council upon request by the manufacturer.

E Companies with Accepted products are subject to the conditions stated in the Agreement Governing Use of ADA Seal of Acceptance.

2. Arrangement of a Submission

A The submission is to be divided into sections and arranged in order as indicated in Part II.

II. INFORMATION TO BE SUBMITTED

1. Cover Page

A Name of company

B Product name

2. Table of Contents

3. Company Information

A Name of company (to be used in official list of Accepted Products)

B Address (to be used in listing)

C Phone Number (to be used in listing)

D Fax Number

E Names of owners, officers and other individuals authorized to furnish information to the Council and represent the firm in dealing with the Council, including the main contact person. (Foreign manufacturers must have an office or branch located in the United States and the product must be available for purchase in the United States).

F Names and qualifications of scientific personnel responsible for formulation and testing of the product.

4. Summary of Submission

Comprehensive summary of the information submitted on safety and effectiveness of the fluoride-containing products used for the control of caries.

5. Product Information

A Name of product (to be used in listing).

B Claims of efficacy.

(i) All claims of efficacy, including all health benefit claims, e.g., caries reduction, and all claims that imply a health benefit, e.g., reduction of plaque acids or enhanced remineralization, must be documented.

NOTE. Requirements for all superiority and equivalency health benefit claims and all superiority and equivalency claims, which imply a health benefit, are included in the ADA Guidelines for Determination of Efficacy in Product Evaluation (1999).

(ii) Claims should avoid disparagement of other products.

C Patent title(s) and patent number(s) relating to the product.

D Product description.

(i) Chemical composition and amounts

E Instructions including indications and contraindications for use, warnings, etc.

F Labeling/packaging – All labeling/packaging must be approved by the Council before use.

G Advertising and Promotional materials – Advertising and promotional materials do not need to be submitted routinely for review and approval before use. Therefore, inclusion of sample advertising and promotional materials in the submission is voluntary. However, all advertising and promotional materials shall comply with the ADA Advertising Standards.

H Council Statement – The Council will provide a statement specifying why the product was granted the ADA Seal of Acceptance. The statement must be used on all packaging/labeling and advertising/promotional materials whenever the Seal is used, or when it is mentioned that the product is ADA Accepted (See example in III. below).

6. Quality Control Procedures for the Manufacturing of the Product

7. Efficacy Data

The following types of dentifrice product will be considered for ADA Acceptance:

A For dentifrice formulations which are identical or similar in chemical composition to previously ADA-accepted products, the manufacturer of these products will be required to submit supporting data in each of the following categories:

1. Total fluoride;
2. Available fluoride in fresh and aged samples;
3. One minute fluoride release rate in fresh and aged samples; and
4. Bioavailability in demineralized enamel.

For additional information, see Appendix I

B New Dentifrice Formulations: When a substantial difference exists in the chemical composition of a new formulation - i.e., a new fluoride species, a new abrasive compound, or a fluoride concentration not approved for marketing in the FDA OTC Monograph on Anticaries Drug Products for OTC Human Use (reference G) - caries clinical trials will continue to be required.

The clinical design requirements for caries studies are presented in Appendix II.

8. Safety Data

All submitted dentifrices must meet ISO 11609 (1995): Dentistry- Toothpastes- Requirements, Test Methods and Marking. In cases where new agents that do not appear on the Generally Recognized As Safe list (reference F) have been introduced into a fluoride dentifrice, animal and/or clinical studies must be submitted which include examinations of oral soft and hard tissues, toxicological studies, and microbiological profiles that should demonstrate that pathogenic or opportunistic microorganisms do not develop over the course of the study. Also there may be occasions where an ingredient has an established record of safe use in the oral cavity but for some reason it does not appear on the GRAS list. In this case, the testing specified in these guidelines may not be required, but the manufacturer should supply supporting data and a rationale for it being considered safe to use in the dentifrice.

A Effect on oral soft tissues: Evidence of the potential effects of any new agents in the product on oral soft tissues should be provided. This includes the development of abnormal conditions, such as candidiasis, oral ulcerations, or other manifestations of opportunistic microorganisms that proliferate and may lead to secondary mucosal lesions.

B Effect on oral hard tissues: Evidence of the potential effects of any new agents in the product on oral hard tissues should be provided.

C Effect on dental restorations: Evidence of potential adverse effects of new agents on dental restorations (e.g. composite resins, porcelain, etc.) should be provided.

D Toxicology: Information submitted for potential effects of new agents in the products shall include assessments of possible toxic effects of these agent(s) or adverse effects of the product formulation. These should include standard toxicological profiles depending on the particular product. All products must submit data on the mutagenicity and the carcinogenicity of all new agents.

E Microbiology: For products with a new agent(s), evidence of the potential effects on oral flora should be provided. Monitoring oral flora should be conducted in patients during the study for the development of opportunistic and pathogenic organisms.

9. **Comprehensive Bibliography**

10. **Copies of Most Significant Articles**

11. **Appendices**

Detailed descriptions of test evaluation methods and any other defined areas should be included.

III. STATEMENT

Statements to be used for products classified under these guidelines including qualifiers for other demonstrated indications.*

"The ADA's Council on Scientific Affairs Acceptance of (Product Name) is based on its finding that the product is effective in helping prevent or reduce tooth decay when used as directed".

*This statement will be modified to include other demonstrated indications, if any, such as reducing plaque and gingivitis, preventing early caries, reducing hypersensitivity or controlling oral malodor.

IV. REFERENCES FOR FURTHER EXPLANATION

The following references were used in the development of these Fluoride Dentifrice Guidelines. They can be consulted for a more detailed discussion of issues addressed in these Guidelines.

1. Guidelines for the Acceptance of Fluoride-Containing Dentifrices. JADA 1985; 110:545-547.

2. Report of workshop aimed at defining guidelines for caries clinical trials: superiority and equivalency claims for anticaries dentifrices. JADA 1988; 117:663-665.

3. Clinical Aspects of De/Remineralization of Teeth, Proceedings of Models Conference 1994. Advances in Dental Research 1995; 9(3).

4. Comparative Attributes for the Description of the Relative Efficacy of Therapeutic Agents: General Concepts and Definitions, and Application to the ADA Guidelines for the Comparison of the Clinical Anticaries Efficacy of Fluoride Dentifrices. J. Clin. Dent. 1995; 3:176-184.

5. ISO Standard No. 11609-1995, Dentistry-Toothpastes-Requirements, Test Methods and Marking. New York: American National Standards Institute.

6. Substances Generally Recognized as Safe. Food and Drug Administration 2004; 21 CFR 182.

7. Anticaries Drug Products for OTC Human Use. Food and Drug Administration 2004; 21 CFR 355.

8. ADA Council on Scientific Affairs. Acceptance Program Guidelines: Determination of Efficacy in Product Evaluation. Chicago: American Dental Association, 1999.

9. ADA Council on Scientific Affairs. Acceptance Program Guidelines: Clinical Trial Protocols. Chicago: American Dental Association, 2003.

Appendix I
Experimental Design Protocol for Dentifrices Identical or Similar to Previously ADA-Accepted Products

This category relates to required laboratory data for dentifrice formulations that do not contain a new fluoride source or abrasive compound. The design of each profile study must either be in accordance with existing recognized research methodology or must be justified by the manufacturer.

1. **Total Fluoride**
   See Test 1.

2. **Available Fluoride in Fresh and Aged Samples**
   Chemical data must be submitted to document that the active fluoride agent is chemically free and available in both fresh and aged samples. At least 90% of the labeled amount of fluoride must be available in both fresh and aged samples. Fresh samples are defined as those prepared and analyzed within one month of formulation. Aged samples are defined as product compositions at the effective end of their expiration period. These samples can be aged either under normal, representative conditions, or by high temperature accelerated aging. In addition, the manufacturer must submit laboratory data to demonstrate the effect of both dilution and of pH on the product as compared with clinically tested formulations of compositional equivalence. See Tests 2a and 2b (Other validated methods will be considered).

3. **One Minute Fluoride Release Rate in Fresh and Aged Samples**
   Chemical data must be submitted to show that at least 80% of the labeled amount of fluoride must be released by the test formulation in fresh and aged samples within one minute of homogenization within a 1:3 dilution with water or saliva (human or artificial). See Tests 3a, 3b and 4 (Other validated methods will be considered).

4. **Bioavailability in Demineralized Enamel**
   Each test dentifrice must demonstrate an ability to deliver and incorporate levels of fluoride into demineralized enamel equivalent to clinically tested formulation(s). In general, demineralized enamel refers to subsurface lesions produced in enamel that are a result of a short-term acidic treatment within a solution partially saturated with respect to hydroxyapatite. The specific methodology to be used in preparing the needed demineralized lesion is optional. However, the rationale for utilizing a particular technique must be justified. Minimally, each study must contain: an appropriate placebo (test product minus active fluoride agent), test product in a formulation equivalent to that to be manufactured, and the clinically tested formulation or ADA Accepted compositional equivalent containing the same active agent/abrasive system.

5. **Statistical evaluation of bioavailability laboratory data**
   The statistical analysis of each study must clearly separate the test product from the placebo and strongly suggest that the test formulation is equivalent to a clinically tested formulation(s). These studies must have adequate statistical power. Manufacturers are responsible for the statistical analysis of their results.

   If the manufacturer should decide to submit “intra-oral" test models, the Proskin-Chilton-Kingman statistical approach (Proskin H. M. J. Dent. Res. 71, 949-952 (1992)) is recommended. This method was proposed with the intent of improving the linkage between the model data and the determination of clinical efficacy. The analysis requires that a test product perform better than the value computed as the average of the 250 ppm product and the gold standard (reject H_0: $u_t<(u_{250}+u_s)/2$ at $p<0.05$, in favor of H_l:$u_t>(u_{250}+u_s)/2$), and that there is a reasonable likelihood that a specific relationship holds between model and clinical response. If these

requirements are met, there is a high likelihood that the test product will perform within at least 90% of the gold standard.

For test profiles, such as fluoride uptake or models that predominately emphasize demineralization or remineralization, which are not intended to simulate the complete caries process, a 90% confidence interval statistical approach similar to that utilized by the FDA in its assessment of bioequivalency will need to be implemented. Two test formulations whose performance in such tests differ by -20%/+25% or less will be considered bioequivalent. In order to verify this rule, two one-sided statistical tests are carried out with either the original or log transformed data. The first test is used to assess if the test product is more than 20% less effective in its performance in the specific test profile, while the second test is used to guarantee that the test product is not more than 25% greater in its performance. In this second case, potential superiority suggests that the chemical and biochemical properties of the test product are substantially different from that of the positive control. This may or may not be beneficial to the overall performance of the product in a clinical setting. It would be the manufacturer's responsibility to support why such differences are not detrimental.

Both one-sided tests will be carried out at the 0.05 level of significance. Computationally, the two one-sided tests are carried out by computing the 90% confidence interval. The manufacturer must demonstrate that a 90% confidence interval for the ratio of the mean response of the test product to that of the positive control ("gold standard") is within the limits of 0.8 to 1.25 using the original or log transformed data.

Test 1

Total Fluoride

Samples:
Three samples from three lots of each formulation (e.g. paste/gel, flavor) should be analyzed.

Chemicals (Use reagent grade chemicals only):
Deionized water
EDTA, 0.1 M (pH=8.0 adjusted by adding NaOH as necessary)
Fluoride standard (commercially available or prepare with NaF)
$HClO_4$, conc. (70-71%)
Sodium hydroxide (NaOH), 0.5 N in ethanol
TISAB II (Total Ionic Strength Adjustment Buffer II, by Orion)

Equipment and instrument:
Balance (readable to 0.01 g)
Centrifuge (at least 16000 *g* force)
Dessicator
Flask (20 to 100 mL)
Fluoride ion selective electrode
Graduated cylinder (15 to 50 mL)
Non-aerating stirrer
Oven (± 5 °C at 60°C)
pH meter with resolution at least 0.1 mV
Pipette (1.0 mL)
Plastic bottle (≥ 100 mL)
Plastic vials (50 × 16 mm, flat bottom)
Polystyrene petri dishes (60 × 15 mm)
Volumetric flasks (100 mL and 10 mL)
Washing bottle

Procedure:

I. Preparation of Standard Solution for Calibration:
Make successive dilutions of the stock fluoride standard to obtain a set of working standards which includes 1, 2, 5, 10, and 20 ppm of fluoride.

II. Preparation of the Fluoride Electrode and Calibration Curve:
Pipette 1.0 mL of standard into a 50 × 16 mm flat-bottom plastic vial. Add 1.0 mL of TISAB II and mix thoroughly.

Insert the fluoride electrode into the vial. Make sure no air bubbles are trapped under the electrode.

Record the mV reading to 0.1 mV after 4 minutes.

Measure every standard at least twice, until the difference of mV reading of the same standard is less than 0.2 mV.

Construct a calibration curve of mV vs. Log [F^-] (ppm).

III. Preparation of the Petri Dish:

Coat the insides of the Petri dish lids with 0.3 mL NaOH ethanol solution.

Put the lids in the desiccator until the lids are completely dry.

Keep the lids in the desiccator until ready to use.

IV. Preparation of Sample:

In duplicate, weigh 1.0 ± 0.10 g (accurate to 0.01 g) of each toothpaste sample in flasks (20 to 100 mL).

Use a graduated cylinder or a pipette to add 15 mL of 0.1M EDTA solution to the flask and homogenize with a non-aerating stirrer.

Transfer all the slurry to a 100 mL volumetric flask.

Use a wash bottle containing 0.1M EDTA to wash off the residual in the flask and on the non-aerating stirrer. Transfer the washing to the volumetric flask containing the slurry.

Dilute to the mark on the volumetric flask with 0.1M EDTA and mix thoroughly.

Transfer all the contents to a plastic bottle.

Centrifuge approximately 5 mL of the liquid for 10 minutes.

Pipette 2 mL of the supernatant into a Petri dish.

Add 4 mL of conc. $HClO_4$ in the Petri dish and immediately cover with NaOH coated lids. <u>This step must be done with extreme care to prevent wetting the lids with the foam that forms after adding the acid.</u>

Place the covered Petri dish with the sample in an oven at $60^{O}C$ for 6 hours.

Remove the Petri dish from the oven and let it cool to room temperature.

Remove the lid and wash it (but not the dish) with 4 mL deionized water twice. Transfer the washing solution to a 10 mL volumetric flask and dilute with deionized water to the mark on the volumetric flask. This is the sample solution for total fluoride measurement.

V. Determination of Fluoride Concentration in Sample Solution:

Pipette 1.0 mL of sample solution into a 50 × 16 mm flat-bottom plastic vial. Add 1.0 mL of TISAB II and mix thoroughly.

Insert the fluoride electrode into the vial. Make sure no air bubbles are trapped under the electrode.

Record the mV reading to 0.1 mV after 4 minutes.

Use the calibration curve of standards to calculate the fluoride concentration in the sample solution.

VI. Calculation of Total Fluoride:

$$\text{Total Fluoride (ppm)} = \frac{\text{Fluoride Concentration of Sample Solution (ppm)} \times 500}{\text{Wt. of Dentifrice (g)}}$$

Report all 18 results from each formulation (3 samples of 3 lots in duplicate).

Test 2a

Measuring Fluoride Availability in Fresh and Aged Samples of NaF and SnF_2 Containing Dentifrices

Samples:
Three samples from three lots of each formulation (e.g., paste/gel, flavor) should be analyzed.

Chemicals (Use reagent grade chemicals only):
Deionized water
Fluoride standard (commercially available or prepared with NaF)
TISAB II (Total Ionic Strength Adjustment Buffer II, by Orion)

Equipment and instrument:
Balance (readable to 0.01 g)
Centrifuge (at least 16000 g force)
Flask (20 to 100 mL)
Fluoride ion selective electrode
Graduated cylinder (15 to 50 mL)
Non-aerating stirrer
pH meter with resolution of 0.1 mV or better
Pipette (1.0 mL)
Plastic bottle (≥ 100 mL)
Plastic vial (50 × 16 mm, flat bottom)
Volumetric flasks (100 mL)
Washing bottle

Procedure:

I. Preparation of Standard Solution for Calibration:

Make successive dilutions of the stock fluoride standard to obtain a set of working standards which includes 2, 5, 10, and 50 ppm of fluoride.

II. Preparation of the Fluoride Electrode and Calibration Curve:

Pipette 1.0 mL of standard into a 50 × 16 mm flat-bottom plastic vial. Add 1.0 mL of TISAB II and mix thoroughly.

Insert the fluoride electrode into the vial. Make sure no air bubbles have been trapped under the electrode.

Record the mV reading to 0.1 mV after 4 minutes.

Measure every standard at least twice, until the difference of mV reading of two same standards is less than 0.2 mV.

Construct a calibration curve of mV vs. log [F^-] (ppm).

III. Preparation of Sample:

In duplicate, weigh 1.0 ± 0.10 g (accurate to 0.01 g) of each toothpaste sample in flasks (20 to 100 mL).

Use a graduated cylinder or a pipette to add 15 mL of deionized water to a flask and homogenize with a non-aerating stirrer.

Transfer all the slurry to a 100 mL volumetric flask.

Use a wash bottle to wash off all the residual in the flask and on the non-aerating stirrer with deionized water. Transfer the washing to the volumetric flask with the slurry.

Dilute to the mark on the volumetric flask with deionized water and mix thoroughly.

Transfer all the contents to a plastic bottle. This is the sample solution.

IV. Determination of Fluoride Concentration in Sample Solution:

Centrifuge approximately 5 mL of the sample solution for 10 minutes.

Pipette 1.0 mL of supernatant into a 50 × 16 mm flat-bottom plastic vial. Add 1.0 mL of TISAB II and mix thoroughly.

Insert the fluoride electrode into the vial. Make sure no air bubbles are trapped under the electrode.

Record the mV reading to 0.1 mV after 4 minutes.

Use the calibration curve of standards to calculate the fluoride concentration in the sample solution.

V. Calculation of Available Fluoride:

$$\text{Available Fluoride (ppm)} = \frac{\text{Fluoride Concentration of Sample Solution (ppm)} \times 100}{\text{Wt. of Dentifrice (g)}}$$

Report all 18 results from each formulation (3 samples of 3 lots in duplicate).

Test 2b

Measuring Fluoride Availability in Fresh and Aged Samples of Na_2FPO_3 (Sodium monofluorophosphate, MFP) Dentifrices

Samples:
Three samples from three lots of each formulation (e.g. paste/gel, flavor) should be analyzed.

Chemicals (reagent grade only):
Deionized water
Sodium hydroxide (NaOH)
Sodium monofluorophosphate (Na_2FPO_3)
Succinic acid ($HO_2CCH_2CH_2CO_2H$)

Equipment:
Balance (readable to 0.0001 g)
Centrifuge (at least 16000 g force)
Flask (20 to 100 mL)
Graduated cylinder (15 to 50 mL)
Non-aerating stirrer
Pipette (1.0 mL)
Plastic bottle (≥ 100 mL)
Syringe filters (0.2 m pore size, PTFE or polyethersulfone)
Volumetric flask (100 mL)
Washing bottle

Instrument:
Ion Chromatography System for Measuring Monofluorophosphate:

System: Suppressed Ion Chromatography
Pump: GD50 Gradient Pump (Dionex)
Autosampler: AS50 Autosampler (Dionex)
Sample Loop: 20 µL
Column: Ion Pack AS17, 4 × 250 mm (Dionex)
Guard Column: Ion Pack AG17, 4 × 50 mm (Dionex)
Column Heater: 35 ± 1 °C (Dionex, LC30)
Eluent: Gradient elution with KOH. From 3.0 mM to 35.0 mM (Dionex, EG40 Eluent Generator).
Flow Rate: 1.5 mL/min
Detector: Conductivity at 35°C (Dionex, CD20)

Procedure:

I. Preparation of Standard Solution for Calibration:

Dissolve 0.7576 g of sodium monofluorophosphate (Na_2FPO_3) in 100 mL of deionized water. This solution contains 1000 ppm fluoride as Na_2FPO_3. This is the stock MFP standard.

Make successive dilution of the stock MFP standard to obtain a set of working standards which includes 5, 10, 20, and 50 ppm of fluoride.

II. Construct Calibration Curve:

In duplicate, inject 20 µL of each MFP standard.

Record the peak area of each MFP standard and take the average of two peak areas.

Construct a calibration curve of peak area vs. [F^-](ppm).

III. Preparation of Sample:

In duplicate, weigh 1.0 ± 0.10 g (accurate to 0.01 g) of each toothpaste sample in flasks (20 to 100 mL).

Add 15 mL of deionized water to the flask and homogenize with a non-aerating stirrer.

Transfer all the slurry to a 100 mL volumetric flask.

Use a wash bottle to wash off the residual in the flask and on the non-aerating stirrer with deionized water. Transfer the washing to the volumetric flask with the slurry.

Dilute to the mark on the volumetric flask with deionized water. Mix thoroughly.

Transfer all the contents to a plastic bottle. This is the sample solution.

IV. Determination of Fluoride Concentration in Sample Solution:

Centrifuge approximately 5 mL of the sample solution for 10 minutes.

Filter 1.0 mL of supernatant through a 0.2 µm pore filter.

Inject 20 µL of filtrate into the ion chromatography system. Record the peak area.

Use the calibration curve of standards to calculate the fluoride concentration in the sample solution.

V. Calculation of Available Fluoride:

$$\text{Available Fluoride (ppm)} = \frac{\text{Fluoride Concentration of Sample Solution (ppm)} \times 100}{\text{Wt. of Dentifrice (g)}}$$

Report all 18 results from each formulation (3 samples of 3 lots in duplicate).

Test 3a

Measuring the One Minute Fluoride Release Rate of NaF & SnF_2 Dentifrices

Samples:
Three samples from three lots of each formulation (e.g., paste/gel, flavor) should be analyzed.

Chemicals (reagent grade only):
Deionized water
Fluoride standard (commercial available or prepare with NaF)
TISAB II (Total Ionic Strength Adjustment Buffer II, by Orion)

Equipment and instrument:
Balance (readable to 0.0001 g)
Centrifuge (at least 16000 g force)
Flask (20 to 100 mL)
Graduated cylinder (15 to 50 mL)
Non-aerating stirrer
Pipette (1.0 mL)
Plastic bottle (≥ 100 mL)
Syringe filters (0.2 µm pore size, PTFE or polyethersulfone)
Volumetric flask (100 mL)
Washing bottle

Procedure:

I. Preparation of Standard Solution for Calibration:

Make successive dilutions of the stock fluoride standard to obtain a set of working standards which includes 50, 100, 200, and 300 ppm of fluoride.

II. Preparation of the Fluoride Electrode and Calibration Curve:

Pipette 1.0 mL of standard into a 50 × 16 mm flat bottom plastic vial. Add 1.0 mL of TISAB II and mix thoroughly.

Insert the fluoride electrode into the vial. Make sure no air bubbles have been trapped under the electrode.

Record the mV reading to 0.1 mV after 4 minutes.

Measure every standard at least twice, until the difference of mV reading of the two same standards is less than 0.2 mV.

Construct a calibration curve of mV vs. Log [F^-] (ppm).

III. Sample preparation:

In duplicate, weigh 4.00 ± 0.10 g accurate to 0.01 g of each toothpaste sample in flasks (20 to 100 mL).

Add deionized water equal to exactly three times the sample weight to the flask.

Mix with a non-aerating stirrer exactly 60 seconds.

Immediately, centrifuge approximately 5 mL of the sample slurry at 16000 *g* for 10 minutes.

Filter 2.0 mL of supernatant through a 0.2 µm pore filter. This is the filtrate of sample solution.

IV. Determination of Fluoride Concentration:

Pipette 1.0 mL of filtrate of sample solution into a 50 × 16 mm flat-bottom plastic vial. Add 1.0 mL of TISAB II and mix thoroughly.

Insert the fluoride electrode into the vial. Make sure no air bubbles have been trapped under the electrode.

Record the mV reading to 0.1 mV after 4 minutes.

Use the calibration curve of standards to calculate the fluoride concentration in the filtrate of sample solution.

V. Calculation of Released Fluoride:

Released Fluoride (ppm) = [F] × Dilution Factor (See Test 4)

[F] = Fluoride Concentration of Filtrate of Sample Solution (ppm)

Report all 18 results from each formulation (3 samples of 3 lots in duplicate).

Test 3b

Measuring the One Minute Fluoride Release Rate of Na_2FPO_3 Dentifrices

Samples:
Three samples from three lots of each formulation (e.g., paste/gel, flavor) should be analyzed.

Chemicals (reagent grade only):
Deionized water
Sodium hydroxide (NaOH)
Sodium monofluorophosphate (Na_2FPO_3)
Succinic acid ($HO_2CCH_2CH_2CO_2H$)
TISAB II (Total Ionic Strength Adjustment Buffer II, by Orion)

Equipment:
Balance (readable to 0.0001 g)
Centrifuge (at least 16000 *g* force)
Flask (20 to 100 mL)
Graduated cylinder (15 to 50 mL)
Non-aerating stirrer
Pipette (1.0 mL)
Plastic bottle (≥ 100 mL)
Syringe filters (0.2 µm pore size, PTFE or polyethersulfone)
Volumetric flask (100 mL)
Washing bottle

Instrument:
Ion Chromatography System for Measuring Monofluorophosphate:

System: Suppressed Ion Chromatography
Pump: GD50 Gradient Pump (Dionex)
Autosampler: AS50 Autosampler (Dionex)
Sample Loop: 5 µL
Column: Ion Pack AS17, 4 × 250 mm (Dionex)
Guard Column: Ion Pack AG17, 4 × 50 mm (Dionex)
Column Heater: 35 ± 1 °C (Dionex, LC30)
Eluent: Gradient elution with KOH. From 3.0 mM to 35.0 mM (Dionex, EG40 Eluent Generator).
Flow Rate: 1.5 mL/min
Detector: Conductivity at 35°C (Dionex, CD20)

Procedure:

I. Preparation of Standard Solution for Calibration:

Dissolve 0.7576 g of sodium monofluorophosphate (Na_2FPO_3) in 100 mL of deionized water. This solution contains 1000 ppm fluoride as Na_2FPO_3. This is the stock MFP standard.

Make successive dilution of the stock MFP standard to obtain a set of working standards, which includes 10, 20, 50, and 100 ppm of fluoride.

II. Construct Calibration Curve:

In duplicate, inject 5.0 µL of each MFP standard.

Record the peak area of each MFP standard and take the average of two peak areas.

Construct a calibration curve of peak area vs. F^- (ppm).

III. Preparation of Sample:

In duplicate, weigh 4.00 ± 0.10 g (accurate to 0.01 g) of each toothpaste sample in flasks (20 to 100 mL).

Add deionized water equal to exactly three times the sample weight.

Mix with a non-aerating stirrer exact 60 seconds.

Immediately centrifuge approximately 5 mL of the sample slurry at 16000 *g* for 10 minutes.

Filter 2.0 mL of supernatant through a 0.2 µm pore filter. This is the filtrate of sample solution.

Dilute 1.0 mL of the filtrate with 4.0 mL of deionized water. This is the test solution.

IV. Determination of Fluoride Concentration in Sample Solution:

Inject 5.0 µL of test solution into the ion chromatography system. Record the peak area.

Use the calibration curve of standards to calculate the fluoride concentration in the test solution.

V. Calculation of Released Fluoride:

Released Fluoride (ppm) = [F] × Dilution Factor (see Test 4)

[F] = Fluoride Concentration of Filtrate of Sample Solution (ppm)
= Fluoride Concentration of Test Solution (ppm) X 5

Report all 18 results from each formulation (3 samples of 3 lots in duplicate).

Test 4

Determining the Dilution Factor for the One Minute Release Rate of Fluoride and Monofluorophosphate from Dentifrices

Samples:
Three samples from three lots of each formulation (e.g. paste/gel, flavor) should be analyzed.

Chemicals (reagent grade only):
Deionized water
Br^- stock standard (1000 ppm, commercially available)
NO_2^- stock standard (1000 ppm, commercially available)

Equipment:
Balance (readable to 0.0001 g)
Centrifuge (at least 16000 *g* force)
Flask (20 to 100 mL)
Graduated cylinder (15 to 50 mL)
Non-aerating stirrer
Pipette (1.0 mL)
Plastic bottle ($\geq$ 100 mL)
Syringe filters (0.2 µm pore size, PTFE or polyethersulfone)
Volumetric flask (100 mL)
Washing bottle

Instrument:
Ion Chromatography System:
System: Suppressed Ion Chromatography
Pump: GD50 Gradient Pump (Dionex)
Autosampler: AS50 Autosampler (Dionex)
Sample Loop: 5 µL
Column: Ion Pack AS17, 4 × 250 mm (Dionex)
Guard Column: Ion Pack AG17, 4 × 50 mm (Dionex)
Column Heater: 35 ± 1 °C (Dionex, LC30)
Eluent: Gradient elution with KOH. From 3.0 mM to 35.0 mM (Dionex, EG40 Eluent Generator).
Flow Rate: 1.5 mL/min
Detector: Conductivity at 35°C (Dionex, CD20)

Procedure:

I. Preparation of Standard Solution:
Dilute the stock standards of Br^- and NO_2^- to obtain a set of combined working standards which includes 5, 10, 20, and 50 ppm of bromide and nitrite.

II. Construct Calibration Curve:

In duplicate, inject 5.0 µL of each combined standards of bromide and nitrite.

Record the peak area of each standard and take the average of two peak areas.

Construct a calibration curve of peak area vs. concentration of both Br^- and NO_2^- (ppm).

III. Preparation of Sample:

In duplicate, weigh 4.00 ± 0.10 g (accurate to 0.01 g) of each toothpaste sample in flasks (20 to 100 mL).

Add the 100 ppm combined working standard of bromide and nitrite in a quantity equal to exactly three times of sample weight to the flask and homogenize thoroughly with a non-aerating stirrer.

Transfer all the slurry to a plastic bottle.

Centrifuge approximately 5 mL of the sample slurry with 16000 *g* for 10 minutes.

Filter 1.0 mL of supernatant through a 0.2 µm pore filter.

Dilute 1.0 mL of the filtrate with 4.0 mL of deionized water. This is the test solution.

IV. Determination of Bromide and Nitrite Concentration:

Inject 5.0 µL of test solution into the ion chromatography system. Record the peak area of both bromide and nitrite.

Use the calibration curve of standards to calculate the concentration of bromide and nitrite in the supernatant of sample solution.

V. Calculate the Dilution Factor:

$$V_1 \times C_1 = V_2 \times C_2$$

$$\text{Dilution Factor} = \frac{V_2 - V_1}{\text{Wt. of Dentifrice (g)}} + 3$$

V_1 = Weight of combined working standard (g) = Volume of combined working standard (mL)
C_1 = Concentration of combined working standard of Br^- and NO_2^- (ppm)
V_2 = Volume of total liquid in the slurry
C_2 = Concentration of Br^- or NO_2^- (ppm) in the supernatant
= Concentration of Br^- or NO_2^- (ppm) in the test solution times 5

The dilution factor can be obtained by using either concentration of Br^- or NO_2^- (ppm) in the supernatant as long as the difference is within 0.2.

Appendix II
Caries Clinical Design Protocol for New Dentifrice Formulations

The following guidelines are given to assist in the design and conduct of clinical studies to be used in the evaluation of new fluoride-containing dentifrices that contain new fluoride compounds, new abrasive systems, combinations of fluoride compounds or fluoride concentrations not contained in the FDA Monograph for Anti-Caries Products for OTC Human Use. These guidelines attempt to define minimal requirements necessary to provide evidence of effectiveness and safety in the reduction of dental caries. Two independent clinical trials of at least two years duration will be required unless one study is waived by Council. This waiver may apply when appropriate clinical data exist that are consistent with these guidelines. Clinical studies must be conducted under conditions that the product will be used. At least one study should be conducted on a normal population expected to use the product, and one may be conducted on a high risk population. For ethical reasons, each study should be undertaken contrasting the test formulation against an ADA Accepted clinically proven positive control. The positive control may only be available as a USP standard. Such studies must demonstrate that the test formulation is at least "as good as" or superior to the clinically established positive control (See the ADA Acceptance Program Guidelines for Determination of Efficacy in Product Evaluation, 1999 and the ADA Acceptance Program Guidelines for Clinical Trial Protocols, 2003).

It is desirable to provide a measure of intra- and inter-evaluator variability. An attempt should be made to assess the level of compliance of the subjects in the study. Manufacturers are encouraged to submit their clinical protocols to the Council for review prior to the start of clinical studies.

A Classical Clinical Caries Studies

Sample Size: A sufficient number of subjects should be enrolled in the study to ensure that appropriate statistical tests can be performed. Sample size is largely influenced by the experimental variance in the population, the level of caries incidence and the degree of effect expected from the test formulation. Justification of sample size which provides adequate power must be given.

Study Duration: The studies will be conducted for a minimum of two years, three years is preferable when cavitated carious lesions are measured. Measurements should be taken at least at baseline (prior to the study), at conclusion of the study, and at an intermediate time period.

Study Design: Each subject will have a complete oral examination to determine eligibility for the study. Both genders should participate; the age of the study populations should be representative of those patients (children are typically used) for whom the product is intended; the frequency of use of the product should be representative of actual use of the product in practice; and the user should be instructed in the proper use of the product but not necessarily supervised. Patients should not be taking medication which might affect bacterial flora during the course of this study.

Caries Assessments: The caries detection criteria used in a trial should be selected based upon the outcome(s) of interest. If the claim for the product focuses on preventing "dental caries", then cavitated carious lesions should be used as an outcome. If the claim focuses on preventing "early carious lesions", then the caries detection criteria should include the assessment of presence or activity status of early (incipient or non-cavitated) carious lesions.

Statistical Analysis: Mean group whole mouth scores for caries (DMFT or DMFS) or other measures of early carious lesions on all surfaces will be compared at baseline, at an intermediate period, and at the termination of the study with either a parametric or non-parametric test. If more than two groups are being evaluated, appropriate adjustments for multiple comparison tests (e.g. Bonferonni) should be employed.

B Early Carious Lesion Clinical Studies

The Council recognizes that research is underway to validate the use of clinical studies on early carious lesions. Such studies may allow for fewer participants and/or shorter duration than classic caries studies that measure frank caries.

The Council is willing to consider such early carious lesion clinical studies. However, it is up to the manufacturer to demonstrate that such studies are valid surrogates for the classic caries studies.

American Dental Association
www.ada.org

211 East Chicago Avenue
Chicago, Illinois 60611-2678