In situ anticaries efficacy of dentifrices with different formulations – a pooled analysis of results from three randomized clinical trials

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Short title: Pooled analysis of in situ anticaries results

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This is the author's manuscript of the article published in final edited form as:

Zero, D. T., Lippert, F., Hara, A. T., Creeth, J., Newby, E., Butler, A., … Bosma, M. L. (2018). In situ anticaries efficacy of dentifrices with different formulations – a pooled analysis of results from three randomized clinical trials. Journal of Dentistry. https://doi.org/10.1016/j.jdent.2018.07.014

Key words: dentifrices, in situ, caries, pooled analysis, fluoride

Declaration of interest

J Creeth, A Butler and P Constantin are employees of GSK Consumer Healthcare; E Newby and ML Bosma were employees of GSK Consumer Healthcare at the time of the studies. They have no further conflicts of interest to declare. DT Zero, F Lippert and AT Hara are employees of OHRI, which has received funding from GSK Consumer Healthcare. DT Zero and F Lippert have received compensation from GSK Consumer Healthcare as independent consultants in the past.

Objectives: Data generated from three similar in situ caries crossover studies presented the opportunity to conduct a pooled analysis to investigate how dentifrice formulations with different fluoride salts and combinations at concentrations of 1400–1450 ppm F, different abrasive systems and in some cases, carbomer (Carb), affect enamel caries lesion remineralization and fluoridation.

Methods: Subjects continuously wore modified partial dentures holding two gauze-covered partially-demineralized human enamel specimens for 14 days and brushed 2×/day with their assigned dentifrice: Study 1: sodium fluoride (NaF)/Carb/silica, NaF/silica, NaF+MFP/chalk; Study 2: NaF/Carb/silica, NaF+MFP/dical, amine fluoride (AmF)/silica; Study 3: NaF/Carb/silica, NaF+ stannous fluoride(SnF_2)/silica/hexametaphosphate (HMP). All studies included Placebo (0 ppm F) and/or dose-response controls (675 ppm F as NaF [675F-NaF]) ±Carb. Specimens were evaluated for percentage surface microhardness recovery (SMHR) and enamel fluoride uptake (EFU).

Results: All 1400–1450 ppm F dentifrices except NaF+SnF_2/silica/HMP provided significantly greater lesion remineralization than Placebo ($p<0.0001$): differences in SMHR ranged from 17.46% (NaF+MFP/dical) to 26.66% (AmF/silica). For EFU (back-transformed log EFU), all 1400–1450 ppm F dentifrices gave significant fluoride uptake compared to Placebo ($p<0.0001$): increases in EFU ranged from 4.95 μg F/cm^2 (NaF+SnF_2/silica/HMP) to 16.32 μg F/cm^2 (NaF/carb/silica). Dentifrices containing NaF or AmF as sole fluoride source provided the greatest remineralization and fluoridation; Carb addition did not alter fluoride efficacy; some excipients appeared to interfere with the cariostatic action of fluoride. Treatments were generally well-tolerated with ≤4 treatment-related adverse events per study.

Conclusion: Commercially available fluoride dentifrices varied greatly in their ability to remineralize and fluoridate early caries lesions.

Clinical significance:

Fluoride dentifrices are the most impactful anticaries modality worldwide. While clinical caries trials have not consistently shown the superiority of one formulation over another, these findings using a sensitive in situ caries model indicated that dentifrices containing NaF or AmF as the sole fluoride source provided the greatest remineralization and fluoridation benefits.

Introduction

It is generally agreed that the anticaries effect of fluoride is predominantly by decreasing the rate of enamel demineralization and enhancing the rate of remineralization [1–3]. However, different formulations of fluoride dentifrices may not have the same anticaries efficacy potential [4]. There has been a controversy over the relative merits of sodium fluoride (NaF) versus sodium monofluorophosphate (MFP), with published reviews reaching different conclusions from basically the same clinical studies [5,6]. Not only can different fluoride salts have intrinsically different anticaries activities, but the formulation environment of the fluoride species can affect its delivery to the oral cavity and its ability to interact with enamel in vivo [4]. Furthermore, other dentifrice ingredients may positively or negative impact the caries process by directly inhibiting demineralization by offering surface protection or by interfering with remineralization.

To examine this further, a series of three studies were carried out using an in situ caries model to evaluate the efficacy of a dentifrice where carbomer (high molecular weight copolymer of acrylic acid crosslinked with a polyalkenyl polyether) had been added to NaF as a possible aid to increase bioavailability of fluoride compared to a variety of commercially available dentifrice formulations. These dentifrices contained fluoride from a number of different sources (NaF, MFP, stannous fluoride [SnF_2], amine fluoride [AmF]) and combinations thereof. The efficacy of these was compared to a variety of dose-response control dentifrices including low fluoride dentifrices (675 ppm F) and fluoride-free dentifrices.

Clinical studies are limited in regard to how many treatment and control groups can be compared. Here we present a pooled analysis of three studies whose similar designs present a unique opportunity to compare the in situ remineralization performance of several commercially available products in a well-characterized model. The three studies carried out here were compared using a network meta-analysis (NMA) technique applied to pooled data. Of note, this is not intended to be a full meta-analysis, as comparison was only within the three studies reported herein. The in situ caries model involving partial denture appliances [7] with partially demineralized enamel specimens used in these studies to evaluate enhancement of net remineralization has been validated based on response to a variety of different dentifrice fluoride concentrations [3,8,9]. This model is advantageous as fluoride is delivered in the presence of physiologically secreted saliva and there are intermittent cycles of demineralization and remineralization during the experimental period as with the natural caries process. The in situ model system is used with the surface microhardness (SMH) test as the primary outcome measure [3,7,9–14]. Here, the hardness of sound enamel is measured and compared with the hardness of enamel after exposure to an in vitro acid challenge and then after intra oral exposure,

simulating the caries process [3]. The in situ model has also been applied to measure fluoride uptake from enamel specimens (enamel fluoride uptake: EFU) [3,9].

Materials and Methods

The three studies followed a similar single center, randomized, examiner-blind, reference-controlled crossover design. They were undertaken as part of an Investigational New Drug (IND) program (IND 75222), with the protocols reviewed and approved by the IUPUI/Clarian Institutional Review Board (Study 1: IRB# 0803-14; Study 2: IRB# 0809-15; Study 3: IRB# 0910-29). All studies were conducted at the Oral Health Research Institute (OHRI), Indianapolis, IN, USA with subjects selected from the OHRI's IRB approved database of previous research subjects, if suitable, or recruited from the area. Prior to study initiation all subjects gave informed consent in accordance with the Declaration of Helsinki. Details of these studies and results can be found at ClinicalTrials.gov (NCT00708097, NCT01005966, NCT01128946). There was one amendment, to Study 3 only, an administration change that did not affect study procedure or outcomes.

Clinical procedures

All studies followed the same protocol with only minor variations, as noted. At the screening visit (Visit 1), demographic details, medical history and concomitant medications were recorded followed by oral soft tissue (OST) and oral hard tissue examinations. Study entry criteria included: healthy volunteers aged 18–80 years with a normal saliva flow rate (unstimulated: ≥0.2 mL/minute; stimulated: ≥0.8 mL/minute) who wore a removable mandibular partial denture able to be adjusted to hold enamel test specimens and lived in the Indianapolis, IN area (with a fluoridated water supply of approximately 1 ppm). Subjects could not be taking fluoride supplements or using fluoride mouthrinse and could not have any clinically significant/relevant abnormalities of medical history or physical examination including current active caries or periodontal disease that could have compromised the study or the subject's health. Exclusion criteria included: pregnant; breast feeding; intolerance to any study material; currently taking antibiotics or had taken antibiotics within 2 weeks prior to screening; participation in another clinical study or receipt of an investigation drug within 30 days of screening.

At Visit 2, 2–3 days before the start of the first treatment period, subjects received a prophylaxis and their partial denture was prepared for enamel specimen placement. They then brushed at home twice-daily with a supplied fluoride-free dentifrice until Visit 3. Eligible subjects were assigned treatments in an order according to a randomization sequence generated by the Biostatistics and Data

Management department of GSK Consumer Healthcare (GSKCH). Details of the test dentifrices can be found in Table 1, including fluoride source and concentration, abrasive, surfactant and viscosity and rheology modifiers. Test dentifrices were supplied in plain white 100 ml tubes; commercially sourced dentifrices were supplied overwrapped with opaque white vinyl to aid in blinding participants and site staff to dentifrice type.

At the start of each treatment period, two partially demineralized enamel specimens covered with Polyester Knit Fabric (Item# 401628, Impra, Tempe, USA) to encourage plaque formation [7] were placed in the buccal flange of the subject's partial denture. Subjects performed their first brushing under site supervision where they applied a full ribbon (Studies 1 and 2) or 1.5 ±0.1 g (Study 3) of dentifrice to a wet toothbrush and brushed their natural teeth for one timed minute, taking care not to brush the enamel specimens, then rinsed for 10 seconds with 10 mL (Studies 1 and 2) or 15 mL (Study 3) water. Subjects continued the study brushing/rinsing regimen at home twice daily for 14 days, recording brushing on a supplied diary card that was used to check compliance. During the treatment period subjects wore their partial denture 24 hours a day except when cleaning it with water. The combination of the fabric-covered specimens and the subjects' normal diet provided a cariogenic environment simulating the caries process [3].

After the 14-day study period, enamel specimens were removed and stored until analyzed. To control for carry-over effects there was a 7-day wash-out period between treatments during which subjects followed their usual dental hygiene regimen for at least 4 days, followed by a 2–3 day lead-in period. During this time the partial denture was re-fitted with new specimens and the OST examination, eligibility check, prophylaxis, and brushing procedure above was repeated. This sequence was continued until all subjects had used all dentifrices within their respective study.

Enamel specimen preparation

Specimens obtained from human permanent teeth were used as the hard tissue study substrate and were prepared as previously described [20] such that each had an enamel surface with a central minimum flattened and polished area of 3×3 mm. For SMH testing, five baseline indentations 100 μm apart were placed in the center of each prepared enamel specimen using a Knoop diamond under a 50 g load. Only specimens with mean baseline indentation lengths of 43 ±3 μm were accepted. Before placement, the enamel specimens were partially demineralized, to simulate early carious lesions, using a modification of the method described by White [21]. The modification involved decreasing the demineralization time from 96 h to 24 h. SMH testing was repeated with five indentations placed to the left of the baseline indentations; only enamel specimens with mean indentation lengths of 120 ±20 μm after demineralization were used. Specimens were sterilized using ethylene oxide gas before

insertion into the subjects' partial dentures where they were mounted such that the enamel surface was flush with the surface of the buccal flange.

Assessment of enamel remineralization

Changes in mineral status of partially-demineralized enamel specimens were evaluated with SMH testing after 14 days intraoral exposure by placing five indentations 100 μm to the right of the baseline indentations. The extent of remineralization, SMH Recovery (SMHR), was calculated as (D2-R/D2-B)×100%, where D2 = indentation length (μm) after in vitro demineralization; R = indentation length (μm) after intra-oral exposure; B = baseline indentation length (μm) [10].

Assessment of fluoride bioavailability in enamel

Fluoride availability of the partially demineralized enamel specimens was carried out after 14 days of intraoral exposure by a microdrill enamel biopsy technique (four cores per specimen) [22]. The diameter of the drill hole was determined using a calibrated microscope interfaced with an image analysis system. Enamel fluoride uptake (EFU) was calculated based on the amount of fluoride divided by the volume of the enamel cores and expressed as μg F/cm^3 (Studies 1 and 2) or divided by the area of the enamel cores and expressed as μg F/cm^2 (Study 3).

Statistical analysis

In Study 1, Study 2 and Study 3 respectively, 57, 65 and 85 subjects were planned to be randomized such that sample sizes of 52, 44 and 72 completed subjects respectively would have 90% (first two studies) and 80% (third study) power at the 0.05 significance level, using two-sided testing, to detect a respective mean difference of 9.0%, 6.5% and 5.0% in SMHR (with within subject standard deviation of the paired differences of 19.32%, 12.89% and 14.90% respectively, taken from GSKCH held data).

Each of the individual studies were analyzed using ANOVA techniques with factors for treatment and study period with subject as a random effect. The pooled analysis of the studies individual subject data were compared using principles of NMA technique [17,18] using analysis of variance (ANOVA) methods. The safety population included all randomized subjects who used at least one of the treatment dentifrices then had at least one safety assessment. The intent-to-treat (ITT) population included all randomized subjects who had used at least one dose of study treatment and provided at least one SMH measurement leading to a SMHR value. The per protocol (PP) population included all randomized subjects who had SMH or EFU values and had no major protocol deviations. All three studies used the PP population as the primary population for analysis hence this pre-defined population was used in the NMA.

Efficacy variables of interest were mean SMHR and mean EFU. For SMHR calculation, for each subject the mean of each series of indentations was taken within each enamel block then the mean of the two blocks was taken. EFU was calculated taking the mean of the two enamel blocks. If a subject was missing an enamel block, data from the remaining block was used alone. As EFU values were reported as drill hole volume (µg F/cm^3) for Studies 1 and 2 but as area (µg F/cm^2) for Study 3, both with a drill hole depth of 100 µm, Study 1 and 2 values were multiplied by 0.01 so that all were expressed as area measures for analysis.

For each variable, the primary objective was to investigate the effect of each treatment compared with NaF/carb/silica and dose response control (675F-NaF). SMHR and EFU were analyzed separately using an analysis of covariance (ANCOVA) model including subject as a random effect, re-coded treatment group, study and period nested within study as factors. A repeated subject effect grouped by study and treatment was also fitted into the model. The Tukey adjustment method was used to control for the alpha level for multiple treatment comparisons. No adjustment for multiple comparisons was used in the analysis of the individual studies, as a primary objective was pre-defined in each of the single studies.

The ANCOVA assumptions were checked for both endpoints and considered to be sufficiently satisfied for SMHR. For EFU, the assumption of homoscedasticity of residuals was violated and data was transformed using log transformation, after which the ANCOVA model assumptions were satisfied. Adjusted means from the analysis were back transformed to geometric means; treatment ratios were back transformed to treatment differences. Approximate 95% confidence limits were constructed for the back transformed treatment differences using the 95% confidence limits of the log ratios. The following equation was used in the calculation of the back transformed treatment differences: Test-Reference = exp[ln(Test/Reference)] × Reference-Reference, where Reference = adjusted geometric mean of NaF/carb/silica or 675F-NaF; ln(Test/Reference) = adjusted log treatment ratio of the corresponding comparison. All analyses were conducted using the PROC MIXED procedure in SAS® software Version 9.2 (SAS Institute Inc., Cary, NC, US).

The assessment of safety and tolerability were based on the safety profiles of the test treatments relative to Placebo with respect to treatment-emergent OST abnormalities and adverse events reported by subjects following the use of study treatments.

Results

The trial profile and baseline characteristics of all subjects are described in Figure 1 and Table 2. From the total number of randomized subjects, 202 (98.5%) were included in the PP population for the pooled analysis: 57 (100%) from Study 1, 63 (96.9%) from Study 2 and 82 (98.8) from Study 3. Overall, subjects mean age at enrollment was 65.4 (SD=9.69) years and 59.9% were female. Study 1 took place between April 2008–August 2008; Study 2 between October 2008–March 2009; Study 3 between November 2009–April 2010.

The network diagram shown in Figure 2 shows how the eight treatments are connected via the three studies. This network shows that out of the ${}^{8}C_{2} = 28$ possible head-to-head comparisons, these studies cover 20 (71%). The figure indicates the eight missing head-to-head comparisons. So, the network is well represented in terms of at least one head-to-head direct comparison. If the network was fully connected and optimized via the three studies, then there would be $3 \times {}^{8}C_{2} = 84$ comparisons. In the network the studies represent ${}^{5}C_{2}+{}^{5}C_{2}+{}^{4}C_{2} = 28$ comparisons, as such, the network is 28/84 = 33% fully represented. Overall the network is fairly well-connected, but at one third of the full optimum. The carbomer and 675 NaF treatments were present in all three studies and act as good anchor reference points.

Results from the individual studies can be found in Supplemental Data Tables 1–4.

SMHR (Table 3, Figures 3–7)

With higher mean values, the NaF/carb/silica group was significantly different from NaF+MFP/dical, NaF+SnF_2/silica/HMP, 675F-NaF and Placebo groups, with no difference compared to the other active dentifrices (Figure 5). The 675F-NaF group also had a statistically significant lower mean SMHR than the NaF/silica and AmF silica groups, but was significantly higher than the Placebo and NaF+SnF_2/silica/HMP groups (Figure 6). Except for NaF+SnF_2/silica/HMP, all other treatments were significantly different from the Placebo group, favoring the active dentifrices. The NaF+SnF_2/silica/HMP group had a significantly lower mean SMHR compared to all the other active treatments (Table 3). Figure 7 shows the SMHR values from each of the studies and from the pooled analysis across the dentifrices investigated in the pooled analysis. The plot shows a very consistent effect from the individual studies and hence when pooled.

EFU (Table 4, Figures 8–12)

The NaF/Carb/silica group was significantly different, in its favor, from all but the NaF/silica and AmF/silica groups (Figure 10). The 675F-NaF group was numerically near the mid-range of all dentifrice groups, significantly different from both the higher values of the NaF/carb/silica, NaF/silica and AmF/silica, and the lower values of the NaF+SnF_2/silica/HMP and Placebo groups (Figures 10

and 11). All other groups were also significantly higher than the Placebo group (Table 4). With the lowest EFU value of the active treatments, the NaF+SnF_2/silica/HMP group was significantly different from all other groups (Table 4). Figure 12 shows the EFU values from each of the studies and from the pooled analysis across the dentifrices investigated in the pooled analysis. Again, there are consistent effects from the individual studies and also when pooled.

Safety Results (Table 5)

In Study 1, 24 (42.1%) subjects reported 38 treatment emergent adverse events (TEAEs) with three of them deemed treatment-related (TR-TEAEs). In Study 2, respective figures were 35 (53.8%) subjects, 55 TEAEs, four TR-TEAEs and, in Study 3, 40 (48.2%) subjects, 68 TEAEs, three TR-TEAEs. No serious adverse events were reported in Studies 1 and 2, in Study 3 one subject had a serious adverse event (coronary artery disease requiring hospitalization), which was unrelated to study treatment. Safety analysis was not performed for the pooled analysis.

Discussion

These three studies were compared using a NMA technique applied to pooled data. Compared to ANOVA and meta-analysis techniques, NMAs are recent developments in the statistical arena. ANOVA has been used since 1918 [15] and meta-analyses since at least 1976 [16]. These are the methods of choice to investigate head-to-head (direct) comparisons. NMA began in 1997, pioneered by Bucher [17] who introduced the idea of indirect comparisons that obtain treatment effects across studies using a treatment that links the studies together (e.g., A vs B and B vs C leads to A vs C via the commonality B). These led into NMA as a combination of direct and indirect treatment comparisons [18]. NMAs are not common in the oral health care arena, and though they are used frequently by the Cochrane collaboration [e.g., 19], they are rare among in situ studies. As this current group of studies were all conducted using the same methodology and at the same study site, this makes the NMA technique an ideal methodology to investigate the comparative efficacy of the eight investigation dentifrices across three studies linking to common controls. Overall, the network pooled across the three studies is fairly well connected but at one third of the full optimum. Two treatments (carbomer and 675 NaF) were present in all three studies and act as good reference points across the studies. Furthermore, the SMHR and EFU results from the individual studies and when pooled from the dentifrices investigated in this pooled analysis are very consistent (Figures 7 and 12, respectively). It's worth noting that the individual study EFU values are arithmetic means, while the values from the pooled analysis are from back-transformed log EFU values, which explains why they are generally slightly lower (Figure 12).

The primary objective of this current pooled analysis of three in situ clinical trials investigating remineralization of enamel caries lesions (SMHR) and uptake of fluoride (EFU) was to investigate the effect of each treatment compared with a NaF dentifrice containing carbomer and silica and a dose-response control dentifrice (675F-NaF). For both measures, a dose-response was shown between a number of the dentifrices with higher levels of fluoride (1400–1450 ppm) and those with medium levels (675 ppm) or no fluoride. Therefore, the present model can be considered of clinical relevance as it matches findings from randomized clinical caries trials, which have shown that higher fluoride concentrations provide greater anticaries benefits [19].

Prior research by some of the current authors suggested carbomer may enhance fluoride delivery [23], hence the hypothesis that the addition of carbomer to a NaF dentifrice would result in improved anticaries efficacy. This, however, was not shown by the pooled analysis to translate to the in situ situation, which is often more complex than can be modeled in vitro. While the NaF/carb/silica dentifrice was significantly different from the NaF+SnF_2/silica/HMP and NaF+MFP/dical dentifrices, and additionally from the NaF+MFP/chalk dentifrice for EFU, it was no different from the NaF/silica and AmF/silica dentifrices on either measure. These findings also highlight the persistent disconnect between laboratory, in situ, and clinical research and that in vitro models alone should be used with caution to determine the possible clinical efficacy of new ingredients included in formulations for functional benefits.

There was a mixed picture of efficacy when comparing between the marketed fluoride dentifrices. The pooled analysis showed that NaF and AmF dentifrices in general provided greater cariostatic benefits than those containing primarily MFP or SnF_2 (with NaF also present). The reason for the varied performance of fluoride dentifrices of similar fluoride concentration can be manifold. MFP dentifrices were considered inferior to their NaF counterparts in a review of the available clinical data in 1993 [24]; however, a more recent study found opposite results when comparing a NaF positive control dentifrice with two MFP dentifrices formulated with 1.5% arginine in either a di-calcium phosphate or calcium carbonate base [25]. This finding was attributed to the arginine and not the different fluoride salts.

Also of interest is that a marketed product (NaF+SnF_2/silica/HMP) with an active excipient, hexametaphosphate (HMP), performed significantly lower for both SMHR and EFU than all other active treatments including the intermediate fluoride dentifrice (675F-NaF). HMP is added for its anti-calculus and stain-removing cosmetic benefits. This finding is in contrast to an earlier in situ study which reported that HMP did not interfere with the anticaries activity of SnF_2 [26], and a clinical caries study that found the SnF_2/HMP combination to be more effective in reducing caries than the NaF positive control [27]. However, it is difficult to determine how the formulation of the marketed

product that was tested in the current study would compare to the dual-phase experimental prototypes tested in these earlier studies. Within the limitations of the study, our findings suggest that fluoride source per se is not the ultimate determinant of dentifrice efficacy, as formulation excipients often interfere with fluoride efficacy, either by, for example, lowering fluoride bioavailability or by providing cariostatic benefits in their own right. The in situ model used to generate the data for the pooled analysis is a remineralization-biased model and suffers the limitations that all models have to one degree or another. The model measured the treatment effect on a surface-softened lesion, which is the initial stage of the caries process, and thus only permits extrapolation of the effect on preventing progression to a more advanced stage of caries (cavitation).

In conclusion, the present pooled analysis using an NMA technique of three independently conducted in situ caries studies has shown that commercially available dentifrices vary in their ability to fluoridate and remineralize early carious lesions. Dentifrices containing NaF or AmF as the sole fluoride source provided the greatest cariostatic benefits, whereas the addition of carbomer did not improve nor diminish fluoride efficacy. While some of the observed differences between dentifrices can be attributed to the type of fluoride salts, the effect of excipients cannot be ruled out. Study products were generally well-tolerated.

Acknowledgements

We acknowledge and thank the many subjects that participated in these studies, without their conscientious participation, these studies would not be possible. We also thank the clinical and laboratory staff of the Oral Health Research Institute for the dedication and hard work in conducting high quality research, in particular Sue Kelly who was the study manager. We would like to thank Eleanor Roberts, Beeline Science Communications, Ltd., funded by GSK Consumer Healthcare, for writing support and Ritu Karwal, previously of GSK Consumer Healthcare, for help with study design.

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Table 1. Study test dentifrices

Dentifrice Name		Fluoride Source and Concentration	Formulation Details (Abrasive; surfactant; viscosity and rheology modifiers [gums only])	Study No		
Individual Study	Pooled Analysis			1	2	3
NaF/carb/silica*	Same	NaF: 1426–1450 ppm F	Silica; SLS; carrageenan, xanthan gum, carbomer[1]	x	x	x
NaF/silica**	Same	NaF: 1400 ppm F	Silica; SLS; carrageenan, xanthan gum	x		
NaF+MFP/chalk***	Same	NaF, MFP: 1450 ppm F	Calcium carbonate; SLS; cellulose gum	x		
NaF+MFP/dical†	Same	NaF (450 ppm F), MFP (1000 ppm F): 1450 ppm F	Dicalcium phosphate dihydrate; SLS; cellulose gum		x	x
NaF+SnF_2/silica/HMP††	Same	NaF (350 ppm F), SnF_2 (1100 ppm F): 1450 ppm F	Silica; SLS; carrageenan, xanthan gum			x
AmF/silica†††	Same	Olaflur: 1400 ppm F	Silica; hydroxyethylcellulose		x	
675F-NaF/silica‡	675F-NaF	NaF: 675 ppm F	Silica; SLS; carrageenan, xanthan gum	x		
675F-NaF/carb/silica‡	675F-NaF	NaF: 675 ppm F	Silica; SLS; carrageenan, xanthan gum, carbomer		x	x
Placebo‡	Same	0 ppm F	Silica; SLS; carrageenan, xanthan gum	x		
Placebo/carb‡	Placebo	0 ppm F	Silica; SLS; carrageenan, xanthan gum, carbomer		x	

[1] Acrylates/C10-30 alkyl acrylate crosspolymer

*Aquafresh® Fresh & Minty Enamelock experimental formulations, GSKCH, Weybridge, UK (formulations were almost identical with minimal differences in fluoride concentration); **Aquafresh® Fresh & Minty, GSKCH, Weybridge, UK; ***Signal® Family Protection, Unilever UK Ltd., Leatherhead, UK; †Colgate® Cavity Protection, Colgate-Palmolive Co., New York, USA (contributions of fluoride sources to total not disclosed); ††Oral-B® Pro-Expert Clean Mint, Procter & Gamble UK Ltd., Weybridge, UK; †††Elmex® Kariesschutz, GABA Schweiz AG, Therwil, Switzerland; ‡Aquafresh Fresh & Minty formula base, GSKCH, Weybridge, UK

NaF=sodium fluoride; Carb=carbomer; MFP=sodium monofluorophosphate; dical=dicalcium phosphate dihydrate; SnF_2=stannous fluoride; HMP=sodium hexametaphosphate; AmF=amine fluoride; SLS= sodium lauryl sulfate (IUPAC name: sodium dodecyl sulfate)

Table 2. Subject disposition (Per Protocol population)

	Study 1 **(N=57)**	**Study 2** **(N=63)**	**Study 3** **(N=82)**	**Overall** **(N=202)**
Male n (%)	23 (40.3)	23 (36.5)	35 (42.7)	81 (40.1)
Female n (%)	34 (59.7)	40 (63.5)	47 (57.3)	121 (59.9)
Mean age (SD)	65.5 (10.32)	67.0 (10.12)	64.0 (8.77)	65.4 (9.69)
Age range	25–80	37–80	38–80	25–80
Ethnicity n (%)				
White	31 (54.4)	36 (57.1)	40 (48.7)	107 (53.0)
Black or African American	26 (45.6)	27 (42.9)	39 (47.6)	92 (45.5)
Other	0	0	3 (3.7)	3 (1.5)

Table 3. Statistical analysis of treatment differences in mean SMHR (%) using pooled data (Per Protocol population)

Adjusted mean (±SE)	Treatment Differences with 95% CI								
Treatment Comparison P-values		**NaF/carb/ silica**	**NaF/silica**	**NaF+MFP/ dical**	**Na+SnF_2/ silica/HMP**	**AmF/silica**	**NaF+MFP/ chalk**	**675F-NaF**	**Placebo**
	NaF/carb/ silica	37.61 (1.507)	-0.53 (-6.63, 5.56)	7.01 (2.00, 12.02)	20.41 (15.79, 25.03)	-2.17 (-9.08, 4.73)	4.40 (-1.48, 10.30)	9.29 (5.14, 13.43)	24.48 (19.21, 29.75)
	NaF/silica	1.0000	38.15 (2.020)	7.55 (0.61, 14.49)	20.95 (14.26, 27.64)	-1.63 (-10.01, 6.74)	4.94 (-1.38, 11.27)	9.82 (3.93, 15.72)	25.02 (18.36, 31.68)
	NaF+MFP/ dical	**0.0007**	**0.0223**	30.59 (1.617)	13.39 (9.04, 17.74)	-9.19 (-16.22, -2.16)	-2.60 (-9.36, 4.14)	2.27 (-2.19, 6.73)	17.46 (11.76, 23.16)
	Na+SnF_2/ silica/HMP	**<0.0001**	**<0.0001**	**<0.0001**	17.19 (1.480)	-22.59 (-29.75, -15.42)	-16.00 (-22.50, -9.51)	-11.12 (-15.01, -7.23)	4.06 (-1.64, 9.78)
	AmF/silica	0.9785	0.9988	**0.0022**	**<0.0001**	39.79 (2.253)	6.58 (-1.63, 14.81)	11.46 (4.85, 18.08)	26.66 (19.60, 33.71)
	NaF+MFP/ chalk	0.3031	0.2492	0.9354	**<0.0001**	0.2217	33.20 (1.950)	4.88 (-0.78, 10.55)	20.07 (13.60, 26.54)
	675F-NaF	**<0.0001**	**<0.0001**	0.7728	**<0.0001**	**<0.0001**	0.1487	28.32 (1.344)	15.19 (10.25, 20.13)
	Placebo	**<0.0001**	**<0.0001**	**<0.0001**	0.3672	**<0.0001**	**<0.0001**	**<0.0001**	13.12 (1.723)

On the main diagonal (shaded) are adjusted means with standard errors (±SEs). Above the diagonal are treatment differences with 95% confidence intervals (CIs); below the diagonal are P-values for treatment comparisons (significant p-values in bold).

Table 4. Statistical analysis of treatment differences in mean EFU (µg F/cm^2) using pooled data (EFU values given are back-transformed log EFU values; Per Protocol population)

Adjusted mean	Treatment Differences with 95% CI								
		NaF/carb/ silica	**NaF/silica**	**NaF+MFP/ dical**	**Na+SnF_2/ silica/HMP**	**AmF/silica**	**NaF+MFP/ chalk**	**675F-NaF**	**Placebo**
Treatment Comparison P-values	**NaF/carb/ silica**	20.92	1.42 (-1.94, 5.44)	6.75 (4.39, 9.40)	11.37 (8.75, 14.36)	0.04 (-3.41, 4.18)	5.76 (2.19, 10.06)	6.65 (4.64, 8.86)	16.32 (12.76, 20.61)
	NaF/silica	0.9220	19.50	5.32 (1.74, 9.71)	9.94 (6.21, 14.56)	-1.38 (-5.57, 3.95)	4.33 (0.56, 9.00)	5.22 (1.95, 9.14)	14.90 (10.86, 19.98)
	NaF+MFP/ dical	**<0.0001**	**<0.0001**	14.17	4.62 (2.71, 6.82)	-6.70 (-9.14, -3.76)	-0.98 (-3.70, 2.36)	-0.09 (-1.72, 1.73)	9.57 (6.97, 12.77)
	Na+SnF_2/ silica/HMP	**<0.0001**	**<0.0001**	**<0.0001**	9.55	-11.33 (-13.13, -9.10)	-5.61 (-7.51, -3.24)	-4.72 (-5.93, -3.32)	4.95 (3.08, 7.28)
	AmF/silica	1.0000	0.9886	**<0.0001**	**<0.0001**	20.88	5.71 (1.09, 11.65)	6.60 (3.16, 10.73)	16.28 (11.91, 21.80)
	NaF+MFP/ chalk	**<0.0001**	**0.0098**	0.9777	**<0.0001**	**0.0031**	15.16	0.89 (-1.78, 4.13)	10.56 (7.32, 14.68)
	675F-NaF	**<0.0001**	**<0.0001**	1.0000	**<0.0001**	**<0.0001**	0.9799	14.27	9.67 (7.20, 12.66)
	Placebo	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	4.60

On the main diagonal (shaded) are back-transformed adjusted means (geometric means). SEs are not shown due to the antilog transformation of SE not compatible with the geometric mean presented. Above the diagonal are back-transformed treatment differences with approximately back-transformed 95% confidence intervals (CIs); below the diagonal are P-values for treatment comparisons (significant p-values in bold).

Table 5. Treatment-emergent adverse events

Study 1	**NaF/carb/ silica (n=50)**		**NaF+MPF/ chalk (n=53)**		**NaF/silica (n=52)**		**675F-NaF/silica (n=53)**		**Placebo (n=51)**		**Overall**	
	n(%)	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*
All AEs	8(16.0)	9	8(15.1)	9	4(7.7)	5	9(17.0)	9	6(11.8)	6	24(42.1)	38
Oral AEs	5(10.0)	5	5(9.4)	6	2(3.8)	3	6(11.3)	6	3(5.9)	3	17(29.8)	23
TR-TEAEs	0	0	1(1.9)	1	1(1.9)	2	0	0	0	0	1(1.9)	2
Oral discomfort	0	0	0	0	1(1.9)	2	0	0	0	0	0	0
Lip dry	0	0	1(1.9)	1	0	0	0	0	0	0	1(1.9)	1
Study 2	**NaF/carb/ silica (n=57)**		**NaF+MPF/ dical (n=59)**		**AmF/silica (n= 57)**		**675F-NaF/ carb/silica (n=59)**		**Placebo/carb (n=59)**		**Overall**	
	n(%)	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*
All AEs	11(19.3)	16	8(13.6)	9	12(21.1)	14	8(13.6)	9	7(11.9)	7	35(53.8)	55
Oral AEs	5(8.8)	6	2(3.4)	2	3(5.3)	3	1(1.7)	1	3(5.1)	3	13(20.0)	15
TR-TEAEs	0	0	1(1.7)	1	2(3.5)	2	0	0	1(1.7)	1	4(6.2)	4
Dry mouth	0	0	1(1.7)	1	1(1.8)	1	0	0	0	0	2(3.1)	2
Oral mucosal exfoliation	0	0	0	0	0	0	0	0	1(1.7)	1	1(1.5)	1
Sensitivity of teeth	0	0	0	0	1(1.8)	1	0	0	0	0	1(1.5)	1
Study 3	**NaF/carb/ silica (n=79)**		**NaF+MPF/ dical (n= 80)**		**NaF+SnF_2/silica/HMP (n=80)**		**675F-NaF/carb/ silica (n=79)**				**Overall**	
	n(%)	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*	*n(%)*	*nAE*			*n(%)*	*nAE*
All AEs	10(12.7)	11	18(22.5)	24	15(18.8)	19	12(15.2)	14			40(48.2)	68
Oral AEs	6(7.6)	6	8(10.0)	12	9(11.3)	9	8(10.1)	10			27(32.5)	37
TR-TEAEs	0	0	1(1.3)	3	0	0	0	0			1(1.2)	3
Burning sensation	0	0	1(1.3)	1	0	0	0	0			1(1.2)	1
Chapped lips	0	0	1(1.3)	1	0	0	0	0			1(1.2)	1
Oral discomfort	0	0	1(1.3)	1	0	0	0	0			1(1.2)	1

n(%) = number (percent) of subjects; nAE = number of adverse events; Treatment-related treatment-emergent adverse event (TR-TEAE)

Figure headings and footers

Figure 1. Study flow

Figure 2. Network diagram of treatments and their connections via the three studies

Figure 3. Individual study mean SMHR (%; adjusted means ±SE) after 14 days of product use

Figure 4. Pooled analysis mean SMHR (%; adjusted means ±SE) after 14 days of product use

Figure 5. SMHR (%) treatment comparisons with NaF/Carb/silica group

A positive treatment difference suggests a beneficial effect for the first-named treatment. The study results are from the individual studies, and not from the pooled analysis. Pooled CIs are adjusted for multiple comparisons; individual study CIs are not adjusted.

Figure 6. SMHR (%) treatment comparisons with the combined 675F-NaF dose response control group

A positive treatment difference suggests a beneficial effect for the first-named treatment. The study results are from the individual studies, and not from the pooled analysis. Pooled CIs are adjusted for multiple comparisons; individual study CIs are not adjusted.

Figure 7. Adjusted mean SMHR values from each study and from the pooled analysis

Data points are offset for clarity.

Figure 8. Individual study mean EFU (adjusted means, ±SE) after 14 days of product use

Figure 9. Pooled analysis mean EFU (adjusted means, ±SE) after 14 days of product use

Figure 10. EFU (µg F/cm^2) treatment comparisons with the NaF/Carb/silica group

A positive treatment difference suggests a beneficial effect for the first named treatment.
The study results are from the results of the individual study not from the pooled analysis. Pooled CIs are adjusted for multiple comparisons; individual study CIs are not adjusted.

Figure 11. EFU (µg F/cm^2) treatment comparisons with the combined 675F-NaF dose response control group

A positive treatment difference suggests a beneficial effect for the first named treatment.
The study results are from the results of the individual study not from the pooled analysis. Pooled CIs are adjusted for multiple comparisons; individual study CIs are not adjusted.

Figure 12. Adjusted mean EFU values from each study and from the pooled analysis

Data points are offset for clarity. Individual study EFU values are arithmetic means; values from the pooled analysis are from back-transformed log EFU values.

Supplemental Data Tables

Supplemental Table 1: Percent Surface Microhardness Recovery (%SMHR) and Enamel Fluoride Uptake (EFU; adjusted means)

Study 1 (ITT population)	**%SMHR (±SE)**	**EFU µg F/cm^3 (±SE)**
NaF/carb/silica (n=50)	34.62 (2.82)	2499.47 (133)
NaF/silica (n=52)	36.06 (2.79)	2513.37 (131)
675F-NaF/silica (n=53)	27.15 (2.75)	1861.80 (130)
NaF+MFP/chalk (n=53)	31.12 (2.77)	1923.76 (130)
Placebo (n=51)	11.10 (2.80)	686.18 (132)
Study 2 (PP population)	**%SMHR (±SE)**	**EFU µg F/cm^3 (±SE)**
NaF/carb/silica (n=56)	38.05 (2.52)	2342.35 (123.65)
675F-NaF/carb/silica (n=59)	29.08 (2.50)	1649.44 (121.93)
AmF/silica (n=57)	41.06 (2.53)	2305.11 (123.83)
NaF+MFP/dical (n=58)	33.48 (2.49)	1809.74 (121.83)
Placebo/carb (n=59)	14.49 (2.48)	462.95 (120.92)
Study 3 (PP population)	**%SMHR (±SE)**	**EFU µg F/cm^2 (±SE)**
NaF/carb/silica (n=78)	40.94 (1.93)	22.33 (0.85)
NaF+SnF_2/silica/HMP (n=79)	17.88 (1.92)	9.13 (0.85)
NaF+MFP/dical (n=78)	30.19 (1.93)	13.54 (0.85)
675F-NaF/carb/silica (n=76)	28.74 (1.94)	14.31 (0.86)

Supplemental Table 2: Study 1 Percent Surface Microhardness Recovery (%SMHR) and Enamel Fluoride Uptake (EFU; adjusted means): Between treatment comparisons

		Difference (Adjusted Mean) [95% CI]	p-value	Percentage change**
		%SMHR*		
NaF/carb/silica vs	NaF/silica	-1.45 [-6.15, 3.26]	0.5451	-4.0
	675F-NaF/silica	7.47 [2.78, 12.16]	**0.0020**	**27.5**
	NaF+MFP/chalk	3.50 [-1.20, 8.19]	0.1433	11.2
	Placebo	23.52 [18.79, 28.25]	**<0.0001**	**212.0**
NaF/silica vs	675F-NaF/silica	8.92 [4.24, 13.59]	**0.0002**	**32.8**
	NaF+MFP/chalk	4.94 [0.29, 9.59]	**0.0373**	**15.9**
	Placebo	24.97 [20.27, 29.66]	**<0.0001**	**225.0**
NaF+MFP/chalk vs	675F-NaF/silica	3.98 [-0.66, 8.61]	0.0925	14.6
	Placebo	20.03 [15.35, 24.70]	**<0.0001**	**180.5**
675F-NaF/silica vs	Placebo	16.05 [11.36, 20.74]	**<0.0001**	**144.6**
		EFU (µg F/cm^3)*		
NaF/carb/silica vs	NaF/silica	-13.90 [-322.47, 294.66]	0.9293	-0.6
	675F-NaF/silica	637.67 [330.30, 945.03]	**<0.0001**	**34.2**
	NaF+MFP/chalk	575.71 [268.24, 883.17]	**0.0003**	**29.9**
	Placebo	1813.29 [1503.03, 2123.54]	**<0.0001**	**264.3**
NaF/silica vs	675F-NaF/silica	651.57 [346.44, 956.70]	**<0.0001**	**35.0**
	NaF+MFP/chalk	589.61 [285.31, 893.92]	**0.0002**	**30.6**
	Placebo	1827.19 [1519.78, 2134.60]	**<0.0001**	**266.3**
NaF+MFP/chalk vs	675F-NaF/silica	61.96 [-241.25, 365.17]	0.6874	3.3
	Placebo	1237.58 [931.46, 1543.70]	**<0.0001**	**180.4**
675F-NaF/silica vs	Placebo	1175.62 [869.15, 1482.10]	0.2432	**<0.0001**

*A positive difference favors the first named treatment

**Second toothpaste used as reference in calculation of % change [(Difference/Reference)*100]

Supplemental Table 3: Study 2 Percent Surface Microhardness Recovery (%SMHR) and Enamel Fluoride Uptake (EFU; adjusted means): Between treatment comparisons

		Difference (Adjusted Mean) [95% CI]	p-value	Percentage change**
		%SMHR*		
NaF/carb/silica vs	AmF/silica	-3.01 [-7.75, 1.73]	0.2117	-7.3
	675F-NaF/carb/silica	8.96 [4.27, 13.66]	**0.0002**	**30.8**
	NaF+MFP/dical	4.57 [-0.11, 9.24]	0.0557	13.6
	Placebo/carb	23.55 18.86, 28.24]	**<0.0001**	**162.5**
AmF/silica vs	675F-NaF/carb/silica	11.97 [[7.31, 16.64]	**<0.0001**	**41.2**
	NaF+MFP/dical	7.58 [2.88, 12.27]	**0.0017**	**22.6**
	Placebo/carb	26.56 [21.88, 31.24]	**<0.0001**	**183.3**
NaF+MFP/dical vs	675F-NaF/carb/silica	4.40 [-0.23, 9.03]	0.0625	15.1
	Placebo/carb	18.99 [14.36, 23.61]	**<0.0001**	**131.0**
675F-NaF/carb/silica vs	Placebo/carb	14.59 [9.95, 19.23]	**<0.0001**	**100.7**
		EFU (µg F/cm^3)*		
NaF/carb/silica vs	AmF/silica	37.24 [-239.62, 314.09]	0.7912	1.6
	675F-NaF/carb/silica	692.91 [418.73, 967.09]	**<0.0001**	**42.0**
	NaF+MFP/dical	532.61 [259.06, 806.16]	**0.0002**	**29.4**
	Placebo/carb	1879.40 [1605.75, 2153.04]	**<0.0001**	**406.0**
AmF/silica vs	675F-NaF/carb/silica	655.67 [382.41, 928.93]	**<0.0001**	**39.8**
	NaF+MFP/dical	495.38 [221.09, 769.66]	**0.0005**	**27.4**
	Placebo/carb	1842.16 [1568.73, 2115.60]	**<0.0001**	**397.9**
NaF+MFP/dical vs	675F-NaF/carb/silica	160.30 [-110.58, 431.18]	0.2448	9.7
	Placebo/carb	1346.79 [1076.46, 1617.11]	**<0.0001**	**290.9**
675F-NaF/carb/silica vs	Placebo/carb	1186.49 [915.38, 1457.60]	**<0.0001**	**256.3**

*A positive difference favors the first named treatment

**Second toothpaste used as reference in calculation of % change [(Difference/Reference)*100]

Supplemental Table 4: Study 3 Percent Surface Microhardness Recovery (%SMHR) and Enamel Fluoride Uptake (EFU; adjusted means): Between treatment comparisons

		Difference (Adjusted Mean) [95% CI]	p-value	Percentage change**
		%SMHR*		
NaF/carb/silica vs	NaF+SnF_2/silica/HMP	10.75 [7.33, 14.17]	**<0.0001**	35.6
	NaF+MFP/dical	23.06 [19.63, 26.48]	**<0.0001**	128.9
	675F-NaF/carb/silica	12.20 [8.74, 15.67]	**<0.0001**	42.5
NaF+SnF_2/silica/HMP vs	NaF+MFP/dical	12.31 [8.90, 15.72]	**<0.0001**	68.8
	675F-NaF/carb/silica	1.46 [-2.00, 4.91]	0.4070	5.1
NaF+MFP/dical vs	675F-NaF/carb/silica	-10.85 [-14.30, -7.40]	**<0.0001**	-37.8
		EFU (µg F/cm^2)*		
NaF/carb/silica vs	NaF+SnF_2/silica/HMP	8.79 [7.04, 10.55]	**<0.0001**	65.0
	NaF+MFP/dical	13.21 [11.46, 14.96]	**<0.0001**	144.7
	675F-NaF/carb/silica	8.03 [6.25, 9.80]	**<0.0001**	56.1
NaF+SnF_2/silica/HMP vs	NaF+MFP/dical	4.41 [2.66, 6.16]	**<0.0001**	48.4
	675F-NaF/carb/silica	-0.77 [-2.54, 1.00]	0.3942	-5.4
NaF+MFP/dical vs	675F-NaF/carb/silica	-5.18 [-6.95, -3.41]	**<0.0001**	-36.2

*A positive difference favors the first named treatment

**Second toothpaste used as reference in calculation of % change [(Difference/Reference)*100]