GlaxoSmithKline
Consumer Healthcare

CLINICAL PROTOCOL

A RANDOMIZED, EXAMINER BLIND, CROSSOVER, *IN SITU* EROSION STUDY TO INVESTIGATE THE EFFICACY OF AN EXPERIMENTAL DENTIFRICE IN REMINERALIZATION OF PREVIOUSLY SOFTENED ENAMEL COMPARED TO A PLACEBO DENTIFRICE

Compound Name:	Sodium fluoride and potassium nitrate
United States (US) Investigational New Drug (IND) Number:	N/A
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Sodium fluoride and potassium nitrate
208166
Clinical Protocol, 08 Aug 2017

Sponsor information

Sponsor Legal Registered Address	**GlaxoSmithKline Research & Development Limited** 980 Great West Road Brentford Middlesex, TW8 9GS UK
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Document History

Document	Version Date	Summary of Changes
Original protocol	08/08/2017	Not applicable (N/A)

Amendments incorporate all revisions to date, including amendments made at the request of country health authorities, institutional review boards/ethics committees (IRBs/ECs), etc.

PRINCIPAL INVESTIGATOR PROTOCOL AGREEMENT PAGE

- I confirm agreement to conduct the study in compliance with the protocol and any amendments and according to the current ICH GCP guidelines.

- I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.

- I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure site staff receives all appropriate information throughout the study.

- I agree to conduct this study in full conformance with the laws and regulations of the country in which the research is conducted and the Declaration of Helsinki.

Investigator Name:	Dr. Anderson Hara
Investigator Qualifications:	DDS, MS, PhD
Investigator Signature:	PPD
Date of Signature/ Agreement:	PPD DD/MMM/YYYY

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SCHEDULE OF ACTIVITIES

The schedule of activities table provides an overview of the protocol visits and procedures.

The investigator may schedule visits (unplanned visits) in addition to those listed on the schedule of activities, in order to conduct evaluations or assessments required to protect the well-being of the subject.

Table 1-1 Schedule of Activities

	Screening		Study period				
Procedure/ Assessment			Period 1		Period 2		Period 3
	Visit 1	Wash out	Visit 2	Wash out	Visit 3	Wash out	Visit 4
Informed consent	X	Minimum 3 days including a 2 days washout using a non fluoride toothpaste		Minimum 3 days including a 2 days washout using a non fluoride toothpaste		Minimum 3 days including a 2 days washout using a non fluoride toothpaste	
Demographics and ethnicity	X						
Medical history	X						
Prior medications	X						
Screening OHT assessment	X						
Screening OST assessment	X						
Unstimulated saliva flow rate	X						
Stimulated saliva flow rate	X						
Inclusion / Exclusion Criteria	X						
Subject Eligibility	X						
Try-in palatal appliance	X						
Dispense wash-out products	X						
Concomitant medication			X		X		X
Continued eligibility			X		X		X
Diary cards review			X		X		X
Pre-treatment OST assessment			X		X		X
Randomize to treatment			X				
Place enamel specimens in			X		X		X

palatal appliance [1]							
Place palatal appliance in subject's mouth			X		X		X
Supervised Treatment			X		X		X
Intra oral phase 2 hours [2]			X		X		X
Intra oral phase 4 hours [3, 4]			X		X		X
Post-treatment OST assessment			X		X		X
Post-treatment OHT assessment							X
Collect wash-out products							X
Adverse Events	X		X		X		X
Incidents	X		X		X		X
Study Conclusion							X

Abbreviations:
OST: Oral soft tissue
OHT: Oral hard tissue
Any serious adverse event assessed as related to study participation that occurs subsequent to the signing of informed consent and any adverse event that occurs subsequent to the screening visit will be recorded.

1. Laboratory analysis of enamel specimens performed prior to placing the specimens in intraoral appliance are specified in Section 7.3

2. Four enamel specimens will be removed at 2 hours ± 10 min from palatal appliance for analysis. Laboratory analysis of enamel specimens described in Section 7.3.

3. Four enamel specimens will be removed at 4 hours (2 hours ± 10 min from previous time point) from palatal appliance for analysis. Laboratory analysis of enamel specimens described in Section 7.3.

4. At the end of the intraoral phase, and after all enamel specimens are removed, the appliance will be disinfected and stored at site until the next treatment visit.

1 INTRODUCTION

Dental erosion is the irreversible loss of tooth substance by chemical processes not involving bacteria. It is a cyclical process, influenced by demineralization and remineralization. Demineralization of the enamel surface can occur by the exposure to acids either from internal origin, such as gastric acids from refluxes, and or external from dietary sources; if of sufficient frequency and severity. The erosive challenges in vivo are brief and it's unlikely they are a major cause of surface loss when occurring on their own (Shellis et al 2011), but this softening of the hard tissues surface makes it more susceptible to further physical wear from abrasion (tooth brushing) and attrition (grinding) resulting in tissue loss. The process of demineralization cycles with remineralization, which is the process of placing minerals back into the previously demineralized hard tissue, ideally to achieve mineral that is more resistant to subsequent demineralization (Cochrane et al 2012).

It is well accepted that fluoride containing dentifrices should be part of an appropriate clinical management strategy for the prevention of caries (Brunelle and Carlos 1990) (Stephen 1993) (Bartizek et al 2001) and to promote tooth remineralization and reduce demineralization (Featherstone et al 1990) (Chow 1990). Fluoride can be administered in many different forms, most commonly sodium fluoride (NaF), sodium monofluorophosphate (NaMFP) and stannous fluoride (SnF2). However, besides the fluoride form and concentration, there are other aspects of a dentifrice that have a strong influence in the uptake of fluoride as has been shown in *in vitro* and *in situ* investigations (Barlow et al 2009, Fowler et al 2006, Fowler et al 2009).

In vitro and *in situ* erosion models are the most commonly used methods to investigate the short term performance of new erosion formulations (Shellis et al 2011). Currently, there is no validated clinical methodology or clinical index to monitor the progression of erosive tooth wear and enamel tissue loss (Shellis et al 2011), and most of the devices used for detection of changes in mineral content can only be performed on specially prepared specimens (Attin 2006). *In situ* erosion models involve the use of appliances or other devices in the human mouth that simulate the process (Zero 1995) and they allow the effect of an erosive challenge to be evaluated under intra oral conditions but with some controlled experimental variables. *In situ* models are placed in between the continuum of *in vitro* models and clinical studies and can provide evidence of the potential effect of new therapies in inhibiting demineralization and promoting remineralization (Zero 1995). However, the extrapolation of their result to clinically relevant evidence must be carefully considered.

An erosion *in situ* model will be used in this study to investigate the efficacy of an experimental dentifrice in promoting enamel remineralization of previously softened enamel.

1.1 Mechanism of Action/Indication

The test dentifrice formulation being investigated contains 0.254% w/w NaF, equivalent to 1150 ppm fluoride and 5% KNO_3. The efficacy of the experimental dentifrice in enhancing enamel remineralization is being investigated in an *in situ* intraoral model with bovine enamel specimens in healthy subjects.

1.2 Background and Rationale

Justification and need to perform the study

The experimental formulation tested in this study is based on the currently marketed Sensodyne Pronamel dentifrice and tested *in vitro* for a superior performance. In order to support the experimental dentifrice market introduction in the US, an *in situ* investigation comparing the experimental dentifrice with a fluoride free placebo dentifrice (primary objective) and with a relevant marketed dentifrice is needed (secondary objective) to investigate the efficacy in enhancing remineralization and preventing demineralization. Therefore, the aim of this study is to investigate the performance of an experimental dentifrice formulation in promoting enamel remineralization and inhibiting post-treatment enamel demineralization in an *in situ* erosion model, in comparison with a fluoride-free placebo and with a marketed competitor dentifrice product.

The *in situ* model to be used in this study has been extensively used to investigate the performance of the currently marketed Sensodyne Pronamel in previously published studies (Barlow et al 2009) and other GSK CH studies (CCI [redacted], GSK-Z3480664 , CCI [redacted], GSK-Z6961385).

Study and dose rationale

This clinical investigation will use a previously published *in situ* remineralization model (Barlow et al 2009) which consists of placing pre-eroded sterilized bovine enamel specimens intra orally using a palatal appliance and testing the enhancement of remineralization and prevention of demineralization performance of the experimental formulation after 2 and 4 hours post treatment application based on surface micro hardness measurements. Details of enamel and palatal appliance preparations are specified in protocol Section 7.3. The palatal appliances (Figure 1) will be made for each eligible subject, and each will carry two plastic holders containing a total of eight bovine enamel specimens (four in each holder).

This will be a randomized, controlled, single center, single- blind (to the dental examiner and specimen analysts), 3 period, 3 treatment, cross-over, *in situ* study design. Each subject will brush their teeth once with each of the study products as described below. At each visit, and under supervision while the intraoral appliance is in place, the subjects will brush the buccal surfaces of their natural teeth using 1.5 g dentifrice and a soft toothbrush for 25 seconds to create a slurry. Subjects will be instructed not to expectorate the slurry. Subjects will then swish the slurry around the mouth for 95 seconds to permit direct contact of the dentifrice slurry with the enamel specimens. The toothbrush however, will not come into contact with the enamel specimens or the appliance as this could cause abrasion on the enamel specimens. This will be followed by an intraoral remineralization period of up to 4 hrs.

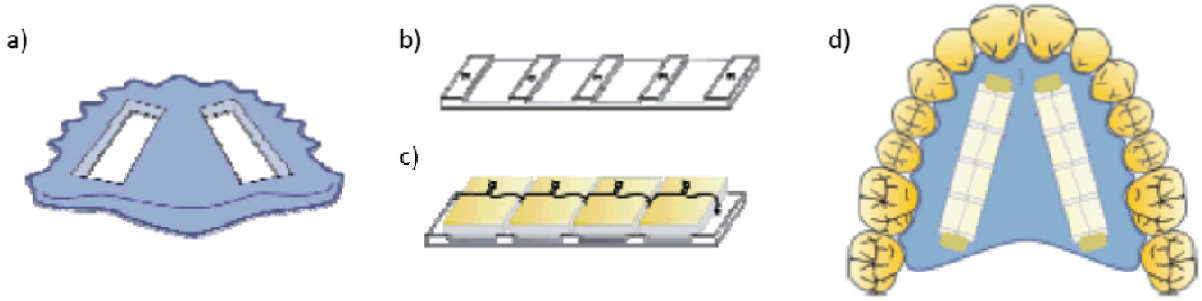

Figure 1: Schematic diagram of a) intraoral appliance, b) enamel specimens' holder, c) enamel specimens placed in the holder and d) palatal appliance with enamel specimens in the mouth.

Oral tolerance

GSK CH has run numerous similar *in situ* clinical investigations with currently marketed and experimental Sensodyne Pronamel formulations. In all those studies, the products were generally well tolerated ([redacted], GSK-Z3480664 , GSK-Z6961385). Oral soft tissue assessments will be carried out at all visits, pre and post intra oral phase and all adverse events will be recorded.

Complete information for this compound may be found in the single reference safety document (SRSD), which for this study is the Safety Statement (SS).

2 STUDY OBJECTIVES AND ENDPOINTS

Table 2-1 Study Objectives and Endpoints

Objective(s)	Endpoint(s)
Primary	
To investigate the efficacy of an experimental dentifrice containing 1150 ppm fluoride and 5% KNO_3 to enhance remineralization of enamel relative to a fluoride free placebo dentifrice.	%SMHR after 4 hrs intraoral phase following a single exposure.
Secondary	
Efficacy	
To investigate the efficacy of an experimental dentifrice containing 1150 ppm fluoride and 5% KNO_3 to inhibit demineralization of enamel relative to a fluoride free placebo dentifrice.	%RER after 4 hrs intraoral phase following a single exposure.
To investigate the efficacy of an experimental dentifrice containing 1150 ppm fluoride and 5% KNO_3 to promote fluoride uptake in enamel relative to a fluoride free placebo dentifrice.	EFU after 4 hrs intraoral phase following a single exposure.
To investigate the efficacy of an experimental dentifrice containing 1150 ppm fluoride and 5% KNO_3 to enhance remineralization, to inhibit demineralization and to promote fluoride uptake in enamel relative to a benchmark comparator containing 1100 ppm fluoride (Crest Pro Health).	%SMHR, %RER and EFU after 4 hrs intraoral phase following a single exposure.
Safety	
To evaluate the oral tolerance of an experimental dentifrice containing 1150 ppm fluoride and 5% KNO_3 following a single brushing event.	Proportion of treatment emergent oral adverse events post 4 hours intraoral exposure.
Exploratory	
To make all paired comparisons of the efficacy of an experimental dentifrice containing 1150 ppm fluoride and 5% KNO_3 after 2 hours of intraoral exposure to enhance remineralization of enamel, to inhibit demineralization of enamel and to promote fluoride uptake in enamel.	%SMHR, %RER and EFU after 2 hrs intraoral phase following a single exposure.
To characterize the efficacy of the experimental dentifrice containing 1150 ppm fluoride and 5% KNO_3; the fluoride free placebo; and the benchmark comparator containing 1100 ppm (Crest Pro Health) after 2 and 4 hours of intraoral exposure on resistance to acid challenge.	Summary statistic table of ARR after 2 and 4 hrs intraoral phase.

Abbreviations: SMHR: Surface micro hardness recovery, RER: Relative erosion resistance, EFU: Enamel fluoride uptake, ARR: Acid resistance ratio

This study will be considered successful if the experimental dentifrice demonstrates a statistically significant enhancement of remineralization, as measured by %SMHR in comparison with the placebo dentifrice after 4 hours intraoral phase.

3 STUDY DESIGN AND SUBJECT POPULATION

This will be a randomized, controlled, single center, single- blind (to the dental examiner and specimen analysts), 3 period, 3 treatment, cross-over, *in situ* design which consists of placing pre-eroded bovine enamel specimens intra orally using a palatal appliance and testing the remineralizing performance of the experimental, comparator and placebo dentifrices 2 and 4 hours post treatment application, based on surface micro hardness measurements. This study will be carried out in healthy adults with a maxillary dental arch suitable for the retention of the palatal appliance. A sufficient number of healthy subjects will be screened so that up to 66 subjects are randomized to participate in the study to ensure 60 evaluable subjects complete the entire study. Subjects will be recruited from an existing panel of subjects pre-fitted with a palatal appliance (IRB approved panel CCI). For a subject whose appliance does not fit adequately and for whom adjustment of the appliance is not sufficient to obtain proper fit, the subject may have a new appliance made. Surface microhardness (SMH) measures changes in the mineral content of enamel, and is used to calculate surface micro hardness recovery (%SMHR), relative erosion resistance (%RER) and acid resistance ratio (ARR). Fluoride uptake of the experimental dentifrice will be assessed by enamel fluoride uptake (EFU) measurements. For each treatment and subject these measurements will be performed on the same enamel specimen, in the four replicates for each time point. The erosive acid challenge, with a commercially available grapefruit juice, will be carried out *ex vivo* and therefore does not pose a risk to the subject's teeth. The overall study design sequence of events is sketched in Figure 2.

For the aim of this study, a fluoride-free placebo dentifrice will be used as comparator to establish if the fluoride present in the experimental dentifrice can provide and enhancement of remineralization and prevention of demineralization in this *in situ* model. Additionally, a relevant commercially available dentifrice containing 1100 ppm fluoride (as stannous fluoride, SnF_2) will be used as a comparator (secondary objectives) to investigate the superior performance of the experimental dentifrice in enhancing remineralization and preventing demineralization.

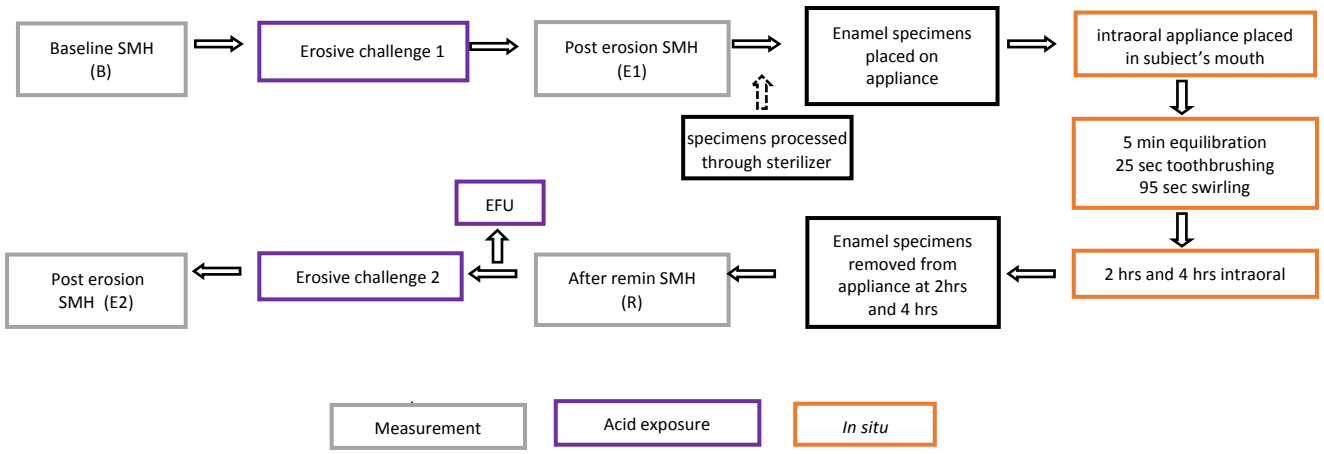

Figure 2: Schematic diagram of the experimental phase for each subject and treatment showing the flow of events at each visit.

In this study there will be 4 visits, 1 screening visit to assess subject eligibility and 3 treatment visits to assess product efficacy, where the treatment product will be dispensed and used under the supervision of a suitably trained study site personnel. Prior to each treatment visit, there will be a washout period of a minimum of 3 days. During this period subjects will use their own dentifrice for at least one day, and a fluoride free dentifrice (provided) for two days prior to the next scheduled visit (including in the morning of the scheduled visit) to minimize any carry-over effects of the fluoride toothpaste. Based on previous studies, two days are sufficient to minimize any carry-over effect.

According to ICH guidelines, for a study to be classed as truly double blind, not only does the examiner (and any appropriate member of staff who may be involved in the dispensing of products, analysis of data etc.) need to be blinded to the treatment the subject receives, but the test products must be identical in every way (colour, flavour, appearance, packaging). Given that it is almost impossible to ensure identical appearance, taste and packaging for the dentifrices evaluated in this study, the level of blindness for this study is described as 'examiner blind' only.

The study statistician and other employees of the Sponsor who may influence study outcomes are blinded to the product allocation of subjects. The examiner and specimen analyst will be blinded to the treatment received. To ensure the examiner and specimen analysts remain blinded throughout the study, the examiner and specimen analysts are not

permitted in the room while product is dispensed. All study products will be overwrapped to conceal any labelling. In addition, subjects will receive treatment in a separate area from where clinical assessments are performed. The dispensing staff will not be involved in any clinical assessments or laboratory analysis during the study. Study site staff that perform study consent can only also dispense the study product but can not perform any other study activity. Laboratory personnel that carry out the specimen analysis will also be blinded to product allocation and not be involved in any clinical assessment.

4 SUBJECT SELECTION

This study can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom participation in the study is considered appropriate. All relevant medical and nonmedical conditions should be taken into consideration when deciding whether a particular subject is suitable for this protocol.

4.1 Inclusion Criteria

Subject eligibility should be reviewed and documented by an appropriate member of the investigator's study team before subjects are included in the study.

Subject must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Evidence of a personally signed and dated informed consent
2. Male and female subjects who, at the time of screening, are between the ages of 18 and 65 years, inclusive.
3. Subject who is willing and able to comply with scheduled visits, treatment plan, and other study procedures.
4. Good general and mental health with, in the opinion of the investigator or medically qualified designee. No clinically significant and relevant abnormalities in medical history or oral examination.
5. Male subject able to father children and female subject of childbearing potential and at risk for pregnancy must agree to use a highly effective method of contraception throughout the study and for 5 days after the last dose of assigned treatment.
6. Good oral health without lesions of the oral cavity that could interfere with the study evaluations.
7. Maxillary dental arch suitable for the retention of the palatal appliance
8. Unstimulated salivary flow rate of at least 0.2 mL/minute and a stimulated salivary flow rate of at least 0.8 mL/minute
9. Understands and is willing, able and likely to comply with all study procedures and restrictions.

4.2 Exclusion Criteria

Subjects with any of the following characteristics/conditions will not be included in the study:

1. Subjects who are investigational site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the investigator, or subjects who are GSK employees directly involved in the conduct of the study.
2. Participation in other studies involving investigational drug(s) within 30 days prior to screening visit.
3. Participation in other studies involving investigational oral care or cosmetic products within 30 days prior to screening visit.
4. Acute or chronic medical or psychiatric condition that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the subject inappropriate for entry into this study.
5. Pregnant female subject (self – reported).
6. Breastfeeding female subject.
7. Known or suspected intolerance or hypersensitivity to the study materials (or closely related compounds) or any of their stated ingredients.
8. Unwilling or unable to comply with the lifestyle guidelines described in this protocol.
9. Medication that may interfere significantly with the saliva flow in the judgment of the investigator. Should new medications that may interfere with the saliva flow be added, a second salivary flow test will be performed.
10. Subject with any condition that would impact on their safety or wellbeing or affect their ability to understand and follow study procedures and requirements.
11. Any sign of grossly carious lesions (active), moderate or severe periodontal conditions, or severe tooth wear. Subject presenting at screening with minor caries may continue in the study if their carious lesions are repaired prior to the first treatment visit of the study.
12. Wears oral appliance or orthodontia (besides subjects wearing permanent lower retainers, which are eligible).
13. Recent history (within the last year) of alcohol or other substance abuse.
14. Subject who has previously been enrolled in this study.

4.3 Randomization Criteria

Subjects will be randomized into the study provided they have satisfied all subject selection criteria.

4.4 Lifestyle Guidelines

During the entire study:

- Subjects will be asked to stop using their own dentifrice two days before Visit 2, 3 and 4, when they will use the provided fluoride-free dentifrice and toothbrush to brush their teeth (including in the morning of the scheduled Visit).
- Subjects will not be permitted to use any fluoride-containing products (including mouthwashes) for 2 days prior to Visit 2, 3 and 4.
- Subjects will be requested not to have any elective dental procedures including teeth professionally cleaned other than those performed within the study (excluding emergency dental treatment).

During Visit 2, 3 and 4:

- Subjects should refrain from talking for the first hour after brushing on each treatment visit.
- Subjects should not leave the study site during the 4 hours treatment phase while wearing the intraoral appliances.

4.4.1 Meals and Dietary Restrictions

- Subjects must abstain from all food and drink (except water) at least 30 minutes prior to their scheduled Visit 2, 3 and 4.

During Visit 2, 3 and 4:

- Subjects will not be permitted to drink water for the first two hours of the intraoral test period (after treatment administration), but may drink water after the first two hours under the supervision of the study personnel.
- Subjects will not be permitted to eat during the four hours' duration of the intraoral test period (after treatment administration).

4.5 Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomized. In order to ensure transparent reporting of screen failure subjects, a minimal set of screen failure information will include demography, screen failure details (e.g., withdrawal of consent), eligibility criteria, and any serious adverse events.

Individuals who do not meet the criteria for participation in this study (screen failure) may not be re-screened.

For a subject whose appliance does not fit adequately and for whom adjustment of the appliance is not sufficient to obtain proper fit, the subject may have a new appliance made. This will not be considered a screen failure. The subject may repeat the appliance try-in portion of the screening visit after fabrication of the new appliance.

4.6 Sponsor's Qualified Medical Personnel

The contact information for the sponsor's appropriately qualified medical/dental personnel for the study is documented in the study contact list in the Trial Master File.

The contact number can be used by investigational staff if they are seeking advice on medical/dental questions or problems; however, it should be used only in the event that the established communication pathways between the investigational site and the study team are not available. It is therefore intended to augment, but not replace, the established communication pathways between the investigational site and the study team for advice on medical/dental questions or problems that may arise during the study. The contact number is not intended for use by the subject directly, and if a subject calls that number, he or she will be directed back to the investigational site.

To facilitate access to appropriately qualified medical/dental personnel on study-related medical/dental questions or problems, subjects are provided with a contact card. The contact card contains, at a minimum, protocol identifiers, subject study numbers, contact information for the investigational site, and contact details in the event that the investigational site staff cannot be reached to provide advice on a medical question or problem identified from the subject's healthcare professional other than the investigator.

5 STUDY TREATMENTS

5.1 Blinding and Allocation to Treatment/Randomization

Blinding

This is a single blind study, with the examiner being blind to the treatment each subject received. Subjects will not be blind to the treatment that they are receiving.

The study statistician and other employees of the Sponsor who may influence study outcomes are blinded to the product allocation of subjects. The examiner will be blinded to the treatment received. To ensure the examiner and specimen analysts remain blinded throughout the study, the examiner and specimen analysts are not permitted in the room whilst product is dispensed. In addition, subjects should be treated in a separate area. The dispensing and laboratory staff will not be involved in any efficacy assessments during the study. Study site staff that perform study consent can only also dispense the study product but can not perform any other study

activity. Laboratory personnel that carry out the specimen analysis will also be blinded to product allocation and not be involved in any clinical assessment.

Allocation to treatment sequence
Subjects will be assigned to study product sequence in accordance with the randomization schedule generated by a Vendor under the supervision of the Biostatistics Department, GSK CH, prior to the start of the study, using a validated program. Subjects will be randomized in a Williams square design balanced for first period carryover.

Randomization
GSK CH will provide a randomization schedule to the investigator and, in accordance with the randomization numbers, subjects are planned to receive all treatments in a pre-determined order as specified by the randomisation schedule.

A unique screening number will identify each subject screened for study participation. Screening numbers will be assigned in ascending numerical order as each subject signs their consent form. Subjects who meet all inclusion and exclusion criteria will be randomized according to the randomisation schedule. Randomisation numbers will be assigned in ascending numerical order as each subject is determined to be fully eligible.

The study site will receive two versions of the randomisation schedule, each in a sealed envelope and clearly marked as either “For Dispensing” or “Emergency Use Only”. The “For Dispensing” schedule will contain the list of randomisation numbers only and will not include any coded description, just a letter A, B or C. The ‘Emergency Use Only’ randomisation schedule will only be removed from the sealed envelope in an emergency situation. This schedule will have a randomisation number followed by a sequence of letters A, B or C. The schedule will have a footnote with a key for A, B or C identifying the three treatment regimen sequences.

However, to maintain the blinding of the study as far as possible, all treatment allocations for all randomisation numbers on this randomisation schedule will be masked with scratch-off panels. Only the panels required for the unblinding the particular subject should be removed.

5.2 Breaking the Blind

At the initiation of the study, the study site will be instructed on the method for breaking the blind. The method will be manual. Blinding codes should be broken only in emergency situations for reasons of subject safety. Whenever possible, the investigator or sub-investigator should consult with a member of the study team prior to breaking the blind unless the delay would endanger the subject’s health. When the blinding code is broken, the reason must be fully documented and entered in the case report form (CRF). When documenting in

the CRF, the site should ensure that no text description has the potential to unblind any other subjects.

Any AE or serious AE (SAE) associated with breaking the blind must be recorded and reported as specified in this protocol. The study site is required to inform the IRB/EC if the blind is broken.

5.3 Subject Compliance

Subjects will complete diary cards during the 2 days of washout period prior to the treatment visit.

Study treatment will be administered under the supervision of investigator site personnel.

5.4 Investigational Product Supplies

	Test Product	Comparator Product	Placebo Product
Product Name	Experimental dentifrice containing 0.254% w/w sodium fluoride (1150 ppm fluoride) and 5% KNO_3; plus 0.25% PVM/MA copolymer and 2.5% sodium lactate	Crest ProHealth Sensitivity & Enamel Shield containing 0.454% w/w stannous fluoride (1100 ppm fluoride)	Fluoride free placebo containing 5% KNO_3 (0 ppm fluoride); plus 0.25% PVM/MA copolymer and 2.5% lactate
Product Formulation Code (MFC)	CCI [redacted]	Commercially available (US marketed)	CCI [redacted]
Dose	1.5 g	1.5 g	1.5 g
Route of Administration	Oral	Oral	Oral
Dosing Instructions	Subjects will apply a full ribbon of the allocated product and brush the buccal surfaces of their natural teeth for 25 timed seconds and then swish the resulting toothpaste slurry around the mouth, without further brushing, for a timed period of 95 seconds. After expectorating the slurry, the subjects will gently rinse their mouths with 15 mL of tap water for 10 seconds before expectorating again		

Sundry items:

- Oral-B Sensi Soft Manual Toothbrush
    - Manual toothbrush to apply test and washout dentifrice
- Fluoride free toothpaste [redacted]
    - Fluoride free dentifrice to brush their teeth during the 2 days prior to treatment visit
- Countdown timer
    - To ensure accurate brushing time
- Diary cards (to be supplied by GSK Warren)
    - Record washout product use.
- Dosing cups
    - For rinsing after brushing.
- Unflavoured gum base
    - For stimulated saliva samples collection

All other items such as saliva sampling collection equipment will be supplied by the study site. Grapefruit juice (commercially available) for the *ex vivo* acid challenge will also be sourced by the study site.

5.4.1 Dosage Form and Packaging

The contents of the label will be in accordance with all applicable regulatory requirements and will be the responsibility of the Clinical Supplies Department, GSK CH.

The fluoride-free washout toothpaste will be supplied in plain white tubes with a study label affixed. Each subject will receive a sufficient number of tubes to cover usage during the washout phase (for 2 days prior to Visit 2, 3 and 4).

The experimental and placebo dentifrices will be supplied in plain white tubes. The comparator dentifrice will be sourced from the US market. All study products (experimental, comparator and placebo dentifrices) will be over-wrapped in white vinyl to maintain the study blind as much as possible.

Each tube will have a study label affixed. Each study label will contain, but not be limited to, protocol number, product code letter (for treatment products only), directions for storage, emergency contact telephone number and “For Clinical Trial Use Only”.

Toothbrushes and countdown timers will be supplied in their commercial packaging.

Care should be taken with the supplied study products and their labels so that they are maintained in good condition. It is important that all labels remain intact and legible for the duration of the trial. Subjects should be instructed to not remove or deface any part of the study label.

5.4.2 Preparation and Dispensing

Test, control and placebo dentifrices and manual toothbrush will be dispensed by qualified unblinded site personnel according to the dosing instructions (Section 5.4).

5.5 Administration

During Visit 2, 3 and 4 and following the pre treatment assessments, a suitably trained study site designee will place the palatal appliance holding eight bovine tooth enamel specimens in the subjects' mouth followed by an equilibration period of at least 5 minutes. Following the equilibration phase, and under the supervision of a trained unblinded study site designee, the subject will brush their own teeth following the dosing instructions (Section 5.4).

5.5.1 Medication Errors

Medication errors may result, in this study, from the administration or consumption of:

- the wrong product,
- by the wrong subject,
- at the wrong time,
- or at the wrong dosage strength

Such medication errors occurring to a study participant are to be captured in the CRF. In the event of medication dosing error, the sponsor should be notified immediately.

Medication errors are reportable irrespective of the presence of an associated AE/SAE, including:

- Medication errors involving subject exposure to the investigational product;
- Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating subject.

Whether or not a medication error is accompanied by an AE, as determined by the investigator, the medication error and, if applicable, any associated adverse event(s) is captured on an adverse event (AE) CRF page.

5.6 Investigational Product Storage

The investigator, or an approved representative, will ensure that all investigational products including any comparator, marketed products and washout products are stored in a secured area with controlled access under required storage conditions and in accordance with applicable regulatory requirements and product label.

Site systems must be capable of measuring and documenting (for example, via a log) minimum and maximum temperatures for all site storage locations. This should be captured from the time of investigational product receipt throughout the study. Even for continuous monitoring systems, a log or site procedure that ensures active evaluation for excursions should be available. The operation of the temperature-monitoring device and storage unit (for example, refrigerator), as applicable, should be regularly inspected to ensure it is maintained in working order.

Any excursions from the product-label storage conditions should be reported upon discovery. The site should actively pursue options for returning the product to the storage conditions as described in the labeling, as soon as possible. Deviations from the storage requirements, including any actions taken, must be documented and reported to the Sponsor.

Once an excursion is identified, the investigational product must be quarantined and not used until the Sponsor provides documentation of permission to use the investigational product. It will not be considered a protocol deviation if the sponsor approves the use of the investigational product after the temperature excursion. Use of the investigational product prior to sponsor approval will be considered a protocol deviation. Specific details regarding information the site should report for each excursion will be provided to the site.

5.7 Investigational Product Accountability

All products supplied are for use only in this clinical study and should not be used for any other purpose.

Study treatments must be received by a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only the staff have access. Upon receipt, all study treatments should be stored according to the instructions specified on the treatment labels. Clinical supplies are to be dispensed only in accordance with the protocol.

The investigative site must maintain adequate records documenting the receipt, use, loss, or other disposition of the investigational product supplies. All study drugs will be accounted for using a drug accountability form/record.

The inventory must be available for inspection by the study monitor during the study. Monitoring of treatments accountability will be performed by the field monitor during site visits and at the completion of the study.

5.7.1 Destruction of Investigational Product Supplies

All investigational study treatments shipped for this clinical trial will be returned to the Sponsor at the termination of the study. At the conclusion of the study, the Principal Investigator or an appropriate designee, and a representative of GSK CH will inventory all used and unused investigational study treatment. The study treatment inventory record for returned study treatment will then be completed. All investigational product for this clinical study (empty containers), as well as all unused study product will be returned to the GSK CH Clinical Supplies Department designated vendor using the return instructions provided.

5.8 Concomitant Treatment(s)

All concomitant treatments taken during the study must be recorded with indication, unit dose, daily dose, and start and stop dates of administration. All subjects will be questioned about concomitant treatment at each clinic visit.

Treatments taken within 30 days before Screening visit will be documented as a prior treatment. Treatments taken after the screening visit will be documented as concomitant treatments.

6 STUDY PROCEDURES

6.1 Visit 1 – Screening

Informed Consent

The investigator, or a person designated by the investigator, will obtain written informed consent from each subject before any study-specific activity is performed. The investigator will retain the original of each subject's signed informed consent document.

The investigator, or designee, must explain to the subjects the aims, methods, objectives, and potential hazards of the study, and that they are completely free to refuse to enter the study or to withdraw from it at any time.

Appropriate forms for documenting a written consent will be provided by the investigator or by GSK CH. The investigator, or designee, should sign and date the consent form to confirm that the consent process was completed correctly. The subject will be provided with a copy of their signed and dated consent form and any other written information which they should be instructed to retain.

If, during a subject's participation in the study, any new information becomes available that may affect the subject's willingness to participate in the study, each ongoing subject should receive a copy of this new information and be re-consented into the study. Subjects should be provided with a copy of the signed and dated amended consent form. The date of consent will be recorded on the CRF.

Demographics and ethnicity
The following demographic parameters will be captured by the Investigator or designee and recorded on the CRF: year of birth, age, gender, race, and ethnicity. Ethnicity will be self-reported and the choices offered will be Hispanic or Latino and Not Hispanic or Latino. Ethnicity will be captured by the Investigator or designee and recorded on the CRF.

Medical History and Prior medications
Medical history will be assessed as related to the inclusion/exclusion criteria by the Investigator or medically qualified designee. Details of any relevant medical or surgical history (within the last year), including allergies or drug sensitivity, will be recorded on the CRF. Any prior treatment taken in the 30 days prior to the Screening Visit will be recorded as per Section 5.8 in CRF.

Screening Oral Hard Tissue assessment
OHT examination will be carried out at the Screening visit as described in Section 7.2 by dental examiner or clinically qualified designee. Assessment will be recorded in the CRF.

Screening Oral Soft Tissue assessment
OST examination will be carried out at the Screening visit as described in Section 7.2 by dental examiner or clinically qualified designee. Assessment will be recorded in the CRF.

Unstimulated salivary flow rate
Unsitumulated salivary flow rate will be measured at the screening visit as described in Section 7.4 by dental examiner or clinically qualified designee.

Stimulated salivary flow rate
Stimulated salivary flow rate will be measured at the screening visit as described in Section 7.4 by dental examiner or clinically qualified designee.

Inclusion and Exclusion criteria
The investigator, or a person designated by the investigator, will review the Inclusion and Exclusion criteria as per Section 4.

Subject eligibility
Subject eligibility will be reviewed as per Section 4.

Try-In of Palatal Appliance
Palatal appliances (Figure 2) will be fitted and checked for comfort at the Screening visit. Subjects will be asked to wear their appliances for up to 15 minutes to determine comfort, fit and wearability. Appliances will be adjusted as needed. As indicated in section 7.3, intraoral

appliances will be fabricated as described in the work instructions document. For a subject whose appliance does not fit adequately and for whom adjustment of the appliance is not adequate to obtain proper fit, may have a new appliance made under IRB approved protocol CCI [redacted]. The subject may repeat the appliance try-in portion of the screening visit after fabrication.

Dispense wash out products and diary cards
Subjects will be dispensed with the washout toothbrush and the fluoride free toothpaste to use during the washout period. Subjects will be instructed to brush their teeth with the fluoride free toothpaste during the 2 days prior to Visit 2, 3 and 4 (including the morning of the treatment visit). Subjects will receive a dairy card to record twice daily toothbrushing with the washout assigned product during the 2 days washout period.

Adverse Events and Incidents

Adverse Events and Incidents will be recorded in the CRF as described in Section 8.

To prepare for study participation, subjects will be instructed on the use of the Lifestyle Guidelines and Concomitant Treatment(s) sections of the protocol.

Information will be recorded in the CRF.

6.2 Study Period

There will be an interval of at least 3 days between study periods (i.e., administration of subsequent doses of investigational product will not occur until at least 3 days after the previous dose of investigational product).

6.2.1 Visit 2 – Period 1

Subjects will attend Visit 2 at least 3 days after Visit 1. Subjects will brush with their own toothpaste for at least one day, and with the provided fluoride free toothpaste for two days prior to the visit.

The following assessments will be conducted in the order written:

Concomitant Medication
Concomitant medications will be assessed as related to the inclusion/exclusion criteria by the Investigator or medically qualified designee. Any concomitant treatment taken throughout the study will be recorded in the CRF.

Continued eligibility
Subject continued eligibility will be reviewed as described in Section 4.

Diary cards review
Investigator or designee will review diary cards of wash out product usage during wash out period.

Pre-Treatment Oral Soft Tissue assessment
OST examination as described in section 7.2 by dental examiner or clinically qualified designee and recorded in the CRF.

Randomize to treatment
Subjects will be randomized to treatment order as described in section 5.1.

Place enamel specimens in palatal appliance
Enamel specimens will be place in the palatal appliance after they are processed through sterilizer as described in Section 7.3.

Place palatal appliance in subject's mouth.
A suitably trained study site designee will place the palatal appliance in the subjects' mouth followed by an equilibration period of at least 5 minutes.

Supervised treatment
Study products will be used following the instructions and under the supervision of study site personnel as described in Section 5.4.

Palatal appliances intra oral phase – 2 hours
After completing the brushing procedures, subjects will wear their palatal appliance for 2 hours ± 10 min. After this period, the study site designee will remove the appliance and four pre-designated enamel specimens will be removed and sent for analysis (Section 7.3 for Laboratory Procedures). External Data will be recorded and transferred to GSK CH as detailed in Section 9.5.

Palatal appliances intra oral phase – 4 hours
After removing 4 specimens at 2 hours, the study site designee will replace the palatal appliance in the subject's mouth for a further 2 hours ± 10 min (4 hours total), after which the study site designee will again remove the palatal appliance. At this point, four pre-designated enamel specimens will be removed and sent for analysis (Section 7.3 for Laboratory Procedures). External Data will be recorded and transferred to GSK CH as detailed in Section

9.5. At the end of the intraoral phase, and after all enamel specimens are removed, the appliance will be disinfected and stored at site until the next treatment visit.

Post-Treatment Oral Soft Tissue assessment
OST examination as described in section 7.2

Adverse Events and Incidents

Adverse Events and Incidents will be recorded in the CRF as described in Section 8.

Information will be recorded in the CRF.

6.2.2 Visit 3 – Period 2

Subjects will attend Visit 3 at least 3 days after Visit 2. Subjects will brush with their own toothpaste for at least one day, and with the provided fluoride free toothpaste for two days prior to the visit.

The following assessments will be conducted in the order written:

Concomitant Medication
Concomitant medications will be assessed as related to the inclusion/exclusion criteria by the Investigator or medically qualified designee. Any concomitant treatment taken throughout the study will be recorded in the CRF.

Continued eligibility
Subject continued eligibility will be reviewed as described in Section 4.

Diary cards review
Investigator or designee will review diary cards of wash out product usage during wash out period.

Pre-Treatment Oral Soft Tissue assessment
OST examination as described in section 7.2 by dental examiner or clinically qualified designee and recorded in the CRF.

Place enamel specimens in palatal appliance
Enamel specimens will be place in the palatal appliance after they are processed through sterilizer as described in Section 7.3

Place palatal appliance in subject's mouth.

A suitably trained study site designee will place the palatal appliance in the subjects' mouth followed by an equilibration period of at least 5 minutes.

Supervised treatment

Study products will be used following the instructions and under the supervision of study site personnel as described in Section 5.4.

Palatal appliances intra oral phase – 2 hours

After completing the brushing procedures, subjects will wear their palatal appliance for 2 hours ± 10 min. After this period, the study site designee will remove the appliance and four pre-designated enamel specimens will be removed and sent for analysis (Section 7.3 for Laboratory Procedures). External Data will be recorded and transferred to GSK CH as detailed in Section 9.5.

Palatal appliances intra oral phase – 4 hours

After removing 4 specimens at 2 hours, the study site designee will replace the palatal appliance in the subject's mouth for a further 2 hours ± 10 min (4 hours total), after which the study site designee will again remove the palatal appliance. At this point, four pre-designated enamel specimens will be removed and sent for analysis (Section 7.3 for Laboratory Procedures). External Data will be recorded and transferred to GSK CH as detailed in Section 9.5. At the end of the intraoral phase, and after all enamel specimens are removed, the appliance will be disinfected and stored at site until the next treatment visit.

Post-Treatment Oral Soft Tissue assessment

OST examination as described in section 7.2 by dental examiner or clinically qualified designee and recorded in the CRF.

Adverse Events and Incidents

Adverse Events and Incidents will be recorded in the CRF as described in Section 8.

Information will be recorded in the CRF.

6.2.3 Visit 4 – Period 3

Subjects will attend Visit 4 at least 3 days after Visit 3. Subjects will brush with their own toothpaste for at least one day, and with the provided fluoride free toothpaste for two days prior to the visit.

The following assessments will be conducted in the order written:

Concomitant Medication
Concomitant medications will be assessed as related to the inclusion/exclusion criteria by the Investigator or medically qualified designee. Any concomitant treatment taken throughout the study will be recorded in the CRF.

Continued eligibility
Subject continued eligibility will be reviewed as described in Section 4.

Diary cards review
Investigator or designee will review diary cards of wash out product usage during wash out period.

Pre-Treatment Oral Soft Tissue assessment
OST examination as described in section 7.2 by dental examiner or clinically qualified designee and recorded in the CRF.

Place enamel specimens in palatal appliance
Enamel specimens will be place in the palatal appliance after they are processed through sterilizer as described in Section 7.3

Place palatal appliance in subject's mouth.
A suitably trained study site designee will place the palatal appliance in the subjects' mouth followed by an equilibration period of at least 5 minutes.

Supervised treatment
Study products will be used following the instructions and under the supervision of study site personnel as described in Section 5.4.

Palatal appliances intra oral phase – 2 hours
After completing the brushing procedures, subjects will wear their palatal appliance for 2 hours ± 10 min. After this period, the study site designee will remove the appliance and four pre-designated enamel specimens will be removed and sent for analysis (Section 7.3 for Laboratory Procedures). External Data will be recorded and transferred to GSK CH as detailed in Section 9.5.

Palatal appliances intra oral phase – 4 hours
After removing 4 specimens at 2 hours, the study site designee will replace the palatal appliance in the subject's mouth for a further 2 hours ± 10 min (4 hours total), after which the

study site designee will again remove the palatal appliance. At this point, four pre-designated enamel specimens will be removed and sent for analysis (Section 7.3 for Laboratory Procedures). External Data will be recorded and transferred to GSK CH as detailed in Section 9.5. At the end of the intraoral phase, and after all enamel specimens are removed, the appliance will be disinfected and stored at site until the next treatment visit.

Post-Treatment Oral Soft Tissue assessment
OST examination as described in section 7.2 by dental examiner or clinically qualified designee and recorded in the CRF.

Post-Treatment Oral Hard Tissue assessment
OHT examination will be carried out by dental examiner or clinically qualified designee. Assessment will be recorded in the CRF.

Collect wash-out products
A suitably trained study site designee will collect the wash-out products from the subject.

Adverse Events and Incidents

Adverse Events and Incidents will be recorded in the CRF as described in Section 8.

Study conclusion
Subjects will be evaluated to determine if they completed all study procedures or if they were discontinued from the study early. If the subject discontinued at any point during the study, the primary reason for withdrawal should be recorded on the study conclusion page of the CRF by selecting one of the options below.

- Subject did not meet study criteria
- Adverse event
- Lost to follow up
- Protocol violation
- Withdrawal of consent
- Other

Information will be recorded in the CRF.

6.3 Subject Withdrawal

Should a subject attend Visit 2, 3 or 4 having used a fluoride oral care product within the previous 2 days, or having consumed food or drinks within 30 minutes prior to the scheduled

visit, or should any other factor, in the opinion of the investigator, be thought to affect study outcomes (e.g. excessive alcohol consumption), every attempt will be made to reschedule the subject. If they cannot be reappointed they will be withdrawn from the study.

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety or, behavioral reasons, or the inability of the subject to comply with the protocol-required schedule of study visits or procedures.

The following circumstances require discontinuation of study treatment and/or premature subject withdrawal:

- Protocol violation that may impact the outcome of the subject's safety
- Withdrawal of informed consent
- Subject lost to follow-up
- Unblinding of the subject
- Pregnancy
- Death

If a subject is discontinued or prematurely withdraws from the study, reasons for discontinuation or withdrawal and associated date must be documented in the relevant section(s) of the CRF.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject. The Investigator or site staff should attempt to contact the subject twice. After two attempts, clinical site staff must send a registered letter. If no response is received from the subject, the subject will be considered lost to follow up. All attempts to contact the subject and information received during contact attempts must be documented in the CRF. In any circumstance, every effort should be made to document subject outcome, if possible. The investigator should inquire about the reason for withdrawal, request that the subject return all unused investigational product(s), request that the subject return for a final visit, if applicable, and follow-up with the subject regarding any unresolved adverse events (AEs).

It may be appropriate for the subject to return to the clinical site for final safety assessments. Subjects should be questioned regarding their reason for withdrawal. Assessments, at the investigator's discretion, will include the following:

- Oral soft tissue examination
- Oral hard tissue examination

Lack of completion of all or any of the early termination procedures will not be viewed as protocol deviations so long as the subject's safety was preserved.

If the subject withdraws from the study and also withdraws consent for disclosure of future information, no further evaluations should be performed and no additional data should be

collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

7 ASSESSMENTS

Every effort should be made to ensure that protocol-required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances, outside the control of the investigator that may make it unfeasible to perform the test. In these cases, the investigator must take all steps necessary to ensure the safety and well-being of the subject. When a protocol-required assessment cannot be performed, the investigator will document the reason for the missed assessment and any corrective and preventative actions that he or she has taken to ensure that required processes are adhered to as soon as possible. The study team must be informed of these incidents in a timely manner.

7.1 Efficacy

In this study there are no clinical efficacy assessments performed. All efficacy assessments will be performed *ex vivo* on the enamel specimens removed at the times defined in the Study Procedures section of this protocol. Enamel specimen preparation and analysis are described in Laboratory Procedures Section 7.3.

7.2 Safety

The following safety assessments will be performed at times defined in the Study Procedures section of this protocol.

Oral Hard Tissue Examination (OHT)

Where possible, this procedure should be conducted by a single dental examiner or clinically qualified designee for all subjects. Subjects with evidence of gross intra-oral neglect or the need for extensive dental therapy will be excluded.

The OHT examination will assess grossly carious lesions or erosive wear, enamel irregularities, tooth fracture, gross decay, decalcification and faulty restorations.

Observations will be listed as “Absent” or “Present” and conditions noted as present will be described. Examination findings will be described and documented in the CRF. Any observation that changes from “Absent” to “Present” from the screening assessment must be recorded as an AE.

Oral Soft Tissue Examination (OST)

Where possible, this procedure should be conducted by a single dental examiner or clinically qualified designee for all subjects for the duration of the study. The examination will be accomplished by direct observation and palpation with retraction aids as appropriate. The examiner will include examination of the labial mucosa (including lips), buccal mucosa, mucogingival folds, gingival mucosa, hard palate, soft palate, tonsilar area, pharyngeal area, tongue, sublingual area, submandibular area and salivary glands.

The results of the examination will be recorded in the CRF as either normal or abnormal, with details of any abnormalities. Any post-treatment soft tissue abnormality, or worsening of a pre-existing condition, observed by the examiner or reported by the subject will be recorded on the CRF. Any abnormalities, or worsening of pre-existing conditions, that occur from Visit 2 onwards will be recorded as AEs.

7.3 Laboratory Procedures

All laboratory procedures will be provided in a separate work instruction document. The work instruction document will be prepared by PI or designee and reviewed by GSK CH (CRS or designee) in eDMS. The work instruction document will be approved by PI or designee and CRS or designee, and stored in eDMS prior to Screening Visit.

The work instruction document will cover, for example, the following elements:

Preparation

- Preparation of enamel specimens
- Preparation of test holders and palatal appliances
- Assembly of the test holder
- *In vitro* erosive challenge

Efficacy measurements

- Surface microhardness (SMH)
- Enamel fluoride uptake (EFU)

Enamel Specimen Storage

All enamel specimen samples will be stored post completing the study and retained for up to six months after laboratory procedures detailed in the work instruction is finalized, as they may be subject to further analysis.

Specimen Retention

Laboratory specimens will be retained by the study site for up to six months following database lock. Before destruction, clinical study site designee will contact the sponsor in writing requesting permission to destroy the specimens. Specimens will be destroyed by autoclaving. The sponsor will provide approval in writing or provide an alternate time period for destruction of specimens. Before destruction, GSK may have the option to require the transfer of a number of specimens after reporting and unblinding the study for further laboratory analysis with the aim to develop new analytical techniques or perform other analytical tests.

7.4 Salivary flow

At the screening visit, both stimulated and unstimulated saliva will be collected for evaluation of flow rates as part of the inclusion criteria. Saliva samples collected will be destroyed inmediatelly after they are measured.

Unstimulated salivary flow

For the unstimulated saliva collection, subjects will sit quietly for five minutes before beginning the treatment period. During the five-minute test period, they will be instructed to allow their saliva to pool, emptying into a collection tube whenever they feel they need to swallow. Samples will be weighed, pre/post collection and weights calculated (1g = 1ml). Unstimulated saliva flow rate must be ≥ 0.2 ml/min. Saliva samples collected will be destroyed immediately after they are measured.

Stimulated salivary flow

For the stimulated saliva collection, subjects will chew on a piece of unflavoured gum base for one timed minute. After one minute, subjects will be instructed to swallow any pooled saliva. They will then chew the gum base for two timed minutes during which time they will empty any pooled saliva into a collection tube. Samples will be weighed, pre/post collection and weights calculated (1g = 1ml). Stimulated saliva flow rate must be ≥ 0.8 ml/min. Saliva samples collected will be destroyed immediately after they are measured.

8 ADVERSE EVENT AND OTHER EVENTS OF SPECIAL INTEREST REPORTING

8.1 Definitions of Adverse Events and Serious Adverse Events

8.1.1 Adverse Event

An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of an investigational or washout product or medical device, whether or not considered related to the investigational or washout product or medical device.

NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study treatment.

Events Meeting the AE Definition:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital sign measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.
- "Lack of efficacy" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events NOT meeting the AE definition:

- Any clinically significant abnormal laboratory findings (if applicable) or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.

- Medical or surgical procedure (e.g., endoscopy, appendectomy) is not the AE. The condition that leads to the procedure is an AE (e.g., appendicitis).
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

8.1.2 Serious Adverse Event

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A serious adverse event is any untoward medical occurrence at any dose that:

- **Results in death**
- **Is life-threatening**
  - The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe;
- **Requires inpatient hospitalization or prolongation of existing hospitalization**
  - In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
  - Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- **Results in persistent or significant disability/incapacity**
  - The term disability means a substantial disruption of a person's ability to conduct normal life functions.
  - This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption
- **Results in congenital anomaly/birth defect**
- **Other situations**
  - Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but

may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

8.2 Reporting Period

8.2.1 Adverse Event

AEs will be collected from the end of the Screening Visit and until 5 days following last administration of the investigational product.

Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.

8.2.2 Serious Adverse Event

SAEs assessed as **related** to study participation (e.g., investigational product, protocol mandated procedures, invasive tests, or change in existing therapy) or related to a GSK concomitant medication will be recorded from the time a subject provides informed consent, which is obtained prior to the subject's participation in the study, i.e., prior to undergoing any study-related procedure and/or receiving investigational product and until 5 days days following last administration of the investigational product.

SAEs assessed as **not related** to study participation (e.g., investigational product, protocol mandated procedures, invasive tests, or change in existing therapy) or not related to a GSK concomitant medication will be recorded from the at least 1 dose of investigational product and until 5 days following last administration of the investigational product.

8.3 Reporting Procedures

The investigator and any designees are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE and remain responsible for following up on AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the study.

The investigator is to report all directly observed AEs and all AEs spontaneously reported by the study subject. In addition, each study subject will be questioned about AEs.

Each AE is to be assessed to determine if it meets the criteria for SAEs. If an SAE occurs, expedited reporting will follow local and international regulations, as appropriate.

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event.

The investigator or site staff will then record all relevant information regarding an AE/SAE in the CRF.

It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.

There may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission to GSK.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms. Clinical AEs will be described by diagnosis and not by symptoms when possible (e.g., upper respiratory tract infection, seasonal allergy, etc. instead of runny nose).

Medical conditions reported prior to the time period for reporting AEs/SAEs should be recorded as part of the subject's medical history.

AEs elicited by the investigator in a standard manner at the study visits should also be recorded in the AE section of the CRF. Care will be taken not to introduce bias when detecting AE and/or SAE. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

8.3.1 Adverse Event

All AEs will be reported on the AE page(s) of the CRF by the investigator or site staff. It should be noted that the form for collection of SAE information is not the same as the AE CRF. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same AE term should be used on both forms. AE should be reported using concise medical terminology on the CRF as well as on the form for collection of SAE information.

8.3.2 Serious Adverse Event

A paper copy of the SAE form provided in the investigator study master file should be completed as fully as possible.

It is essential to enter the following information:

- Protocol and subject identifiers
- Subject's demography
- Description of events, with diagnosis if available

- Investigator opinion of relationship to study product
- Criterion for seriousness.

The following are desirable and are of particular relevance for investigator and GSK CH assessment of the SAE report:

- Date of onset of AE
- Date AE stopped, if relevant
- Study product start date
- Study product end date if relevant
- Action taken on study product
- Outcome if known

The SAE form, completed as fully as possible, must be e-mailed to the GSK CH Clinical Operations Safety Reporting email box with the study number and subject number in the subject line of the email **immediately and under no circumstance should this exceed 24 hours** after study site personnel learn of the event. The investigator will submit any updated SAE data to the sponsor, **immediately and under no circumstance should this exceed** 24 hours of it being available. The GSK CH Study Manager should also be notified of the situation by telephone or email.

Email Serious Adverse Events to:

PPD

The GSK CH Study Manager or designee will be responsible for forwarding the SAE form to the Case Management Group, Global Clinical Safety and Pharmacovigilance mailbox PPD

The initial report will be followed up with more information as relevant, or as requested by the GSK CH study manager.

8.3.3 Sponsor's Reporting Requirements to Regulatory Authorities and Ethics Committees

GSK has a legal responsibility to notify, as appropriate, the local regulatory authority and other regulatory authorities about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to GSK is essential so that legal obligations and ethical responsibilities towards the safety of subjects are met.

GSK will comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/EC and investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE or other specific safety information eg, summary or listing of SAE) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

8.4 Evaluating Adverse Events and Serious Adverse Events

8.4.1 Severity Assessment

The investigator or designee will make an assessment of severity for each AE and SAE reported during the study and will assign it to one of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities
- Severe: An event that prevents normal everyday activities.

Note: An AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. For example, a headache may be severe (interferes significantly with the subject's usual function) but would not be classified as serious unless it met one of the criteria for SAEs, listed above.

8.4.2 Causality Assessment

The causality assessment is one of the criteria used when determining regulatory reporting requirements. For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. The investigator's assessment of causality must be provided for all AEs (serious and non-serious); the investigator must record the causal relationship in the CRF, as appropriate, and report such an assessment in accordance with the SAE reporting requirements if applicable.

A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. Generally, the facts (evidence) or arguments to suggest a causal relationship should be provided.

The investigator will use clinical judgment to determine the relationship and will also consult the Investigator Brochure (IB), Safety Statement and/or Product Information, for marketed products, in the determination of his/her assessment. Alternative causes, such as underlying

disease(s), concomitant therapy, other risk factors, and the temporal relationship of the event to the study product will be considered and investigated.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. **However, it is very important that the investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK.** The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.

8.5 Withdrawal Due to an Adverse Event and Serious Adverse Events

Withdrawal due to AEs should be distinguished from withdrawal due to other causes, according to the definition of AE noted earlier, and recorded on the appropriate AE CRF page.

When a subject withdraws because of an SAE, the SAE must be reported in accordance with the reporting requirements defined below.

8.6 Pregnancy

8.6.1 Time Period for Collecting Pregnancy Information

Pregnancy information will be collected on all pregnancies reported following administration of any investigational product or washout product and until 5 days after the last treatment.

8.6.2 Action to be Taken if Pregnancy Occurs

The investigator will collect pregnancy information on any subject who becomes pregnant while participating in the study after administration of the investigational product or washout product. The investigator will record pregnancy information on the appropriate form and e-mail it to the GSK CH Clinical Operations Safety Reporting email box PPD within 24 hours of learning of the subject becoming pregnant. The GSK CH Study Manager or designee will be responsible for forwarding the SAE form to the Case Management Group, Global Clinical Safety and Pharmacovigilance mailbox PPD.

The subject will be followed to determine the outcome of the pregnancy. Information on the status of the mother and infant / neonate (including concomitant medications taken by the mother during the pregnancy) will be forwarded by the investigator to the GSK CH Clinical Operations Safety Reporting email box and the GSK CH Study Manager or designee will forward this information to the Case Management Group, Global Clinical Safety and Pharmacovigilance group mailbox at GSK PPD Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE, abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered to be and should be recorded as an SAE.

Any female participant who becomes pregnant while participating will discontinue study treatment or be withdrawn from the study.

8.7 Follow-up of Adverse Events and Serious Adverse Events

After the initial report, the investigator is required to proactively follow up with each subject and provide further information on the subject's condition.

All AEs/SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

New or updated information will be recorded in the originally completed CRF.

The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Investigators are not obliged to actively seek AEs or SAEs in former subjects. However, if the investigator learns of any SAE, including the death, at any time after a subject has been discharged from the study, and considers the event reasonably related to the investigational product or study participation, the investigator will promptly notify GSK by emailing the information to the GSK CH Clinical Operations Safety Reporting email box PPD . The GSK CH Study Manager or designee will be responsible for forwarding the information to the Case Management Group, Global Clinical Safety and Pharmacovigilance group mailbox at GSK PPD .

The investigator will submit any updated SAE data to GSK within the designated reporting time frames.

8.8 Definition of and Procedure for Reporting Medical Device Incidents

Medical devices are being provided by GSK CH for use in this study; the medical device in this study is the washout manual toothbrush (Class I medical device).

8.8.1 Definition of an Incident

A medical device incident is any malfunction or deterioration in the characteristics and/or performance of a device as well as any inadequacy in the labeling or the instructions for use

which, directly or indirectly, might lead to or might have led to the death of a participant/user/other person or to a serious deterioration in his/her state of health.

Not all incidents lead to death or serious deterioration in health. The nonoccurrence of such a result might have been due to other fortunate circumstances or to the intervention of health care personnel.

It is sufficient that:

- An **incident** associated with a device happened and
- The **incident** was such that, if it occurred again, might lead to death or a serious deterioration in health.

A serious deterioration in state of health can include any of the following:

- Life-threatening illness
- Permanent impairment of body function or permanent damage to body structure
- Condition necessitating medical or surgical intervention to prevent one of the above
- Fetal distress, fetal death, or any congenital abnormality or birth defects

Examples of incidents:

- A participant, user, caregiver, or healthcare professional is injured as a result of a medical device failure or its misuse.
- A participant's study treatment is interrupted or compromised by a medical device failure.
- A misdiagnosis due to medical device failure leads to inappropriate treatment.
- A participant's health deteriorates due to medical device failure.

8.8.2 Reporting of an Incidents and Malfunctions

- **All incidents must be reported to GSK within 24 hours (or sooner if possible) of the investigator or designee becoming aware of the situation.**
- Any medical device incident occurring during the study will be documented in the subject's medical records, in accordance with the investigator's normal clinical practice, and on the appropriate Incident Report Form. In addition, for incidents fulfilling the definition of an AE or an SAE, the appropriate AE CRF page or SAE form will be completed and reported as per the AE and SAE reporting sections.
- The Incident Report Form will be completed as thoroughly as possible and signed by the investigator before transmittal to GSK CH. It is very important that the investigator describes any corrective or remedial actions taken to prevent recurrence of the incident.

- The completed Incident Report Form should be emailed to the GSK CH Clinical Operations Safety Reporting email box with the study number and subject number in the subject line of the email as soon as possible, **but not later than 24 hours** after study site personnel learn of the event. If there is an SAE, the completed SAE pages should be sent together with this report form. However, if a copy of the SAE report is sent with this form, this does not replace the procedure to report an SAE. The original Incident Report Form will remain with the subject's records.

- The GSK CH Study Manager should be notified of the situation by telephone or email.

Email the Incident Report Forms to:
PPD

- The GSK CH Study Manager or designee will be responsible for forwarding the Incident Report Form to the Case Management Group, Global Clinical Safety and Pharmacovigilance group mailbox PPD, responsible for the study and other GSK CH personnel as appropriate.

- The initial report will be followed up with more information as relevant, or as requested by the GSK CH study manager.

The investigator will follow the following directions regarding the reporting of a device failure (malfunction):

- Notify GSK CH immediately (by following the process described above).
- Schedule the subject to return to the site promptly to return the failed device.
- Record any incidents on the CRF and Incident Report Form following instructions given in the section above.
- Return the failed device to the sponsor as soon as possible, including documentation of the details of the failure

8.8.3 Follow-up of Medical Device Incidents

- All medical device incidents involving an AE will be followed and reported in the same manner as other AEs. This applies to all participants, including those who discontinue study treatment or the study.
- The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the incident.
- New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

8.8.4 Regulatory and Ethics Reporting Requirements for Incidents

- In order to fulfill regulatory reporting obligations worldwide, the investigator is responsible for the detection and documentation of events meeting the definitions of incident or malfunction that occur during the study with such devices. Medical device

incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.

- The investigator will promptly report all incidents occurring with any medical device provided for use in the study in order for the sponsor to fulfill the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.
- The investigator, or responsible person according to local requirements (eg, the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of incidents to the IRB/IEC.

9 DATA MANAGEMENT

As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this study.

For this study subject data will be entered into an electronic CRF using a validated data system.

9.1 Source Documents/ Data

The source documents, such as diary cards, which contain the source of data recorded in the CRF should be specified in Section 6. The CRF can be used as a source document at the discretion of data management.

Each subject will be assigned and identified by a unique Screening Number. Any reference made to an individual subject within the study must be done using the unique Screening Number.

9.2 Case Report Form

A CRF is a printed, optical, or electronic document designed to record all of the protocol required information to be reported to the sponsor on each trial subject. Refer to the appropriate CRO handbook and study-specific CRF specifications.

For each subject who has given informed consent/assent and has been screened, CRF must be completed and signed by the Principal Investigator (or authorized designee) to certify that the data are complete and correct.

Management of clinical data will be performed in accordance with Third Party Biostatistics and Data Management vendor applicable standards and data cleaning procedures with oversight by GSK CH to ensure integrity of the data e.g., removing errors and inconsistencies in the data.

In order to protect the privacy of subjects, no Personally Identifiable Information (PII) (including the subject's name or initials or full birth date) is to be recorded in the CRF or as part of the query text.

All CRF pages should be completed during a subject assessment when the CRF has been designated as the source. Data that is sourced elsewhere should be entered into the CRF in an agreed upon timeframe between the Investigator and Sponsor.

GSK CH will obtain and retain all CRFs and associated study data at the completion of the study.

9.3 Data Handling

Documentation of all data management activities should allow step-by-step retrospective assessment of data quality and study performance.
Any changes or corrections to data will be performed in the Electronic Data Capture (EDC) System, and it will include rationale for changes. The EDC system has an audit trail, which will provide a complete record of the changes and corrections endorsed by the Investigator.

Adverse events will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and concomitant medications terms (if applicable) using an internal validated medication dictionary, GSKDrug.

9.3.1 Queries

Programmed edit checks will be generated automatically, as the data is being entered into the system. Data Management will also run reports and listings on the CRF data, in addition to the queries already programmed and generated by the system, to raise manual queries as needed for site clarification or correction. The Clinical Dictionary Development and Management Group will raise queries as needed on safety data to code the terms (Adverse Events and Drugs) appropriately.

The study monitor at the study site will review the CRFs in accordance with the monitoring plan, and any queries will be generated in the EDC System to the Investigator or designee, enabling the errors to be addressed in parallel with Data Management review. The study monitor can also run reports and listings on the CRFs, to raise manual queries as needed for site clarification or correction.

9.4 Processing Patient Reported Outcomes

Patient reported outcome (PRO) data may be collected from diary cards, questionnaires, etc, and entered into the sponsor's clinical data management system (DMS). In instances where the PRO data is entered into the DMS by GSK CH, the PROs will be anonymized as agreed and documented prior to study initiation. PROs that are source will be retained by the investigator and certified copies will be sent to GSK CH.

In order to protect the privacy of subjects, no Personally Identifiable Information (PII) (including the subject's name or initials or birth date) is to be recorded on all PRO's that will be forwarded to GSK CH.

9.5 External Data

External Data are subject data obtained externally to the CRF. These data are generated from laboratory instruments, computers or other sources and then transcribed into a file and format agreed upon by GSK CH to identify the subject and time point referenced in the CRF and/or protocol.

An agreed upon quality control process is performed against the transcribed data to the source to ensure the accuracy of the transcription. The transcribed data is transmitted in an agreed upon format to GSK CH.

Reconciliation will be performed between the transcribed data and the clinical database to ensure subject and time point referenced in the Clinical Database.

10 STATISTICAL CONSIDERATIONS AND DATA ANALYSES

The endpoints of this study are described below and in Section 10.2

Primary end point:

%SMHR

The extent of remineralization will be calculated as the % recovery in SMH, which is calculated from the enamel indentation length at baseline, after the first erosive challenge and after the *in situ* remineralization phase after 4 hrs of intraoral phase.

Secondary end point:

%RER

This end point aims to test whether the formulations prevents enamel demineralization after the 4 hours intra oral remineralization phase as per a second erosion challenge. The %RER is calculated based on the indentation length at baseline, after the first and the second erosive challenge after 4 hrs.

EFU

EFU is assessed by the microdrill enamel biopsy, and its determined after 4 hours *in situ* remineralization period but before the second extra-oral erosive challenge. This measurement will provide with micrograms of fluoride per square centimeter (µg F/cm).

Exploratory end point:

%SMHR, %RER and EFU after 2 hours of intraoral exposure will be evaluated as exploratory end point.

The ARR measures acid resistance of the treated enamel after 2 and 4 hours intraoral phase. It is calculated based on the indentation length after the second erosive challenge and the *in situ* remineralization phase, and after the first erosive challenge and baseline.

10.1 Sample Size Determination

A sufficient number of healthy subjects will be screened so that up to 66 subjects are randomized to participate in the study to ensure 60 evaluable subjects complete the entire study. With a sample size of 60 subjects that complete the study, the study will have 90% power to detect a difference in means of 5.0 in %SMHR at 4 hours, assuming a standard deviation of differences of 11.92 (estimated from GSK study Z2560490, which was a study of similar design), using a paired t-test with a 0.05 two-sided significance level.

A sample size of 60 subjects will be large enough to also detect a difference of 7.4 in RER with 80% power.

A sufficient number of healthy adult subjects will be selected from the Oral Health Research Institue's IRB approved database of previous research studies or from persons expressing interest in participating in research, and screened for participation.

10.2 Statistical Methods and Analytical Plan

Additional details of the proposed statistical analysis will be documented in the statistical analysis plan (SAP), which will be written following finalization of the protocol and prior to study unblinding.

Treatment differences in the study variables will be tested under the null hypothesis:

H0: there is no treatment difference versus the alternate hypothesis

H1: there is a treatment difference.

10.2.1 Demographic and Baseline Characteristics

Descriptive statistics (number of subjects, mean, standard deviation, median, minimum and maximum for continuous variables, and frequency and percentage for categorical variables) will be provided for demographic, ethnicity, baseline characteristics, concomitant medications, medical history and compliance.

10.2.2 Primary Analysis(es)

The primary efficacy variable is %SMHR after 4 hours of remineralization.

Definition of primary variable:

% SMH recovery = [(E1-R)/(E1-B)] * 100

B = indentation length (µm) of sound enamel at baseline

E1 = indentation length (µm) after first erosive challenge

R = indentation length (µm) after in situ remineralization

Per subject %SMHR will be determined from the %SMHR calculated for each specimen using five indentations measurements per specimen, averaged across the four specimens at 4 hrs. Therefore, a single observation per treatment for each subject will be used in the statistical analyses. If a subject is missing an enamel specimen, the mean will be computed over the available enamel specimens.

The ANOVA model will include fixed factors for study period and treatment, and a random effect for subject. The baseline SMH and first demineralization level will not be included in the model as covariates as the samples will be preselected so that each sample has a measurement of 43 +/-3 µm (at baseline, B) and 120 ± 20 µm (after the first erosive challenge, E1).

The primary comparison of this study is the 1150 ppm fluoride experimental dentifrice versus the fluoride free placebo (0 ppmF) and must be statistically significant to meet the success criteria. The treatment difference and 95% CI will be presented for the primary comparison and all other %SMHR paired comparisons (classed as secondary and exploratory objectives). P-Values for these secondary and exploratory comparisons will only be presented if the primary comparison is statistically significant.

The assumptions underlying the ANOVA will be examined, and if necessary, a suitable transformation or a non-parametric analysis will be performed (eg paired Wilcoxon Sign Rank test).

10.2.3 Secondary Analysis(es)

The secondary efficacy variables are:

i) % Relative Erosion Resistance (RER) after 4 hours intraoral phase.

ii) Enamel Fluoride Uptake (EFU) 4 hours intraoral phase.

Definition of secondary variables

% Relative Erosion Resistance = [(E1-E2) / (E1-B)] * 100

B= Indentation length (µm) of sound enamel at baseline

E1= Indentation length (µm) after first erosive challenge

E2= Indentation length (µm) after second erosive challenge

Per subject % RER will be determined for each specimen using five indentations measurements per specimen, averaged across the four specimens at 4 hours. Therefore, a single observation per treatment for each subject will be used in the statistical analyses. If a subject is missing an enamel specimen, the mean will be computed over the available enamel specimens.

Per subject EFU score will be calculated as follows: microdrill samples from each enamel specimen will be pooled and a value for fluoride content per enamel specimen determined. These values will be averaged across the four enamel specimens for each subject and time point to produce the subject-wise mean enamel fluoride uptake.

Analysis of secondary variables

The same ANOVA model and testing procedure outlined for the primary variable will be conducted on the two secondary variables.

The treatment difference and 95% CI will be presented for all of the comparisons however the p-values for these secondary comparisons will only be presented if the primary comparison is statistically significant.

The assumptions underlying the ANOVA will be examined, and if necessary, a suitable transformation or a non-parametric analysis will be performed (eg paired Wilcoxon Sign Rank test).

10.2.4 Safety Analysis(es)

All assessments of safety will be based on the safety population, defined as all subjects who are randomised and receive at least one dose of study treatment during the study. Safety analyses will be performed according to the treatment that the subject received (using variable ATRT). All adverse events (AEs) will be reviewed by the Clinical Research Scientist or Designee prior to database lock and unblinding and will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). During this review stage, AEs will be further categorized as oral or non-oral. AEs will be listed and summarized by treatment received. Serious AEs will also be listed. AEs will be regarded as treatment emergent if they

occur on or after the first treatment application at the baseline visit. The following AEs tables split by treatment will be produced:

- Listing of all AEs (including Non-treatment emergent AEs from all subjects)
- Listing of all AEs for screened subjects
- Treatment emergent AEs by Oral/Non-Oral Preferred Term (PT)
- Treatment emergent AEs by System Organ Class (SOC) and PT
- Treatment emergent AEs by intensity and PT
- Treatment emergent treatment related AEs by Oral/Non-Oral
- Treatment emergent treatment related AEs by SOC
- Listing of serious AEs (if there are none a null listing will be produced; if there are more than 5 treatment emergent serious AEs (SAEs) a table will be produced instead by SOC and PT)
- Non-serious treatment emergent AEs by SOC and PT (only produced if there are more than 5 SAEs).
- Listing of incidents (if there are none a null listing will be produced).

Further information related to safety also includes a table and listing of OST data. The table will show changes in abnormality pre and post treatment. A table of exposure not needed in this study as it is a single use study.

10.2.5 Other Analysis(es)

Exploratory efficacy variables are:

i) %SMHR after 2 hours intraoral phase

ii) % RER after 2 hours intraoral phase.

iii) EFU after 2 hours intraoral phase.

iv) ARR after 2 and 4 hours intraoral phase

Definition of exploratory variables

%SMHR as defined above per primary variable, and %RER and EFU as defined above per secondary variables.

The ARR will be calculated as follows:

ARR = 1- [(E2-R) / (E1-B)]

B= Indentation length (µm) of sound enamel at baseline

R= Indentation length (µm) after in situ remineralization

E1= Indentation length (µm) after first erosive challenge

E2= Indentation length (µm) after second erosive challenge

Analysis of exploratory variables

For %SMHR, %RER and EFU variables the same ANOVA model and testing procedure outlined for the primary and secondary variables will be conducted on these exploratory variables. All pair-wise treatment comparisons will be estimated form the respective models for the variables. The treatment difference and 95% CI will be presented for all of the comparisons made however the p-values for these exploratory comparisons will only be presented if the primary comparison is statistically significant.

For ARR, summary statistic table by treatment and time point will be provided.

10.2.6 Definition of Analysis Populations

All assessments of safety will be based on the safety population, defined as all subjects who are randomised and receive at least one dose of study treatment during the study. Safety population summaries will be presented by treatment received.

The primary population for efficacy assessment will be the intent-to-treat (ITT) population, defined as all subjects who are randomized, receive the study treatment at least once and provide at least one post-baseline (post treatment) assessment of efficacy. All ITT population summaries and analyses will be presented by treatment randomized.

10.2.7 Exclusion of Data from Analysis

Exclusion of any data from the analyses will be determined during a Blind Data Review Meeting prior to database lock. Any reasons for exclusion from an analysis population will be listed, if applicable.

The per protocol (PP) population is defined as all subjects in the ITT population who have at least one assessment of the primary endpoint efficacy considered unaffected by protocol violations.

PP analysis will be performed only on those data considered unaffected by protocol violations.

Efficacy analysis on the PP population will be performed only on the primary variable (%SMHR) only if there is more than 10% difference in the number of subjects per treatment between PP and ITT populations.

A decision on whether a PP analysis will be performed will be made prior to study unblinding

10.2.8 Handling of Dropouts and Missing Data

Subjects who withdraw from the study early will be included in the study analysis up to the point of withdrawal. Subjects who withdraw will not be replaced. No data will be imputed in the case of dropouts or missing data.

10.2.9 Interim Analysis

No interim analysis is planned for this study

11 STUDY GOVERNANCE CONSIDERATIONS

11.1 Quality Control

In accordance with applicable regulations including GCP, and GSK procedures, GSK or designee (i.e. third party vendor) monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements.

When reviewing data collection procedures, the discussion will include identification, agreement and documentation of data items for which the CRF will serve as the source document.

GSK or designee will monitor the study and site activity to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The extent and nature of monitoring will be described in a written monitoring plan on file at GSK CH. The investigator (or designee) agrees to allow the monitor direct access to all relevant documents and agrees to co-operate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

11.2 Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance assessment and/or audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study.

In the event of an assessment, audit or inspection, the investigator (and institution) must agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any findings/relevant issues and to implement any corrective and/or preventative actions to address any findings/issues identified.

The investigator(s) will notify GSK CH or its agents immediately of any regulatory inspection notification in relation to the study. Furthermore, the investigator will cooperate with GSK CH or its agents to prepare the study site for the inspection and will allow GSK CH or its agent, whenever feasible, to be present during the inspection. The investigator will promptly apply copies of the inspection finding to GSK CH or its agent. Before response submission to the regulatory authority, the investigator will provide GSK CH or its agents with an opportunity to review and comment on responses to any such findings.

The sponsor will be available to help investigators prepare for an inspection.

11.3 Regulatory and Ethical Considerations

11.3.1 Institutional Review Board/ Ethics Committee

It is the responsibility of the investigator to have prospective approval of the study protocol, protocol amendments, informed consent documents, investigator brochure/safety statement (including any updates) and other relevant documents, e.g., recruitment advertisements, if applicable, from the IRB/EC. All correspondence with the IRB/EC should be retained in the investigator file. Copies of IRB/EC approvals should be forwarded to GSK CH prior to the initiation of the study, and also when subsequent amendments to the protocol are made.

The only circumstance in which an amendment may be initiated prior to IRB/EC approval is where the change is necessary to eliminate apparent immediate hazards to the subjects. In that event, the investigator must notify the IRB/EC and GSK CH in writing immediately after the implementation.

11.3.2 Ethical Conduct of the Study

The study will be conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), guidelines for GCP (ICH 1996 and revision 2), and the Declaration of Helsinki (World Medical Association 2013).

In addition, the study will be conducted in accordance with the protocol, the ICH guideline on GCP, and applicable local regulatory requirements and laws.

11.3.3 Subject Information and Consent

All parties will ensure protection of subject personal data and will not include subject names or other identifiable data in any reports, publications, or other disclosures, except where required by laws.

When study data are compiled for transfer to GSK CH and other authorized parties, subject names, addresses, and other identifiable data will be replaced by numerical codes based on a numbering system provided by GSK CH in order to de-identify study subjects.

The study site will maintain a confidential list of subjects who participated in the study, linking each subject's numerical code to his or her actual identity. In case of data transfer, GSK CH will maintain high standards of confidentiality and protection of subjects' personal data consistent with applicable privacy laws.

The informed consent documents must be in compliance with ICH GCP, local regulatory requirements, and legal requirements, including applicable privacy laws.

The informed consent documents used during the informed consent process must be reviewed and approved by the sponsor, approved by the IRB/EC before use, and available for inspection.

The investigator must ensure that each study subject, is fully informed about the nature and objectives of the study and possible risks associated with participation.

The investigator, or a person designated by the investigator, will obtain written informed consent from each subject before any study-specific activity is performed. The investigator will retain the original of each subject's signed informed consent document.

11.3.4 Subject Recruitment

Advertisements approved by IRBs/ECs and investigator databases may be used as recruitment procedures. Use of ethics committee approved, generic, prescreening questionnaire to assess basic subject characteristics to determine general eligibility for this study is allowed. This generic questionnaire may be used by sites as a phone script and/or to review internal databases to identify subjects.

GSK CH will have an opportunity to review and approve the content of any study recruitment materials directed to potential study subjects before such materials are used.

11.3.5 Reporting of Safety Issues and Serious Breaches of the Protocol or ICH GCP

Within a GSK CH a serious breach is defined as a breach likely to affect to a significant degree the safety and rights of a subject or the reliability and robustness of the data generated in GSK CH- sponsored human subject research studies.

In the event of any prohibition or restriction imposed (i.e., clinical hold) by an applicable competent authority in any area of the world, or if the investigator is aware of any new information that might influence the evaluation of the benefits and risks of the investigational product, GSK CH should be informed immediately.

In addition, the investigator will inform GSK CH immediately of any urgent safety measures taken by the investigator to protect the study subjects against any immediate hazard, and of any serious breaches of this protocol or of ICH GCP that the investigator becomes aware of.

11.4 Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins in accordance with applicable GSK processes.

11.5 Provision of Study Results to Investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

11.6 Records Retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.

The records (study/ site master file) must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.

Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.

The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a

hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

The investigator must assure that the subject's anonymity will be maintained. On CRFs or other documents submitted to GSK CH, subjects should not be identified by their names or initials, but by an identification code. The investigator should keep a separate log of subjects' codes, names and addresses. Documents not for submission to GSK CH, e.g. subjects' written consent forms, should be maintained by the investigator in strict confidence.

Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report (CSR)/ equivalent summary unless local regulations or institutional policies require a longer retention period. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements.

No study document should be destroyed without a prior written agreement between GSK CH and the investigator. The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

11.7 Conditions for Terminating the Study

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the IRB/EC, or investigational product safety problems, or at the discretion of GSK CH. In addition, GSK CH retains the right to discontinue development of the experimental dentifrice at any time. For multicenter studies (if applicable), this can occur at one or more or at all sites.

If a study is prematurely terminated, GSK CH will promptly notify the investigator. After notification, the investigator must promptly contact all participating subjects and should assure appropriate therapy/ follow-up for the subjects. As directed by GSK CH, all study materials must be collected and all CRF completed to the greatest extent possible. Where required by the applicable regulatory requirements, GSK CH should inform the regulatory authority(ies) and the investigator should promptly inform the IRB/EC and provide the IRB/EC a detailed written explanation of the termination or suspension.

If the IRB/EC terminates or suspends its approval/favorable opinion of a trial, the investigator should promptly notify the GSK CH and provide GSK CH with a detailed written explanation of the termination or suspension.

Upon completion or premature discontinuation of the study, the GSK CH monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK CH Standard Operating Procedures.

11.8 Definition of Study End/ End of Study

The End of Study (EOS) is defined as the date of the last laboratory testing related to primary and secondary endpoints, to be achieved no later than 8 months after the date of the last subject last visit.

12 REFERENCES

Attin T (2006). *Methods for assessment of dental erosion*, vol. 20. Karger Monographs in Oral Science.

Barlow AP, Sufi F, Mason SC (2009). Evaluation of different fluoridated dentifrice formulations using an in situ erosion remineralization model. *J Clin Dent* **20:** 192-198.

Bartizek RD, Gerlach RW, Faller RV, Jacobs SA, Bollmer BW, Biesbrock AR (2001). Reduction in dental caries with four concentrations of sodium fluoride in a dentifrice: a meta-analysis evaluation. *J Clin Dent* **12:** 57-62.

Brunelle JA, Carlos JP (1990). Recent trends in dental caries in U.S. children and the effect of water fluoridation. *J Dent Res* **69 Spec No:** 723-727; discussion 820-723.

Chow LC (1990). Tooth-bound fluoride and dental caries. *J Dent Res* **69 Spec No:** 595-600; discussion 634-596.

Cochrane NJ, Zero DT, Reynolds EC (2012). Remineralization models. *Adv Dent Res* **24:** 129-132.

Featherstone JD, Glena R, Shariati M, Shields CP (1990). Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. *J Dent Res* **69 Spec No:** 620-625; discussion 634-626.

Fowler C, Willson R, Rees GD (2006). In vitro microhardness studies on a new anti-erosion desensitizing toothpaste. *J Clin Dent* **17:** 100-105.

Fowler CE, Gracia L, Edwards MI, Willson R, Brown A, Rees GD (2009). Inhibition of enamel erosion and promotion of lesion rehardening by fluoride: a white light interferometry and microindentation study. *J Clin Dent* **20:** 178-185.

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GSK-Z3480664 GSK Clinical Study (2013): Evaluation of a Test Mouthwash and Dentifrice Regimen in an In-Situ Model of Dental Erosion.

GSK-Z3480664 GSK Clinical Study (2013): Evaluation of a Test Mouthwash and Dentifrice Regimen in an In Situ Model of Dental Erosion.

CCI [redacted]

GSK-Z6961385 GSK Clinical Study (2013): A Placebo Controlled Study to Evaluate the Effectiveness of Two Gel to Foam Toothpastes Using a Modified In situ Model of Dental Erosion and Remineralization.

Shellis RP, Ganss C, Ren Y, Zero DT, Lussi A (2011). Methodology and models in erosion research: discussion and conclusions. *Caries Res* **45 Suppl 1:** 69-77.

Stephen KW (1993). Dentifrices: recent clinical findings and implications for use. *Int Dent J* **43:** 549-553.

Zero DT (1995). In situ caries models. *Adv Dent Res* **9:** 214-230; discussion 231-214.

13 APPENDIX

13.1 ABBREVIATION

The following is a list of abbreviations that may be used in the protocol.

Table 13-1 Abbreviation

Abbreviation	Term
AE	adverse event
ANOVA	analysis of variance
ARR	Acid resistance ratio
CI	confidence interval
CRF	case report form
CRO	contract research organization
CSR	clinical study report
CRS	clinical research scientist
DMS	data management system
EC	ethics committee
EDC	electronic data capture
EFU	Enamel fluoride uptake
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
gr	grams
GSK CH	GlaxoSmithKline Consumer Healthcare
hrs	hours
IB	investigator's brochure
ICH	International Conference on Harmonisation
ID	identification
IRB	institutional review board
ITT	intention to treat
MedDRA	medical Dictionary for Regulatory Activities
min	minutes
mL	milliliters
N/A	not applicable
OHT	oral hard tissue
OST	oral soft tissue
PI	principal investigator
PII	personally identifiable information
PP	per protocol
PRO	patient reported outcome
RER	relative erosion resistance
PT	preferred term

Abbreviation	Term
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
sec	seconds
SMH	surface micro hardness
SMHR	surface micro hardness recovery
SOC	system organ class
SRSD	single reference study document
US	United States
µg	micrograms

SIGNATURE PAGE

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