Total Salivary Fluoride Concentrations of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing Water Rinsing

A Double Blinded Randomised Controlled Trial

Marwah M M H S Albahrani

Submitted in accordance with the requirements for the degree of
Doctor of Paediatric Dentistry (DPaedDent)

The University of Leeds
Department of Paediatric Dentistry
School of Dentistry

October, 2017

Intellectual property and publication statements

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

Acknowledgements

A father and a mother are the fuel to keep someone running forward. Mom and dad have always been very supportive and expressing how proud they were every time I hit a new mile stone. It would not be easy to write what they have given me or provided for me to succeed in life because they have given so much that I cannot count or even remember. I can never know how hard it was to be a parent of seven kids but I know having one kid is hard enough.

I am proud to say that my mother is a house wife. She has always been with us. Mom did not have the chance to finish her degree but today she has two degrees in dental surgery, a degree in electrical engineering, a degree in medicine and two degrees in English education. She is the power of the Universe that, secretly, keeps everything moving.

An army salute to retired Colonel M. Albahrani, my father. There have been days where you had not been around because you were involved in a holy duty. You are intrepid, strong, sure and brave. Thank you for keeping us safe. You are one man of a kind, Sir.

Safa, Shahad, Mohammed, Zainab, Shimaa and Ali. Thank you for being great and supportive siblings. Thank you for taking care of baby Sarah when I was not around.

Dear husband Ahmed Altimimi, thank you. You have been my teddy bear when I needed a hug and a high quality radiator during winter times, except you do not burn. I have always thought I would never meet a man who is better than me but you proved me wrong. You are a gentleman who is nicer, smarter and bigger of course. Our first pregnancy was not ideal but you were very supportive from day one and until this moment. Ahmed you are an amazing father to baby Sarah. I love you unconditionally just the way you are.

I would also like to dedicate this to my teacher in Ashton outreach clinics during university times Dr. Steven Legg. A teacher is a word of God, and you were a teacher I would tip my hat to you. Your directions and advice have taught me a lot in only thirteen weeks in Ashton. You have been a dentist, a teacher, a father and role model. I still, to this day, remember everything I have learnt from you.

Gillian, I know I keep thanking you for helping me throughout the whole project. We worked as a brilliant team from day one till the day we recruited participant number 120. This research was a very organised piece of art, thanks to you.

And when science meets fashion you get the one and only Marina. Dr Malinowski, you are a woman with a fist of steel. I am very grateful for all the help you have provided and the directions you have given.

Everyone who was mentored / supervised by professor Toumba would agree that words can never describe the real human resides inside you. It is very hard to think of anything to write because having your name as my supervisor, in itself, is a great honour. You have trusted me and always pushed me to go forward. You have never put me down, even when you thought it would be difficult for a beginner researcher to recruit 120 participants, you encouraged me to go ahead. This is only one example out of many.

I have read so many dedications that thank you for your help and support. This time I would like to thank your parents for creating such a magnum opus of humanity, professionalism and morals; a very rare thing to find these days.

A special thanks to Dr Lynn Mei Woo and Precious Dental Care (20 Gledhow Avenue, Roundhay, Leeds, West Yorkshire, LS8 1NU) for offering to put up my research poster in their practice. Thank you, this was very generous and thoughtful.

And finally, thanks to everyone who participated or even considered participating in my research project to make it happen.

Abstract

Background: Caries prevalence has declined significantly since the introduction of fluoridated toothpastes. There have been a number of developments with regards to specific active fluoride ingredients but not enough evidence to support one over the other.

Aim: To compare the salivary fluoride concentrations of different fluoride formulations in the form of toothpaste: fluoride-free (0 ppmF), sodium fluoride (1,450 ppmF), sodium monofluorophosphate (1,450 ppmF), sodium fluoride and monofluorophosphate combined (1,450 ppmF), stannous fluoride and sodium fluoride combined (1,450 ppmF) and amine fluoride (1,400 ppmF) with and without post-brushing water rinsing.

Design: Study registered with ClilincalTrials.gov public database (NCT02740803). In vivo double-blinded randomised controlled trial measuring salivary fluoride concentrations following brushing with six toothpaste formulations. Power calculation was performed using PASS11.0 software and the total sample size of 120 was recruited in this study. Participants brushed with 1.0g of one of six different formulations of toothpastes either with or without water rinsing post-brushing. Participants were randomly assigned to groups using an online random team generator. Saliva was collected at six different times (baseline and at 1, 15, 30, 60 and 90 minute(s) post-brushing]. Samples were analysed using a fluoride ion-specific sensitive electrode connected to an ion analyser. Codes were broken after data analysis. Data analysis was performed using IBM SPSS 23 software.

Results: Demographic characteristics were not significant variables ($p>0.05$). Time, toothpaste formulation and rinsing methods had significant effects ($p<0.05$). In general, amine fluoride toothpaste resulted in significantly higher salivary fluoride concentrations at 90 minutes than control groups, in both rinsing and non-rinsing groups. Sodium

monofuorophosphate toothpaste did not result in significant difference compared to control group at any time point, in both rinsing and non-rinsing groups.

Conclusion: The results of this study supports the current recommendation of no rinsing post-brushing. It also supports the previous literature in that amine fluoride resulted in significantly higher fluoride concentrations.

Table of Contents

List of Tables

List of Figures

Abbreviations

Acronym	Definition
AmF	Amine fluoride
ASA	American Society of Anesthesiologists physical status classification
At	Astatine
Br	Bromine
C	Degree Celsius
CASP	Critically Appraised Skills Programme
CDTA	Cyclohexylenedinitrilotetraacetate
CI	Confidence interval
Cl	Chlorine
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DenTCRU	Dental Translational and Clinical Research Unit
DMFS	Decayed, missing and filled surfaces of permanent teeth
DMFT	Decayed, missing, filled permanent teeth
dmft	Decayed, missing, filled permanent teeth
DS	Decayed surfaces of permanent teeth
DT	Decayed permanent teeth
F	Fluoride
FS	Filled surfaces of permanent teeth
FT	Filled permanent teeth
F-test	F-distribution test
g	Gram(s)
g/mol	Gram per mole
HF	Hydrogen fluoride
H_n	Null hypotheses
I	Iodine
LCI	Lower bond confidence interval
M	Mean

Acronym	Definition
Max. value	Maximum value
MFP	Sodium monofluorophosphate
mg/L	Milligrams per litre
mgF/kg/day	Milligrams of fluoride per kilograms per day
Min.	Minute(s)
Min. value	Minimum value
ml	Millilitre(s)
MS	Missing surfaces of permanent teeth
MT	Missing permanent teeth
mV	millivolts
NaCl	sodium chloride
NaMFP	Sodium monofluorophosphate
NaOH	sodium hydroxide
PASS	Power Analysis and Sample Size Software
pH	Potential of hydrogen
PICO	Population, intervention, control or comparison and outcome(s)
ppmF	Parts per million fluoride
P-value	Calculated probability
S. mutans	Streptococcus mutans
SD	Standard deviation
SE	Standard error
SIGN	Scottish Intercollegiate Guidelines Network
SnF	Stannous fluoride
SRFD	Slow-releasing fluoride devices
TISAB	Total Ionic Strength Adjustment Buffering solution
U.S.	United States
UCI	Upper bond confidence interval
µg	Microgram
PTD	Probably toxic dose

Chapter 1 **Introduction**

1.1 General Introduction

Dental caries is the most common dental disease worldwide. According to the oral health survey of five-year-old children (2012) conducted for the National Dental Epidemiology Programme in England; almost one third of five-year-olds in England are suffering from dental caries and it is the most common reason why paediatric patients are admitted to hospital. The report also highlighted significant regional inequalities with the highest level of dental disease tending to be seen in the most deprived areas.

Dental caries prevalence has declined significantly since the introduction of fluoridated toothpastes. There have been a number of developments with regards to specific active fluoride ingredients but not enough evidence to support one over the other (Scottish Intercollegiate Guidelines Network, 2014).

1.2 The element fluorine

Fluorine (F) is the 9th chemical element in the periodic table. It is a naturally occurring, widely distributed mineral and belongs to the halogen group along with chlorine (Cl), bromine (Br), iodine (I) and astatine (At). They are all highly reactive, with fluorine being the most electronegative and reactive of all elements and combines with most elements. Fluoride occurs in ionic form or combined with other chemicals and compounds (Facer, 2013; Burrows et al., 2009).

As atomic number increases, reactivity of the halogens decreases; that is, astatine is the least reactive of all the naturally occurring halogens. Fluoride is the ionic form of fluorine. Fluoride ions react in order to achieve stability of the outer shell by sharing outer shell electrons. When halogens are combined with other elements in the periodic table, the resulting product is called a halide, ie. sodium fluoride ($Na^{+}F^{-}$) (Facer, 2013; Brady and Senese, 2009).

1.3 Fluoride toxicity

Historically, most cases of serious fluoride toxicity have followed an accidental ingestion. If ingested in high concentrations, fluoride could cause serious harm to the human body. It can induce early acute symptoms that include vomiting, nausea, diarrhoea, hypersalivation, pain in the gastrointestinal tract and headaches (Whitford 1994; 1992). Fluoride toxicity can also manifest itself with late chronic symptoms such as neurological, respiratory and cardiovascular failures, convulsions and even death (Whitford 1994; 1992). When fluoride is absorbed in the blood stream, it binds with plasma calcium ions. If calcium levels fall below 2.54 mmol /L, the subject would start to have convulsions/ tetany (Shulman and Wells, 1997; Heifetz and Horowitz, 1984). Hence, it is recommended to give milk in case of accidental fluoride ingestion, since it is rich in calcium and magnesium ions (Drummond and Curzon, 1990; Ekstrand and Ehrnebo, 1980).

Prolonged exposure of high levels of fluoride would also cause different types of fluorosis. Dental fluorosis is associated with ingestion of fluoride (1–2 ppmF) on a chronic basis (Whitford, 1992). Other types of fluorosis such as skeletal fluorosis may appear with higher levels of fluoride intake (8–10 ppmF) over a period of 10 or more years (Whitford, 1992).

Water fluoridation has also been associated with an increased incidence of hip / bone fractures in women of menopause age (Jacobsen et al., 1990). This study however, was performed in rural areas with high poverty prevalence. Meaning that other factors such as malnutrition could have confounded the results. Although a relationship was found between water fluoridation and the increased incidence of bone fractures, the evidence is weak (Whitford, 1992).

Dental fluorosis is a qualitative and / or a quantitative enamel defect resulting from high exposure of fluoride during tooth formation. Central incisors are most prone to this during a 4-months-period between 22 and 26 months (Evans and Stamm, 1991). It was suggested however, that the central incisors are at increased risk of developing clinically visible fluorosis between 22 months and up to the age of 3 years (Department of Health and British Association for the Study of Community Dentistry, 2017; Scottish Intercollegiate Guidelines Network, 2014).

The U.S Public Health Service (2015) recommends a daily fluoride intake of 0.7 mg/L. It also recommended not to exceed 0.1 mgF/Kg/day to avoid the risk of fluorosis for children up to 8 years old. For older children and adults, the maximum daily dose of ingested fluoride should not exceed 10 mg/day, regardless of weight (Scottish Intercollegiate Guidelines Network, 2014).
Estimates of toxic and safe levels of fluoride vary substantially. According to Hodge and Smith (1965), the certainly lethal dose was estimated to be between 32 – 64 mgF/Kg of body weight. A dose of 8 mgF/Kg of body weight of fluoride can be safely tolerated (Heifetz and Horowitz, 1984), while doses up to 5 mgF/Kg of body weight was defined as the probably toxic dose (PTD) (Whitford, 1987).

1.4 Cariostatic mechanisms of fluoride

There are several mechanisms that have been proposed for how fluoride could reduce susceptibility to caries. It was suggested that teeth that were formed in fluoridated environments tend to be smaller and have shallower pits and fissures than those formed in non-fluoridated environments (Lovius and Goose, 1969). This evidence, however, is weak. The cariostatic activity of fluoride has been attributed mainly to its topical rather than systemic effect (Featherstone, 1999). Hydroxyapatite enamel crystals [Ca_{10} $(PO_4)_3$ $(OH)_2$] can incorporate various extraneous ions substituting for calcium, phosphate and hydroxyl groups (Elliott, 1964). This can either occur during tooth formation or after tooth development (Robinson, 2009).
There is very little evidence on the antimicrobial action of fluoride in relation to the inhibition of oral bacteria (Rosin-Grget et al., 2013). When the intraoral pH falls below the critical level (pH 5.5), fluoride exists in the saliva as hydrogen fluoride (HF). Hydrogen fluoride is a weak acid (pH 3.15) and can interfere with the bacterial acid production pathway by inhibition of the enzyme enolase (Bowden, 1990). Recent literature, however, concluded that low levels of fluoride are insufficient to have a significant effect on the antimicrobial activity of bacteria (Lynch, et al., 2004). This supports the practice of the placement of a highly concentrated fluoride varnish, producing a transient effect on disturbing the ability of plaque to release acid into the oral environment (Rosin-Grget et al., 2013). Other recent reviews

found that the fluoride in plaque had a biological effect on S. mutans action, but this is yet to be supported by high quality evidence (Koo, 2008).

It has also been suggested that enamel demineralisation is inhibited by the constant presence of fluoride in the saliva during cariogenic challenge (Rosin-Grget et al., 2013). It was therefore suggested, that the constant introduction of low levels of fluoride over a prolonged period of time is better than the application of high-dose fluoride a few times a year (Rosin-Grget et al., 2013; Crommelin et al., 1983).

Fluoride also plays an important role in remineralisation enhancement (Rosin-Grget et al., 2013). At a pH value of 7, demineralised enamel crystals would start incorporating fluoride ions substituting for hydroxyl ions, transforming it from a hydroxyapatite structure into a fluorapatite crystal (Brown et al., 1977). Fluorapatite crystals are larger than hydroxyapatites, more stable and more resistant to acid dissolution. This secondary acquired resistance is developed as the fluoride ions adhere to the outer surface of the partially demineralised hydroxyapatite attracting calcium ions (Rosin-Grget et al., 2013; Koulrides, 1983). Toumba and Curzon (2001) showed that salivary fluoride in caries-free children (mean 0.13 mg/L fluoride) was higher when compared to caries-prone children (mean 0.05 mg/L fluoride).

Brown et al. (1977) showed that the fluoroapatite is soluble at pH values as low as 3.7.

1.5 Available topical fluoride interventions

1.5.1 Fluoridated toothpaste

Toothpaste is a paste or a gel that is regarded as an important part of daily dental hygiene routine to maintain health and aesthetics of teeth. Different toothpastes are comprised of different ingredients including active ingredients, flavourings, detergents, humectants and abrasives (Centre of Scientific Information, 2017).

Fluoride is regarded as an active ingredient for dental caries prevention. Potassium nitrate is considered as the main ingredient for reducing sensitivity in sensitive toothpastes. A Cochrane systematic review by Poulsen et al. (2006) has concluded that there was no strong evidence to

support the efficacy of potassium nitrate for the treatment of dentine hypersensitivity.

Low dose hydrogen and carbamide peroxides are added to whitening toothpastes to improve colour perception of teeth. In an in vitro study testing the efficacy of toothpastes with chemical whitening agents in reducing extrinsic stating, the whitening toothpastes did not outperform the ordinary toothpastes in extrinsic stain removal (Soares, et al., 2015).

Mild abrasives, such as magnesium carbonate, hydrated aluminum oxides and calcium carbonate, are small particles that remove surface stains and food debris.

Saccharin, sorbitol are sweetening agents that are added to improve the flavour of the toothpaste and make it more acceptable (Centre of Scientific Information, 2017). Some children's toothpastes even come fruit-flavoured. Bland toothpastes such as Oranurse® are very helpful for patients who cannot tolerate the toothpaste's taste or who suffer from oral mucosal disease such as mucositis.

Humectants play a role in keeping the moisture in the toothpaste and prevent water loss. Examples of humectants include sorbitol, glycol and glycerol (Centre of Scientific Information, 2017).

Foaming agents are added in toothpastes as detergents. One of the most common detergents in toothpastes is sodium lauryl sulphate (Centre of Scientific Information, 2017). Some studies suggested that the presence of sodium lauryl sulphate can also increase the availability of fluoride ions by preventing fluoride ions from reacting with silica abrasives forming insoluble fluorosilicates (Carey, 2014).

Fluoridated toothpastes have proven efficacy in reduction in caries prevalence through several proposed mechanisms of action as discussed in Section 1.3.

Several high quality review articles (systematic reviews and meta-analyses) demonstrated the successful role of fluoride in significantly reducing the development of new carious lesions. Strong evidence was found relating daily use of fluoride toothpaste and significant caries reduction when compared to a placebo (Twetman, 2009). When compared to the placebo or no intervention, fluoridated toothpastes with fluoride concentrations of

1,000–1,500 ppmF resulted in caries reduction in primary teeth (Marinho et al., 2003).

The maximum concentration of fluoride allowed in toothpastes, by the UK and Europe in the community law, before it is classified as a medicine is 1,500 ppmF (Scottish Intercollegiate Guidelines Network, 2014). The maximum fluoride concertation of toothpastes listed in the Department of Health Prevention Toolkit and British Association for the Study of Community Dentistry (2017) that is available over-the-counter is 1,450 ppmF.

The use of fluoridated toothpastes follows a dose-response relationship between the concentration of fluoride in the toothpaste and caries reduction (Walsh et al., 2010). There is a greater caries preventive effect of toothpastes containing at least 1,000 ppmF when compared with toothpastes containing only 250 ppmF (DenBesten and Ko, 1996).

In light of the strong evidence supporting the efficacy of high concentrations of fluoride, patients at increased risk of developing dental caries are advised to use 1,450 ppmF as part of their daily oral hygiene regimen. Higher concentrations of fluoridated toothpastes (2,800 ppmF and 5,000 ppmF) are also available and are classified as medicinal products and are only provided on prescription (Department of Health and British Association for the Study of Community Dentistry, 2017).

It is also recommended, that a smear layer or a pea-sized amount is dispensed, especially for younger children (Department of Health and British Association for the Study of Community Dentistry, 2017; Scottish Intercollegiate Guidelines Network, 2014). A research study investigated the relationship between the ingestion of fluoride and both the concentration and the amount of the fluoridated toothpaste used by the children. The study showed that increased amounts (full toothbrush head's length) of 1,450 ppmF fluoridated toothpastes were associated with a 20-fold increase in the ingestion of fluoride (1.02 mg). Low amounts (pea-size) of 1,450 ppmF toothpastes, however, were associated with only a 4-fold increase in the ingestion of fluoride (0.05 mg) (Bentley et al., 1999).

1.5.2 Topical fluoride varnish

Fluoride varnish is a professionally applied adherent material, which contains high concentrations of fluoride at 22,600 ppmF.

Fluoride varnishes have proven effectiveness in preventing dental caries in both primary and permanent dentitions (Marinho et al, 2013; Poulsen 2009). A systematic review showed that fluoride varnish helped in substantially decreasing the DMFT index by 43% and the dmft index by 37% (Department of Health and British Association for the Study of Community Dentistry, 2017; Marinho et al, 2013). It is important to highlight, however, that the fluoride varnish in most of these studies reduced caries increments in target populations. Those studies were classified to be of low to moderate quality (Scottish Intercollegiate Guidelines Network, 2014).

No consistent evidence has been found on the recommended frequency of fluoride varnish application. Most of the studies involved twice yearly applications, while a small number involved four applications a year (Scottish Intercollegiate Guidelines Network, 2014). The Department of Health and British Association for the Study of Community Dentistry (2017) recommended that fluoride varnish is applied at least twice a year for patients at increased risk of developing dental caries.

Where the application of a colophony-based fluoride varnish is contraindicated, alternatives (e.g. 3M Clinpro™ White Varnish) exist. Alcohol-based fluoride varnishes are recommended to be considered as an alternative (Department of Health and British Association for the Study of Community Dentistry, 2017).

1.5.3 Fluoride drops and tablets

There is insufficient evidence to recommend use of fluoride drops and tablets for caries prevention (Tubert-Jeanning et al., 2011; Ismail and Hasson 2008). The Scottish Dental Effectiveness Programme (2017) recommended that the use of additional fluoride tablets and / or supplements should no longer be recommended. The Department of Health and British Association for the Study of Community Dentistry (2017) states that the use of fluoride tablets / drops requires compliance which includes either over-use or under-use. Over-use carries a risk of fluorosis for children under 6 years of age. Other sources of fluoride are more preferable and should be considered first (Department of Health and British Association for the study of Community Dentistry, 2017; Scottish Intercollegiate Guidelines Network, 2014).

1.5.4 Slow-release fluoride beads

Fluoride beads work by slowly releasing low and steady levels of fluoride over a prolonged period of time. This ensures that fluoride ions are always available during the acid cariogenic challenge which would help in reducing demineralisation and promoting early remineralisation preventing dental caries.

A Cochrane review (Chong et al., 2014) reviewed a single randomised controlled trial with an initial sample size of 174 comparing slow-release fluoride beads to a placebo over a period of two years. The majority of the children lost the fluoride bead within the two years and only 36% of the data was analysed as a result (Scottish Intercollegiate Guidelines Network, 2014). This evidence is therefore minimal and unreliable and further studies are required.

1.5.5 Fluoride mouthwash

The Department of Health and British Association for the Study of Community Dentistry (2017) recommended daily fluoride mouth rinses (225 ppmF) for patients who are at least eight years old and are able to comply with the instructions provided. These are advised to be used at different times than brushing to maximise the topical effect of both the fluoridated mouth rinse and the fluoridated toothpaste.

In patients undergoing fixed orthodontic treatment, daily fluoridated mouth rinses have shown efficacy in reducing the severity of early enamel demineralisation around orthodontic brackets (Benson et al., 2013).

A systematic review compared the use of fluoridated mouth rinse in the presence of other sources of fluoride. The use of fluoridated mouth rinse significantly decreased the development of caries where there was no background exposure of fluoride. However, when fluoridated mouth rinses were used in conjunction with other fluoridated products (i.e. toothpastes), the results were inconclusive (Twetman et al., 2004). A meta-analysis by Marinho et al. (2016) found no association between rinsing frequency or concentration of the fluoridated mouth rinse and estimates of DMFS.

1.5.6 Fluoride gels

A clear caries-inhibiting effect of fluoride gels when compared to placebo groups has been demonstrated through several systematic reviews (Marinho et al., 2015). Increased frequency of fluoridated gel applications (10 times a year) can provide greater protection against caries (Ammari et al., 2007).

Fluoride gels are usually applied using either custom made trays or pre-constructed trays. High frequency of professional applications is required to achieve the desired caries prevention effect. The time and cost associated with the application of fluoridated gels are a barrier to their use (Scottish Intercollegiate Guidelines Network, 2014).

1.6 Toothbrushing practices

1.6.1 Frequency of toothbrushing

Literature has revealed a direct relationship between brushing frequency and caries reduction. Chestnutt et al. (1998) concluded that brushing at least twice a day when compared to brushing less than twice a day resulted in significant reduction in caries experience.

Kumar et al. (2016) conducted a systematic review of 25 studies. Thirteen studies were of a good quality, 14 were classified as having moderate quality and 6 were rated as poor. The systematic review concluded that individuals who reported infrequent toothbrushing were at higher risk of developing caries than those who brushed frequently. This paper also discussed that brushing frequency could be related to socio-economic status, education level, type of diet and degree of motivation. Since caries is a multifactorial disease, the pronounced effect of infrequent brushing in caries progression could be indirectly related to other associated factors.

The current guidelines recommend brushing at least twice a day last thing at night and at least one other occasion during the day (Department of Health and British Association for the Study of Community Dentistry, 2017; Scottish Intercollegiate Guidelines Network, 2014).

1.6.2 Duration of toothbrushing

No evidence is available that looked at the duration of toothbrushing in relation to the development and / or progression of dental caries. Current

guidelines do not specify minimum duration for toothbrushing (Department of Health and British Association for the study of Community Dentistry, 2017; Intercollegiate Guidelines Network, 2014).

Twetman (2009) advises toothbrushing for at least 2 minutes.

1.6.3 Post-brushing rinsing practices

Chestnutt et al. (1998) examined the effect of rinsing methods post-brushing to caries experience and increment. The study concluded that rinsing method following brushing is strongly correlated to the reduction in caries experience. This study also reported that the children who rinsed their mouth following brushing using a beaker had significantly higher caries than those who did not (Chestnutt et al., 1998).

A randomised controlled trial concluded that using the toothpaste slurry for rinsing following brushing as opposed to water rinsing resulted in developing 26% less proximal caries (Sjögren et al., 1995).

A prospective study conducted in Lithuania (Machiulskiene et al., 2002) however, concluded that the rinsing protocol had no statistically significant effect on caries progression. The study did not follow a randomisation and blinding protocol. About 407 children started the study but only 276 were available for examination at the end of the study (drop-out rate of 32%). This study also mainly depended on the blinded radiographic comparison between baseline and final bitewing radiographs to assess caries progression. Out of 407 children, three children refused having initial radiographs taken. At the end of the study, more drop-outs were seen as only 225 children had final radiographs taken. Therefore, this study had poor methodological quality and is associated with a high risk of bias.

An updated review by Twetman (2009) was published in the European Archives of Paediatric Dentistry examining the caries-preventive effect of fluoride toothpaste in children. This study concluded that evidence regarding the post-brushing practices was poor and conclusions could not be drawn.

Current guidelines discourage post-brushing water rinsing as this washes away the fluoride and reduces the caries preventive effect of the fluoridated toothpastes (Department of Health and British Association for the Study of Community Dentistry, 2017; Scottish Intercollegiate Guidelines Network, 2014.

1.7 Toothpaste formulations

There are multiple different fluoride formulations available on the market. No evidence was found relating a specific chemical formulation to caries prevention (Scottish Intercollegiate Guidelines Network, 2014).

The European Commission has approved 20 fluoride compounds as ingredients in oral hygiene products subject to restriction conditions. These compounds are not allowed to be used, as over-the-counter products, in concentrations higher than 1,500 ppmF.

Table 1.7-1 Fluorine compounds approved by the European Commission to be used in oral health products (From: European Commission, 2016; SCCPNFP, 2003) .

Annex III Number	Substance	Empirical formula and molecular weight (g/mol)
26	Ammonium monofluorophosphate	
27	Sodium monofluorophosphate Disodium monofluorophosphate	Na_2PO_3F 143.95
28	Potassium monofluorophosphate Dipotassium fluorophosphate	K_2PO_3F 176.17
29	Calcium monofluorophosphate Calcium fluorophosphate	$Ca\ F\ H_2\ O_3\ P$ 138.05
30	Calcium fluoride	$Ca\ F_2$ 78.08
31	Sodium fluoride	Na F 41.99
32	Potassium fluoride	K F 58.10
33	Ammonium fluoride	NH_4F 37.05
34	Aluminium fluoride	$Al\ F_3$

Annex III Number	Substance	Empirical formula and molecular weight (g/mol)
		83.98
35	Stannous fluoride Tin difluoride	SnF_2 156.69
36	Hexadecyl ammonium fluoride Cetylamine hydrofluoride Hetaflur	$C_{16}H_{35}NHF$ 261.53
37	3-(N-hexadecyl-N-2-hydroxyethyl-ammonio)propylbis (2-hydroxyethyl) ammonium dihydrofluoride, Olaflur	$C_{27}H_{60}N_2O_3F_2$ 498.89
38	N,N',N'-tris(polyoxyethylene)-N-hexadecyl-propylenediamine dihydrofluoride	
39	Octadecenyl-ammonium fluoride	
40	Sodium fluorosilicate Disodium hexafluorosilicate	F_6-Si-Na_2 188.07
41	Potassium fluorosilicate Dipotassium hexafluorosilicate	F_6K_2Si- 220.29
42	Ammonium fluorosilicate Ammonium hexafluorosilicate	F_6 $Si(NH_4)_2$- 178.19
43	Magnesium fluorosilicate Magnesium hexafluorosilicate	$MgSiF_6$ 166.40
47	Nicomethanol hydrofluoride	C_6H_8FNO 129.13
56	Magnesium fluoride	MgF_2 62.31

1.7.1 Sodium fluoride (NaF)

Sodium (Na) is a soft, silvery metal, made up of sodium atoms. Fluoride exists in the environment as a gas and consists of two fluoride ions paired together (F_2). The resulting compound of the reaction between the two is sodium fluoride (NaF) (Burrows et al., 2009). Sodium fluoride is arranged in a strong ionic structure, which is reflected on its high melting point (993 degree Celsius) (Rennie, 2016). Sodium fluoride can either exist in the form of a white powder or a colourless crystalline solid (Rumble, 2017). Sodium fluoride is odorless and soluble in water, hence, it is used to fluoridate water supplies. It instantly dissolves in saliva releasing free fluoride and sodium ions.

1.7.2 Stannous fluoride (SnF_2)

Stannous fluoride is a white crystalline solid. It has a low melting point (213 degree Celsius) compared to sodium fluoride (Rennie, 2016).

Stannous fluoride is an ionic compound that is chemically unstable. In water-based formulations stannous ions are not stable. Historically, older formulations of stannous fluoride caused golden brown discolouration of teeth (Ellingsen et al., 1982). The first stannous fluoride toothpaste was Crest® Fluoristan™ which was manufactured by Procter & Gamble. The clinical use of the toothpaste however was limited due to its astringent taste and the formulation of extrinsic staining on the teeth; this resulted in the withdrawal of the original stannous fluoride toothpaste.

The formula of stannous fluoride was later stabilised by the addition of sodium hexametaphosphate in a low-water formulation toothpaste (Sensabaugh and Sagel, 2015). Unlike sodium fluoride, calcium-based abrasives are more compatible with stannous fluoride and sodium monofluorophosphate toothpastes (Sensabaugh and Sagel, 2015; Hattab, 1989).

Sodium hexametaphosphate is a chemical whitening agent which also protects against new stain formation and has anti-calculus properties.

The stabilised combination formula for stannous fluoride and hexametaphosphate has improved the aesthetic quality over the original preparation of stannous fluoride dentifrices (Sensabaugh and Sagel, 2015).

Older studies have compared the caries protection effect of single applications of sodium fluoride and stannous fluoride. Nevitt et al. (1958) compared 2% stannous fluoride and 2% sodium fluoride. Different participants received different active interventions but the control group followed a split-mouth design. No significant difference was found between sodium fluoride and stannous fluoride. The study also concluded that treated quadrants had less carious lesions than untreated quadrants. The methodology of this study however, could be rated as poor.

Another study conducted in Indiana, USA (Mercer and Muhler, 1972) studied single applications of (8% stannous fluoride, 4% stannous fluoride, 0.4% stannous fluoride, 2% sodium fluoride and distilled water) at six monthly intervals over a 24 month period. This clinical trial concluded that stannous fluoride was more effective as an anti-cariogenic agent than sodium fluoride.

The evidence comparing the anti-cariogenic effectiveness of stannous fluoride and sodium fluoride is inconclusive.

1.7.3 Sodium monofluorophosphate (Na_2PO_3F)

In a study conducted on Syrian hamsters, sodium monofluorophosphate was reported to be 7–8 times less toxic than sodium fluoride (Shourie et al., 1950). This study introduced an amount of 40 ppmF in the drinking water of rats weighing between only 200 – 300 grams.

It was suggested that sodium monofluorophosphate undergoes rapid hydrolysis in the oral environment (Bruun et al., 1984; Gron et al., 1971). This decomposition is suggested to be caused by bacterial phosphatases in saliva (Ericsson, 1967). The enamel uptake of fluoride from sodium monofluorophosphate was higher at pH 5 than at pH 7 (Gron et al,. 1971).

A two-year-trial was conducted on children in New Jersey and Puerto Rico (Sabporito et al., 2000) comparing the caries prevention efficacy of both sodium fluoride (1,100 ppmF) and sodium monofluorphosphate (1,000 ppmF). The study did not find any significant difference in term of the caries preventive efficacy between both tested formulations.

This argument supported an earlier study conducted by Depaola et al. (1993) and a critical review by Holloway and Worthington (1993).

1.7.4 Amine fluoride ($C_{27}H_{60}N_2O_3F_2$)

The caries inhibitory effect of amine fluoride has been related to its tensio-active and anti-glycolytic properties. Amine fluoride reduces plaque adhesion to the enamel surface by the self-alignment of its hydrophilic particles towards the enamel surface. This leads to fluoride accumulation around the tooth surface (Priyadarshini et al., 2013).

No studies comparing amine fluoride, stannous fluoride, sodium fluoride and sodium monofluorophosphate in term of the anti-caries efficacy were found in the literature.

An in vitro study was conducted comparing the remineralisation effect of several compounds including amine fluoride, sodium fluoride and sodium monofluorophosphate on caries-like enamel lesions (Arnold et al., 2006). The study concluded that amine fluoride was associated with a marked increase in remineralisation when compared to sodium fluoride and sodium monofluorophosphate.

Chapter 2 **Literature Review**

2.1 Introduction

Before the present study was designed the literature was systematically searched and reviewed in a critical manner to identify similarly conducted studies, identify their strengths and weaknesses and plan a more improved study design with less risk of bias and increased methodological quality.

2.2 Research Question

An answerable research question was formulated according to the PICO format as follows:

What is the salivary fluoride concentration of healthy subjects following toothbrushing with variable formulations of fluoridated toothpastes with and without rinsing?

2.3 Search methods and strategy

Relevant literature was identified using the following search databases:

(1) EMBASE classic + EMBASE via OVID (1947 to August 2017).
(2) MEDLINE via OVID (1946 to 2017 August week 2).
(3) BIOSIS via OVID (1969 to 2017 week 38).
(4) Web of Science (1969 – August 2017).
(5) PUBMED via The National Centre of Biotechnology Information (August 2017).
(6) Leeds University's Library's Journals Via Ovid (full text).
(7) All EMB reviews via OVID:
(8) Cochrane Database of Systematic Reviews (2005 to August 2017).
(9) Cochrane Central Registers of Controlled Trials (June 2017).
(10) Clinicaltrials.gov registry and results database of publicly and privately supported clinical studies of human participants conducted around the world.

Research methods involved identifying the key search terms for the research question but it did not include identification of unpublished grey literature. Cross-referencing was also used to identify additional articles. These

activities commenced on the 30th of January 2015 and were researched quarterly throughout the review until around the time of the final submission of this dissertation (20th August 2017).

The following search terms were identified and were searched manually using the previous databases:

1) Fluoride$
2) Toothpaste$ **OR** Dentifrice$
3) Salivary clearance **OR** Fluoride retention **OR** fluoride concentration
4) Rins$

2.4 Selection criteria and identification of studies

Studies comparing salivary fluoride concentration between at least two different toothpaste fluoride formulations were included in this review. Studies comparing salivary fluoride concentration between at least two different toothpaste fluoride concentrations were also included to review the behaviour of the studied toothpaste formulation at different concentrations.

The results were limited to in vivo trials that were performed in either adult or child populations and were published in English. Only full text articles were considered and double publications, abstracts, letters, short communications and textbooks were discarded. No restrictions were placed on date of publication or type of study when searching the electronic databases.

2.5 Search results

The electronic search retrieved 241 articles. The abstracts of these 241 articles were reviewed and irrelevant abstracts that did not match the inclusion criteria were excluded. After duplicate citations were removed, six articles remained which were included in this review.

Two additional articles were identified using cross-referencing. In total, eight articles were included in this systematic review of the literature.

2.6 Quality assessment

The quality of each paper was assessed independently with the aid of the Consolidated Standards of Reporting Trials (CONSORT), the Critically Appraised Skills Programme (CASP) and the Scottish Intercollegiate Guidelines Network (SIGN) checklists.

In general the assessment included examiner blinding, randomisation, sample size calculation, inclusion and exclusion criteria, the presence of a control group and whether confounding factors were taken into consideration (Appendices A.1 and A.2).

2.7 Final search results:

The following studies / research papers were identified:

1. Bruun, C. et al. 1984. Whole saliva fluoride after toothbrushing with NaF and MFP dentifrices with difference F concentrations. Caries Research. 18, pp.282-288.
2. Duckworth, R. and Morgan, S. 1991. Oral fluoride retention after use of fluoride dentifrices. Caries Research. 25, pp.123-129.
3. Paul, S. et al. 1993. Effect of fluoride dentifrices on salivary fluoride levels in children. Indian Journal of Dental Research. 4, pp.95-101.
4. Attin, T. and Hellwig, E. 1996. Salivary fluoride content after toothbrushing with a sodium fluoride and an amine fluoride dentifrice followed by different mouthrinsing procedures. Journal of Clinical Dentistry. 7, pp.6-8.
5. Campus, G. et al. 2003. Fluoride concentration in saliva after use of oral hygiene products. Caries Research. 37(1), pp.66-70.
6. Issa, A. and Toumba, K. 2004. Oral fluoride retention in saliva following toothbrushing with child and adult dentifrices with and without water rinsing. Caries Research. 38(1), pp.15-19.
7. Hirose M. et al. 2015. Fluoride retention in saliva following toothbrushing using different types fluoridated dentifrices containing 1500 ppm F of NaF and MFP. Pediatric Dental Journal. 25 (2), pp.45-49.

8. Nazzal, H. et al. 2016. Comparison of residual salivary fluoride retention using amine fluoride toothpastes in caries-free and caries-prone children. European Archives of Paediatric Dentistry, 17, pp.165–169.

2.8 Critical appraisal and analysis of literature

Limited literature has been published comparing salivary fluoride concentrations post-brushing using different fluoride compounds and/or different fluoride concentrations. The literature was reviewed in a chronological order and standardised forms (data template) were used for data extraction from the full versions of the articles (Appendices A.1 and A.2).

Bruun et al. (1984) was one of the earliest published studies that compared the effect of toothpastes of variable fluoride compounds and concentrations of salivary fluoride. It adapted a crossover study design of nine dental students who brushed with a controlled size of five different intervention toothpastes over five experimental sessions. The dentifrices tested contained the following fluoride compounds and concentrations: sodium fluoride (NaF): 1,500 ppmF, 1,000 ppmF and 500 ppmF and sodium monofluorophosphate (NaMFP): 1,500 ppmF and 1,000 ppmF. Subjects were instructed to rinse out 5 times post-brushing and salivary samples were collected mid-brushing (0.5 minute) and at 3, 10, 15, 30, 60 and 120 minutes intervals post-brushing. Both the total fluoride concentration and the total fluoride ions were measured.

The study concluded that, initially, the majority (at least 96%) of fluoride in NaF toothpastes was in ionic form compared to only 3 % of total fluoride in NaMFP toothpastes. The article referred to previous literature suggesting that NaMFP compound is subjected to rapid hydrolysis in the oral environment, which explained the rapid increase of fluoride ion concentration 10 minutes post-brushing with NaMFP (Figure 2-1). The hydrolysis of NaMFP is caused by bacterial phosphatases enzymes in saliva (Ericsson, 1967).

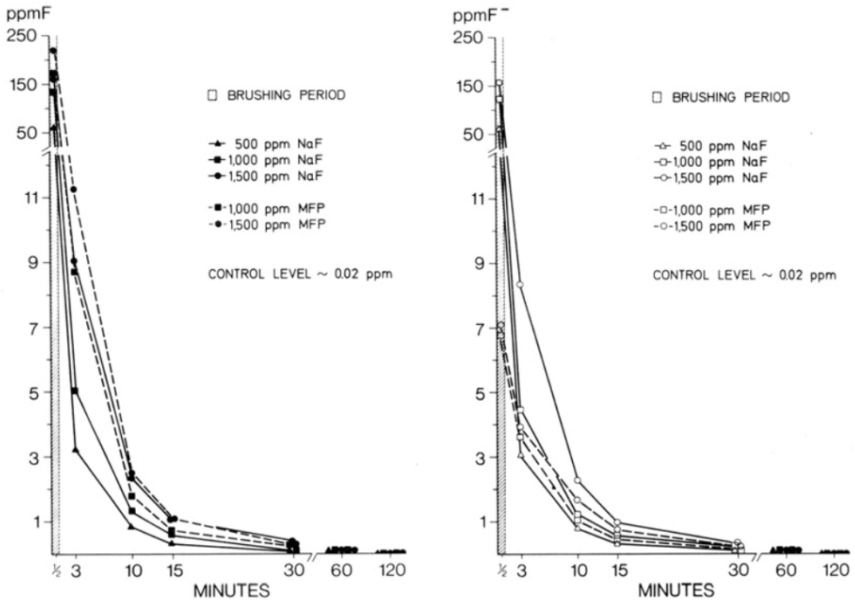


Figure 2-1(A) Total fluoride concentration in whole saliva during and after brushing. (B) Total fluoride ion concentration in whole saliva during and after brushing. (From: Bruun et al., 1984)

Toothpaste fluoride concentration on the other hand showed a direct positive relationship to the fluoride ion concentration and the total fluoride concentration in saliva at all sampling intervals.

This paper is considered as poor evidence due to the lack of a control arm, randomisation, examiner blinding and no mention of whether confounding factors were taken into consideration. This paper however, has raised crucial questions on the behaviour of NaF and NaMFP after toothbrushing as the former provides an instant and constant increase in salivary fluoride ions concentration while the later increases the ionic fluoride concentration as the NaMFP compound is decomposed intraorally. This leaves an unanswered question; will combined NaF and NaMFP toothpaste cause a significant increase in salivary fluoride concentration over an extended period of time compared to NaF or NaMFP toothpastes alone?

Similar results were obtained by Duckworth and Morgan's crossover study (1991). This study tested the differences between oral fluoride concentrations after brushing with NaMFP toothpaste 1,000, 1,500 and 2,500 µg fluoride per gram. This study introduced two types of measurements to investigate the salivary fluoride. Oral clearance studies monitor the drop in fluoride concentration with time after a single topical

fluoride application (i.e. toothpastes). Equilibrium studies on the other hand monitor the levels of fluoride in saliva during regular, repeated use of the fluoridated toothpaste.

To test oral fluoride clearance, 7-10 adult subjects were instructed to refrain from fluoride containing foods, drinks and toothpastes for 10 days prior and during the experimental period. The amount of toothpaste used for brushing, the rinsing time post-brushing and the amount of water used for rinsing were all controlled between subjects and during different experimental visits. Fluoride concentration was measured prior to toothpaste application and at regular intervals for up to several hours post-brushing.

Two distinct phases were identified in the results: a rapid decrease in the salivary fluoride concentration in the first 40–80 minutes followed by a slower phase. The mean fluoride concentration after using 2,500 µg fluoride per gram was significantly higher than toothpastes with lower fluoride amounts (Figure 2-2).

Na_2FPO_3 (F, µg/g)	Parameter B (F, µmol/l)[a]	AUC (mmol F/l · min)[a]	$t_{1/2}(1)$ min	$t_{1/2}(2)$ min
1,000	1.48 (+ 0.73) (− 0.47)	1.91 (+ 0.36) (− 0.30)	9.1 ± 2.2	244.9 ± 84.3
1,500	1.88 (+ 0.85) (− 0.57)	2.22 (+ 0.42) (− 0.35)	9.0 ± 2.4	197.2 ± 50.0
2,500	3.03 (+ 1.43) (− 0.98)	3.87 (+ 0.73) (− 0.61)	6.5 ± 2.8	141.9 ± 34.8

[a] Values are antilogs of logarithmic mean values of the individual data. The 2,500-µg/g values are significantly different from the values connected by the bar ($p < 0.02$; analysis of variance).

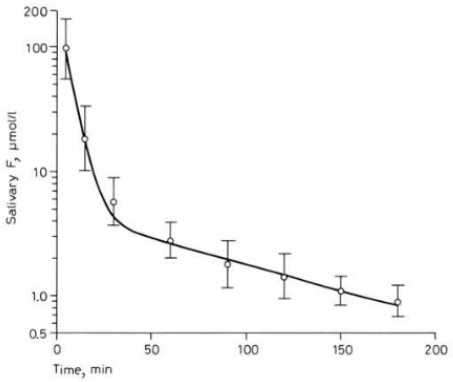


Figure 2-2 (A) A table showing dependence of the salivary clearance curve on the fluoride content of toothpaste. (B) Mean salivary fluoride clearance after using 1,500 µg F/g MFP toothpaste. (From: Duckworth and Morgan, 1991)

In the equilibrium study, salivary fluoride concentration increased markedly during the study and returned back to baseline once the fluoridated toothpaste use had ceased. Mean equilibrium salivary fluoride concentration tended to plateau at high fluoride concentration doses.

Since no randomisation and blinding were identified in this study, the results were more likely to be associated with a high risk of bias, thus negatively impacting on the reliability of the results of this study.

In spite of the low methodological quality of this study, it has discussed the possible fate of fluoride during and after toothpaste applications. It suggested that the fluoride clearance curve was consistent with a two-compartment open pharmacokinetic model. That is, the elimination of the therapeutic agent from one compartment and the update of the agent to, and release from, a second compartment. Five stages were identified as follows and are summarised in (Figure 2-3).

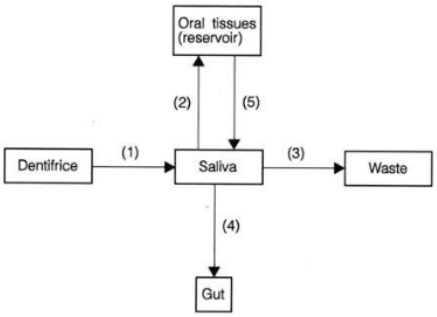


Figure 2-3 The fate of fluoride during and after toothpaste application. (From: Duckworth and Morgan, 1991)

- During the brushing, toothpaste becomes mixed with saliva in the mouth.
- Fluoride ions are taken up by the oral tissues.
- After 30-60 seconds, the majority of fluoride is lost when the bulk of toothpaste slurry is spat out and/or the mouth is rinsed with water.
- The remaining fluoride is mostly lost by uptake to the oral tissues or by swallowing.
- As the salivary fluoride concentration is decreased, fluoride release from the different oral tissues is favoured.

According to the previous theory, and in light of the dynamic theory of remineralisation and demineralisation of the hard dental tissues in the oral cavity, the majority of fluoride that would remain several hours post-brushing would be re-released from the oral tissues (including teeth, soft tissues and plaque) rather than the retained fluoride in the saliva. In other words, the oral cavity acts as a fluoride reservoir, and “oral fluoride retention after use of dentifrices” is a misleading title and does not fully describe the accurate fate of fluoride in the oral cavity.

Details of Paul et al’s (1993) study will not be included in the summary table but will be discussed here for completeness. The aim of this study was to clinically evaluate the salivary fluoride retention of toothpastes containing low-fluoride concentrations in 50 children aged between 7–9 years. According to this paper, four different concentrations were prepared by using

different quantities of the same toothpaste tube (A-D) and a fifth toothpaste that contained very low fluoride concentration was used as a control. (Table 2-1)

Table 2.8-1 Weight of toothpaste and concentration of fluoride (From: Paul et al, 1993).

Group	Length of Ribbon	Weight	Approximate F1.concentration	Mean Fluoride Concentration
	Cibaca Fluoride (125 gms)			
A	1/4 ribbon	0.3341 gms	278 - 301	289
B	1/2 ribbon	0.6567 gms	576 - 607	591
C	3/4 ribbon	1.1308 gms	704 - 742	723
D	Full ribbon	1.4217 gms	893 - 925	909
	Cibaca Top (125 gms)			
E	1/4 ribbon	0.3732 gms	76 - 93	

Increasing the quantity of the toothpaste, however, increased the amount of the fluoride rather than the concentration itself; meaning that the study was of poor methodological quality.

In a study comparing salivary fluoride content after toothbrushing with NaF and amine fluoride (AmF) of the same concentrations (0.125% F), AmF toothpaste has shown to result in a significantly higher salivary fluoride concentration than NaF toothpaste (Attin and Hellwig, 1996). Both toothpaste formulations had significantly increased salivary fluoride content compared to the non-fluoridated control toothpaste. This study also compared between two post-brushing rinsing regimens; rinsing versus not-rinsing. Rinsing after brushing was demonstrated to significantly reduce the salivary fluoride level after brushing, regardless of the formulation used (Figure 2-4).

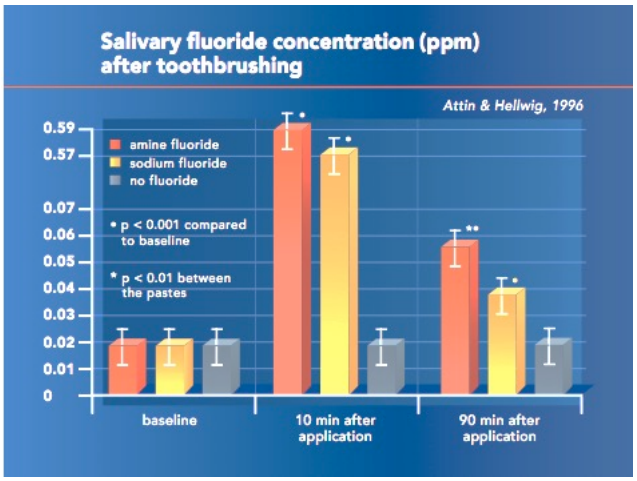


Figure 2-4 Salivary Fluoride concentration after brushing with amine fluoride, sodium fluoride and no fluoride toothpastes (From: Attin and Hellwig, 1996).

Attin and Hellwig's (1996) crossover trial had a sample size of 24 subjects, which was larger than earlier conducted studies. It also introduced the concept of a control group with participants using non-fluoridated toothpaste as part of the trial rather than just using it prior to the experimental days. Although this study cannot be considered as the best available evidence as it lacks randomisation and examiner blinding, it can be considered as being of higher evidence than previously published literature (Paul et al, 1993; Duckworth and Morgan, 1991; Bunn et al, 1984), since it adapted an improved methodology.

Randomisation and blinding was first implemented in post-brushing salivary fluoride concentration studies by Campus et al. (2003). This was an in vivo study with a total sample size of 104 volunteers who were randomly assigned to five intervention groups as illustrated in (Table 2-2).

Table 2.8-2 A table showing the different intervention groups. It also shows fluoride concentrations in saliva before the use of the intervention products (t0), after 20 days (t1) and 24 hours after cessation of using fluoridated products (t2). (From: Campus et al, 2003)

	Product	Saliva fluoride concentration, ppm			p
		t_0	t_1	t_2	
A	NaMFP dentifrice, 1,250 ppm F	1.99 (1.01)	8.26 (0.33)	7.41 (0.28)	! 0.001
B	AmF dentifrice, 1,250 ppm F	1.35 (0.07)	8.50 (0.43)	7.35 (0.19)	! 0.001
C	AmF dentifrice, 1,250 ppm F	1.52 (0.09)	7.20 (0.19)	6.67 (0.27)	! 0.001
D	AmF dentifrice, 1,250 ppm F + AmF mouthrinse, 248 ppm F	1.98 (1.00)	8.69 (0.39)	9.88 (3.002)	! 0.001
E	NaMFP dentifrice, 1,250 ppm F + NaMFP varnish, 1,250 ppm F	1.15 (0.08)	8.14 (0. 43)	6.99 (0.42)	! 0.001
		p 1 0.05	p 1 0.05	p 1 0.05	

Results presented as means of triplicate measurements, with standard errors in parentheses. Statistical comparisons were performed on log-transformed data.

Both groups B and C tested an identical intervention with aiming to assess the reproducibility of the analysis methods in the study.

Campus, et al. (2003) adapted a similar experimental design as Duckworth and Morgan (1991) in terms of looking at both the oral salivary clearance and the equilibrium studies. For equilibrium studies, subjects ranged between 19 - 22 subjects per group, with a mean age ranging between 23–24 years. Each volunteer brushed with the assigned intervention toothpaste for 3 minutes, 3 times a day for a period of 20 days. Unstimulated salivary samples were collected at baseline, after 20 days and 24 hours post-cessation of the intervention toothpaste. For all groups, the average concentration of fluoride was significantly higher than baseline, but no

statistically significant difference was observed between intervention groups. Group D showed the highest salivary fluoride. Even 24 hours after cessation, the salivary fluoride concentrations remained higher than baseline readings (Table 2-2).

To measure the salivary fluoride clearance, unstimulated salivary samples were collected from five participants per group immediately after the use of the fluoride product and at 30, 60, 90 and 120 minutes intervals. As shown in Figure 2-5, fluoride concentrations were significantly different at each time interval, with AmF products having higher fluoride concentration post-brushing than after the use of NaMFP toothpaste.

Randomisation and blinding decreases the operator/investigator bias and ensures that the results of the study are tangible to the general population. It is of paramount importance how randomisation and blinding was generated and implemented, which was not mentioned in this article. This study however, recruited larger sample sizes than historically conducted studies; the larger the sample size, the narrower the confidence interval, leading to a higher accuracy of the results.

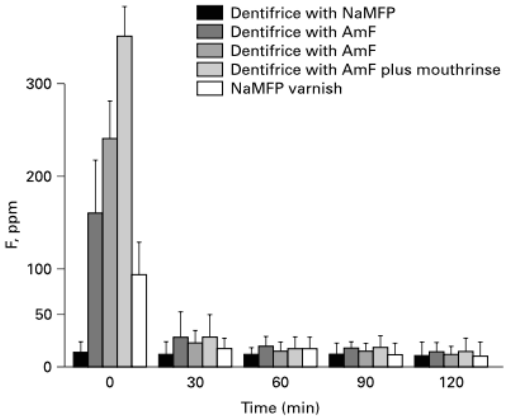


Figure 2-5 Clearance curve of fluoride concentration in saliva. The first sampling was made immediately after the use of fluoride products. (From: Campus et al, 2003)

In 2004, Issa and Toumba conducted an 18-arm double-blinded randomised controlled crossover trial aimed at comparing fluoride retention in saliva in vivo following brushing with different fluoride concentrations and formulations with and without water rinsing. Ten healthy volunteers were recruited, and brushed for one minute with 9 different toothpastes and were finally asked to either rinse or not rinse. Unstimulated saliva samples were collected at 0, 1, 15, 30, 60, 90 and 120 minutes. Amine fluoride toothpastes (1,400 ppm F) resulted in the highest fluoride content of saliva without rinsing at 120 minutes. Salivary fluoride content of AmF and NaF were still higher than baseline levels after 120 minutes.

There was no mention of exclusion or inclusion criteria nor a sample size calculation was conducted in the study. The study however included the largest sample size (10 volunteers in each leg) than historically conducted crossover oral clearance studies. Although participant blinding is less likely to have an effect in this study, this study was both double-blinded and randomised. The study did not express the results in term of significance difference in means but rather higher and lower which makes it difficult to interpret whether any increase is likely to have had an effect on caries prevention.

A Japanese single-blinded two-arm randomised control trial was conducted comparing salivary fluoride level post-brushing between NaF toothpaste (1,500 ppmF) and NaMFP toothpaste (1,500 ppmF) (Hirose et al., 2015). All participants in this study were also asked to rinse post-brushing with 15 ml of distilled water for 5 seconds. It was not clear who was the blinded part in this study. Eight healthy volunteers participated in the study but there was no mention of whether sample size calculations were performed. Saliva samples were collected at baseline and at the following time points: 3, 5, 10, 15, 30, 60, 90 and 180 minutes. The study concluded that NaF toothpaste resulted in significantly higher fluoride retention when compared to NaMFP toothpaste.

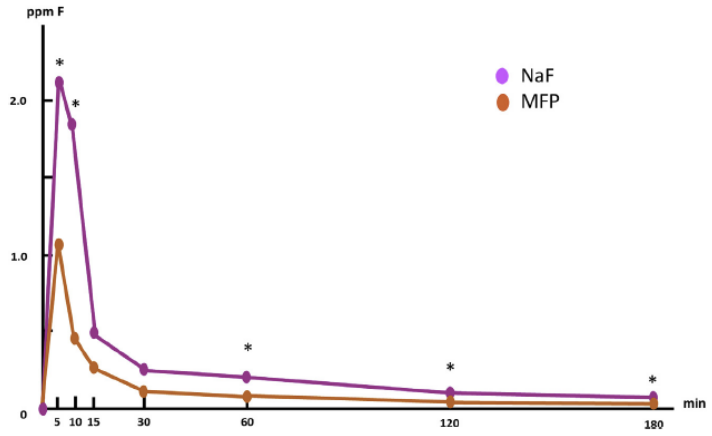


Figure 2-6 Time-dependence of fluoride retention curves during 180 min for each dentifrice. Fluoride concentrations in saliva following toothbrushing with NaF and NaMFP (From: Hirose et al., 2015).

Nazzal et al. (2016) conducted a salivary clearance study on 32 children in the primary dentition only, but it was not clear from the article whether a sample size calculation was performed. The total sample was divided into two groups: caries-prone (dmft>5) and a caries-free group (dmft=0). This was a six-arm crossover study. Each participant was seen six times to brush with different concentrations of AmF toothpaste (250, 500 and 1,250 ppmF) with and without post-brushing water rinsing. All participants rinsed with Leeds tap water (F<0.1 mgF/L). Saliva samples were collected at baseline and at 1, 15, 30, 60 and 90 minutes post-brushing. The study concluded that higher concentrations of AmF resulted in higher salivary fluoride concentrations. No significant difference was found between the caries-prone and the caries-free groups. Salivary fluoride levels returned to baseline at 90 minutes. This study has also supported the previously conducted studies in that rinsing post-brushing resulted in significantly less salivary fluoride concentrations compared to no-rinsing (Figure 2-7).

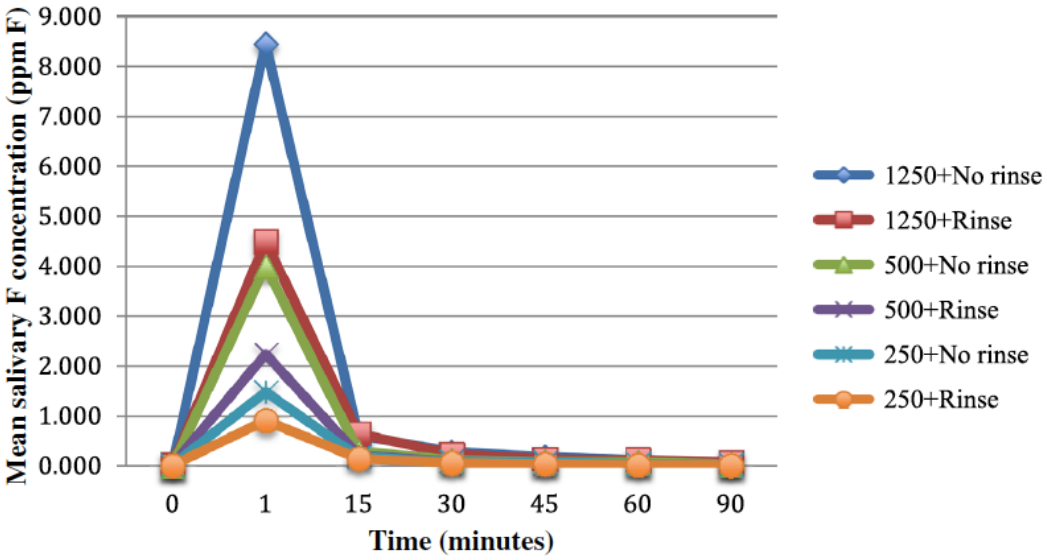


Figure 2-7 Line graph showing mean residual salivary fluoride concentrations after brushing with three amine fluoride concentrations with rinsing and no rinsing over time (From: Nazzal et al., 2016).

2.9 Conclusions

- The majority of previously conducted oral clearance studies were of a crossover design with small sample sizes and with no reference to whether sample size calculations were performed.
- Earlier studies were associated with a high risk of bias and were of poor methodological quality.
- Amine fluoride toothpastes resulted in higher salivary fluoride concentrations than the other tested toothpastes (sodium fluoride and sodium monofluorophosphate) (Issa and Toumba, 2004; Campus, et al., 2003; Attin and Hellwig, 1996).
- Significant difference was found between sodium fluoride and sodium monofluorophosphate in terms of salivary retention post-brushing (Hirose et al., 2015).
- Fluoridated toothpastes showed significant differences in salivary fluoride compared with non-fluoridated toothpastes (Issa and Toumba, 2004; Attin and Hellwig, 1996).
- There was a direct relationship between fluoride toothpaste concentration and salivary fluoride concentration. (Nazzal et al., 2016; Issa and Toumba, 2004;Duckworth and Morgan, 1991; Bruun, et al., 1984).
- Rinsing post-brushing resulted in significantly lower salivary fluoride concentrations when compared to no-rinsing (Nazzal, et al., 2016; Issa and Toumba, 2004; Campus, et al., 2003).

Therefore, there is a need for methodologically sound randomised controlled trials that are reported according to the Consolidated Standards of Reporting Trials (CONSORT) statement. Further research is also needed to explore additional commercially and non-commercially available toothpastes including NaF toothpastes (2,800 ppmF and 5000 ppmF), stannous fluoride and sodium fluoride (SnF and NaF) and sodium fluoride and sodium monofluorophosphate (NaF and MFP) combined toothpastes.

Chapter 3 **Salivary Clearance Study**

3.1 Introduction

No studies were found in the literature comparing over-the-counter toothpastes with all the different fluoride formulations.

The clinical part of this research study took place at the Dental Translational and Clinical Research Unit (DenTCRU) based at the Leeds Dental Institute (Level 5, The Worsley Building, Clarendon Way, Leeds, LS2 9LU). Mrs. G. Dukanovic (a clinical research assistant) was present during all the clinical sessions.

Several meetings took place and a log was completed for each session to ensure accuracy of the paper work. Training of equipment and for taking volunteer consents were also completed using the appropriate reading logs / training.

3.2 Aims of the study

This in vivo study aimed to compare the salivary fluoride concentrations following brushing with different fluoride formulations of toothpastes with and without post-brushing water rinsing.

Secondary aims included assessing the interaction between salivary fluoride concentrations and the gender, age, caries, calculus, DMFT and DMFS.

3.3 Null hypotheses

3.3.1 Primary aim hypothesis:

H_{01}: All of the following toothpaste formulations of similar fluoride concentrations have no significant difference in terms of salivary clearance concentrations of fluoride when measured at different time intervals:

- Non-fluoride toothpaste
- Sodium fluoride toothpaste
- Sodium fluoride and sodium monofluorophsophate combined toothpaste
- Stannous fluoride and sodium fluoride combined toothpaste

- Amine fluoride toothpaste
- Sodium monofluorophosphate toothpaste

H_{02}: No significant difference exists between rinsing and non-rinsing post-brushing with regard to salivary fluoride concentrations amongst all the toothpaste formulations at any time point.

3.3.2 Secondary aim hypotheses:

H_{03}: No significant interaction exists between the gender of the participants and the salivary fluoride concentrations.

H_{04}: No significant interaction exists between caries status of the participants and the salivary fluoride concentration.

H_{05}: No significant interaction exists between the presence of calculus and the salivary fluoride concentrations.

H_{06}: No significant interaction exists between the age of the participants and the salivary fluoride concentrations.

H_{07}: No significant interaction exists between the DT, MT, FT and DMFT scores and the salivary fluoride concentrations.

H_{08}: No significant interaction exists between the DS, MS, FS and DMFS scores and the salivary fluoride concentrations.

3.4 Study objectives

The primary objective was to compare the salivary fluoride concentrations of different fluoride toothpaste formulations. Toothpaste formulations included NaF, Amf, NaMFP, NaF and NaMFP combined, and SnF and NaF combined. Fluoride-free toothpaste served as a control in this study. The fluoride concentrations were standardised for all the formulations.

The secondary objective was to compare the effect of two rinsing regimens post-brushing (with and without water rinsing) on the salivary fluoride concentrations.

The results of this study would reflect on the oral hygiene instructions given to patients in terms of tooth brushing frequency, fluoride-containing dentifrices of different formulations and post-brushing rinsing practices.

3.5 Study type

Double blinded controlled randomised trial. The chief investigator who also performed the statistical analysis was blinded to the toothpaste formulations and the rinsing method used. All of the participants were blinded to the toothpaste formulations but were aware of the rinsing method of their groups. This was achieved by preparing concealed envelopes containing a card with either rinsing or non-rinsing and labelled with the group number on the outside. These were kept with Mrs. Dukanovic at all times.

3.6 Materials under investigation

The toothpastes used for the study were as follows:

- **<u>Control toothpaste - Total fluoride ion concentration: 0 ppmF as stated on the packaging</u>**

  Kingfisher Natural Toothpaste ® Fennel-fluoride free - 100 ml

- **<u>Sodium fluoride toothpaste – Total fluoride ion concentration: 1,450 ppmF</u>**

  Colgate Total ® Original Care ™ - 125 ml

- **<u>Sodium monofluorophosphate toothpaste - Total fluoride ion concentration: 1,450 ppmF</u>**

  Colgate Sensitive ® Pro-Relief ™ Extra strength - 75 ml

- **<u>Sodium monofluorophosphate (1,000 ppmF) and sodium fluoride (450 ppmF) combined toothpaste - Total fluoride ion concentration: 1,450 ppmF</u>**

  Colgate ® Cavity protection ™ – 75 ml

- **<u>Stannous fluoride (1,100 ppmF) and sodium fluoride (350 ppmF) combined toothpaste - Total fluoride ion concentration: 1,450 ppmF</u>**

  Oral-B ® Pro-Expert ™ – 75 ml

- **<u>Amine fluoride toothpaste - Total fluoride ion concentration: 1,400 ppmF</u>**

  Elmex ® Protezione carie – 75 ml

Batch number details and expiry dates were logged in the investigator site file to ensure that the use of the toothpastes were within the recommended time frame. This was also to allow tracking batch numbers in case of adverse effects.

3.7 Inclusion criteria

1. Participants in this study should be ASA I and ASA II adult volunteers.
2. Resting salivary flow rate of 0.1 ml / minute or more.
3. Caries-free and caries-prone are to be included.

3.8 Exclusion criteria

1. Edentulous patients.
2. Participants who are ASA III or higher.

3. Allergy to any of the materials used in the study.
4. Participants with orthodontic devices / braces.
5. Participants with resting salivary flow rate of/ or below 0.1 ml / minute.
6. Participants who are incapable of fasting for 4 hours.
7. Participants who refuse to use fluoride-free toothpaste, or those who would want to have control over which toothpaste to use.
8. Participants who cannot retain toothpaste and have to rinse after tooth brushing (i.e. gagging due to toothpaste taste).

3.9 Materials necessary for the study

The materials that were used in the study were as follows:

- Disposable individually wrapped mouth mirrors
  Used for initial screening of participants
- Disposable individually wrapped toothbrushes
  Used for participants to brush with as part of the study
- Disposable 15 ml graduated centrifuge sterile plastic tubes
  Used for saliva collection at six different time intervals for each participant
- Disposable plastic funnels
  Used for saliva collection at six different time intervals for each participant
- Disposable plastic test-tubes
  Used for measurement of fluoride in saliva samples.
- De-ionised distilled water:
  Used for rinsing post-brushing for rinsing groups.
- Digital electronic balance for weighing the amount of toothpaste
  Used to measure an amount of 1.0 g of toothpaste to standardise the amount of toothpaste used in brushing.
- Fluoride ion standard solutions 0.01, 0.1, 1.0, 10.0, 100.0 and 1000 ppmF.
  Used for calibration of ion electrode and to assess reproducibility of measurements.
- Thermo Scientific Orion^{TM} Fluoride ion selective combination electrode 9609BNWP

Used for fluoride ion measurement in saliva samples.

- Metrohm™ ion analysis meter 781 pH / Ion meter
  Used for ion analysis during measurements.
- Thermo Scientific Orion™ Optimum results A for ion selective electrode filling solution catalogue number 900061
- Thermo Scientific Total ionic strength adjustment buffering solution with cyclohexylenedinitrilotetraacetate (TISAB II Low level with CDTA)
- Disposable Gilson™ sterile plastic tips of pipettor
- Gilson™ Pipettes

3.10 Sample size and power calculation

Statistical advice was sought from Mrs. J. Kang (Department of Oral Biology, School of Dentistry, University of Leeds, UK) and sample size calculation was performed using Power Analysis and Sample Size 11.0 (PASS). The study aimed to test 12 groups; each group was tested at 6 different time intervals. For this study confidence intervals were set to be 95%, with 100% power.

Sample size calculations were performed using raw data from the research study: Issa and Toumba (2004).

A sample of at least 3 participants was needed for each group to achieve significant difference. The study aimed to test 12 different groups, with at least 10 participants in each group, giving a total number of at least 120 participants.

3.11 Investigator site file

An investigator site file was produced as a hard copy and an electronic copy to aid in the smooth progression of the research project as well as for auditing purposes. Appendix C.1 shows the investigator site file contents.

3.12 Randomisation and blinding toothpaste groups

A trained research dental assistant (Mrs. G. Dukanovic) helped in concealing the toothpaste tubes and labelling them (G01 – G12).

A step-by-step guide was produced (Appendices C.2 and C.3) to help Mrs. G. Dukanovic in the randomisation and blinding of the toothpaste tubes before the concealment and labelling process.

3.13 Random assignments of toothpastes to participants

Random assignment of participation numbers of participants (1 – 120) to the groups (G01 – G12) was performed by the chief investigator (Mrs. M. Albahrani) with the aid of the following website (Appendix C.4):
https://www.randomlists.com/team-generator

3.14 Publicity and recruitment

Recruitment of participants was achieved with the aid of recruitment flyers and circular emails (Appendix C.5).
Flyer posters were displayed across the different schools and libraries of the University of Leeds, through the Leeds Dental Institute (Level 5, The Worsley Building, Clarendon way, LS2 9LU) and Precious Dental Care dental clinic (20 Gledhow Avenue, Roundhay, Leeds, West Yorkshire, LS8 1NU). Posters displayed across the University of Leeds were replaced regularly.
Circular emails were sent to students across the University of Leeds every 2 months starting from September 2016 until April 2017.
The study was also registered with a public clinical trials data base (ClinicalTrials.gov Identifier: NCT02740803).

3.15 Elective assessment of the participants

When participants contacted the lead examiner (responding to the email or posters) about participating in the research study, they were asked several questions about their medical history using a medial questionnaire illustrated in Appendix C.6 to determine whether they were within ASA I and ASA II categories. Other ASA categories (ASA III or higher) were excluded from the study. No further contact was made with excluded participants.
For participants who showed interest over the phone, they were given the option of whether they wanted the participant information sheet (Appendix

C.1) mailed to them, emailed or if they preferred to pick it up at their convenience.

Date of birth, contact phone number and participants initials and gender were obtained over the phone or via email.

Participant information sheets were sent out at least 48 hours prior to the experimental appointment to aid in the informed decision to participate in the study.

3.16 Sending out appointments to participants

The appointment date and time were agreed with the participant who showed interest to take part in the study and was deemed eligible to participate as per the inclusion and exclusion criteria explained in sections 3.7 and 3.8.

A confirmation appointment email or text was sent to the participants (Appendix C.7).

Participants were advised that they could bring something to read, listen to or work on as they would be need to be in the research clinic for approximately two hours.

3.17 Appointment reminders

A reminder message / email was sent out to participants one day prior to their appointment. This included instructions such as fasting at least 2 hours prior to their appointment and tooth-brushing instructions (not to brush their teeth on the day of the research; the latest they could brush their teeth was the night before). It also included the location details of the clinic for the research study (Appendix C.8).

3.18 Anonymising participants

Each person who showed interest to take part in the study was assigned a unique identification number (Screen number i.e. S001).

Participants were invited to attend the research study which was divided into two parts: A screening part and research study part.

Once the participant passed the screening part, they were assigned a participation number. This number was assigned in the order of screening of participants.

The date of birth, participant's initials, age and gender were also collected.

3.19 Obtaining informed consent

Information sheet (Appendix C.9) and a copy of the consent sheet (Appendix C.10) were sent to participants by email, post or by collection in person. Information sheets were given to participants at least 48 hours prior to their appointment.

On the appointment day, consent sheets were explained and an informed consent was obtained from all participants by either the chief investigator (Mrs. M. Albahrani) or the research assistant (Mrs. G. Dukanovic). The original copy of the consent sheet was kept in the investigator site file and the participants retained a copy of their signed consent form.

3.20 Screening of participants

Participants underwent a screening process and the salivary clearance study was performed in the Dental Translational and Clinical Research Unit (DenTCRU) based at the Leeds Dental Institute (Level 5, The Worsley Building, Clarendon Way, Leeds, LS2 9LU).

Each participant who was deemed eligible to participate had been invited to take part in the screening process to complete the process of inclusion and elimination of participants. A case record form (CRF) and an appointment checklist were produced to aid in the screening and research process (Appendices C.11 and C.12).

For each participant, after informed consent was obtained, they were asked to drool into a sterile tube for two whole minutes to determine the salivary flow rate. This sample was also used as a baseline pre-brushing sample if the salivary flow rate was 0.1 ml / minute or more. Each tube was labelled with the participant's screening number, date of appointment and time interval.

Any participant who had a salivary rate below 0.1 ml / minute at this stage was excluded from the study. No further analysis was performed on samples given by participants excluded from the study.

Caries examination procedure followed a systematic approach in examining each surface / tooth using a teeth chart as illustrated in the CRF from (Appendix C.11). Teeth were dried thoroughly using a 3in1 syringe. This was followed by visual inspection using a dental mirror and the dental chair light. Participants were inspected sitting on the dental chairs at the DentCRU department.

WHO criteria for assessment of oral health status were followed for caries detection and obtaining both DMFS and DMFT scores (WHO, 2013).

Table 3.20-1: Caries scoring system

Score	Interpretation
Positive (+)	Signs of shadowing or clinical cavitation visible clinically
Negative (-)	No signs of shadowing or clinical cavitation visible clinically

Calculus detection was based on visual inspection only and a thorough drying of the teeth surfaces with a 3in1 syringe. Teeth were examined for the presence of supra-gingival calculus that was visible on visual inspection. No aiding tools were used to detect small traces of calculus or sub-gingival calculus.

Table 3.20-2: Calculus scoring system

Score	Interpretation
Positive (+)	Supragingival calculus present
Negative (-)	No calculus present

The screening process included the following steps and the roles were divided as illustrated in Table 3-1.

Table 3.20-3 Summary of screening process.

Screening process steps	Chief investigator	Research assistant
Confirmation of participants' details	√	√
Confirmation of medical history	√	
Confirmation of recipient of participant information sheet 48 hours prior to appointment	√	√
Confirmation that participants followed pre-appointment instructions	√	√
Obtaining informed consent	√	√
Collection and measurement of pre-brushing baseline unstimulated saliva sample for 2 minutes.	√	√
Charting dentition of participants	√	
Completing inclusion / exclusion criteria checklist	√	√
Determining eligibility of participant to take part in the study	√	

3.21 Salivary fluoride study

Participants who passed the screening process and were signed off as eligible to take part in the study were assigned a participation number. Each participation number had been previously randomly assigned to one of the study groups (G01 – G12) (Appendix C.4).

Participants were then asked to brush their teeth with a pre-weighed amount of toothpaste (≈ 1.0 g) for 2 full minutes. An sensitive electronic scale was used to measure an amount of 1.0 g of toothpaste for all participants. Measurement of the weights of the toothbrushes prior and after toothpaste dispensing were taken to increase accuracy of measurements. Each

toothpaste was dispensed using a spatula for more controllability over the dispensed amounts.

Depending on which group they were in, participants were either asked to spit the excess toothpaste and not rinse their mouth for the entire appointment or to rinse their mouth following brushing with 10 ml of distilled water for 5 seconds. The amount of distilled water was measured using a 5 ml pipette.

Following brushing, unstimulated saliva samples were collected five times at the following time intervals: 1, 15, 30, 60 and 90 minutes. Each saliva sample was collected over a period of two minutes.

Participants remained in the clinic under the direct supervision of the research staff (Mrs. M. Albahrani and Mrs. G. Dukanovic) and were instructed to refrain from eating or drinking throughout the entire appointment.

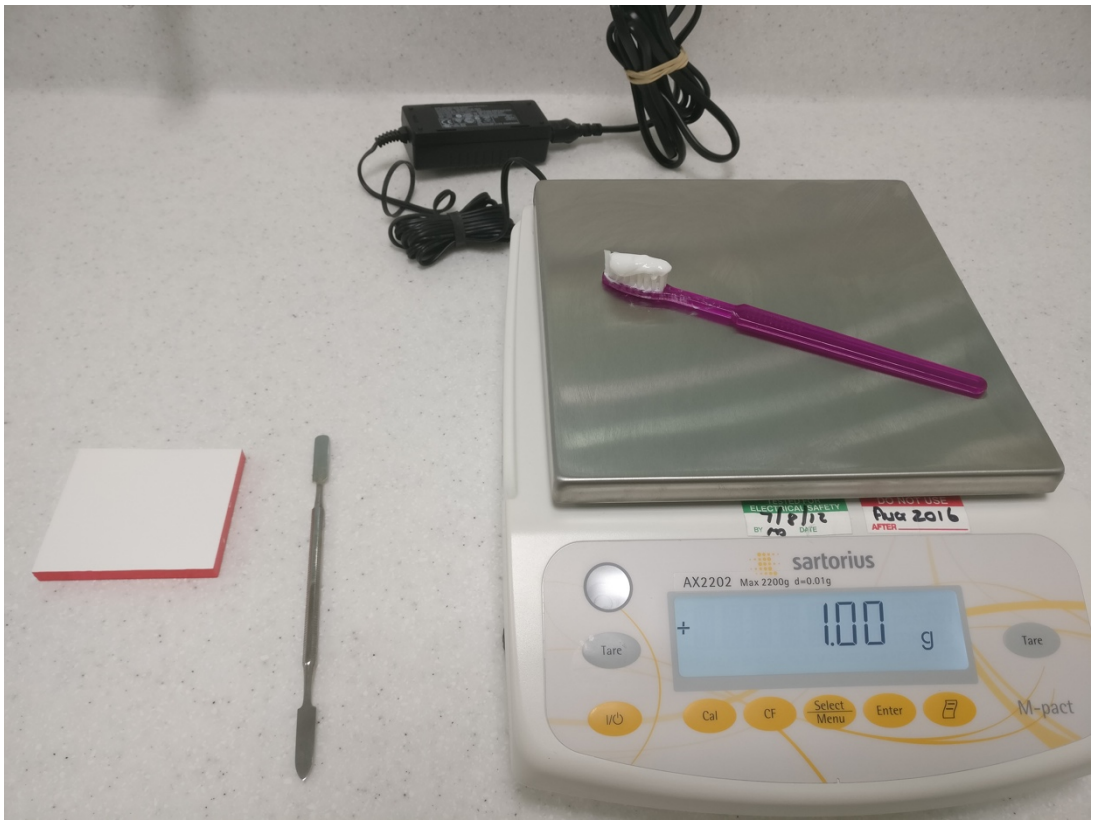


Figure 3-1 Measurement of ≈ 1.00 g of toothpaste onto the toothbrush using an electronic sensitive scale.

Table 3-2 summarises the steps of the salivary fluoride clearance process and the roles of both the chief investigator and the research assistant.

Table 3.21-1 Salivary fluoride clearance process summary.

Steps of the salivary fluoride study process	**Chief investigator**	**Research assistant**
Confirmation of participants' group number	√	√
Measuring ≈ 1.0 g of toothpaste onto the toothbrush using an electronic sensitive scale	√	
Instructions to participants on the toothbrushing process and salivary collection process	√	√
Supervising toothbrushing of participants for two minutes		√
Supervising participants rinsing. Rinsing should be with 10 ml distilled water for 5 seconds.		√
Collection of saliva samples at the 1 minute time interval		√
Collection of saliva samples at 15, 30, 60 and 90 minutes time intervals.	√	√

3.22 Sample collection

Saliva samples were collected using sterile tubes that were labelled with the time interval, participants screening number and the appointment date. Each tube was also colour coded so that it corresponded to the time interval. A colour coded chart was produced to aid in the identification of the colour codes with regard to the corresponding time intervals.

Participants were instructed to passively drool into a disposable funnel which was placed into the saliva collection tubes. A funnel was used to facilitate saliva collection from participants. Each sample was collected for 2 minutes with the aid of a digital timer.

Participants who were excluded following the collection of the baseline saliva sample had their saliva samples discarded at that time and no further samples were collected.

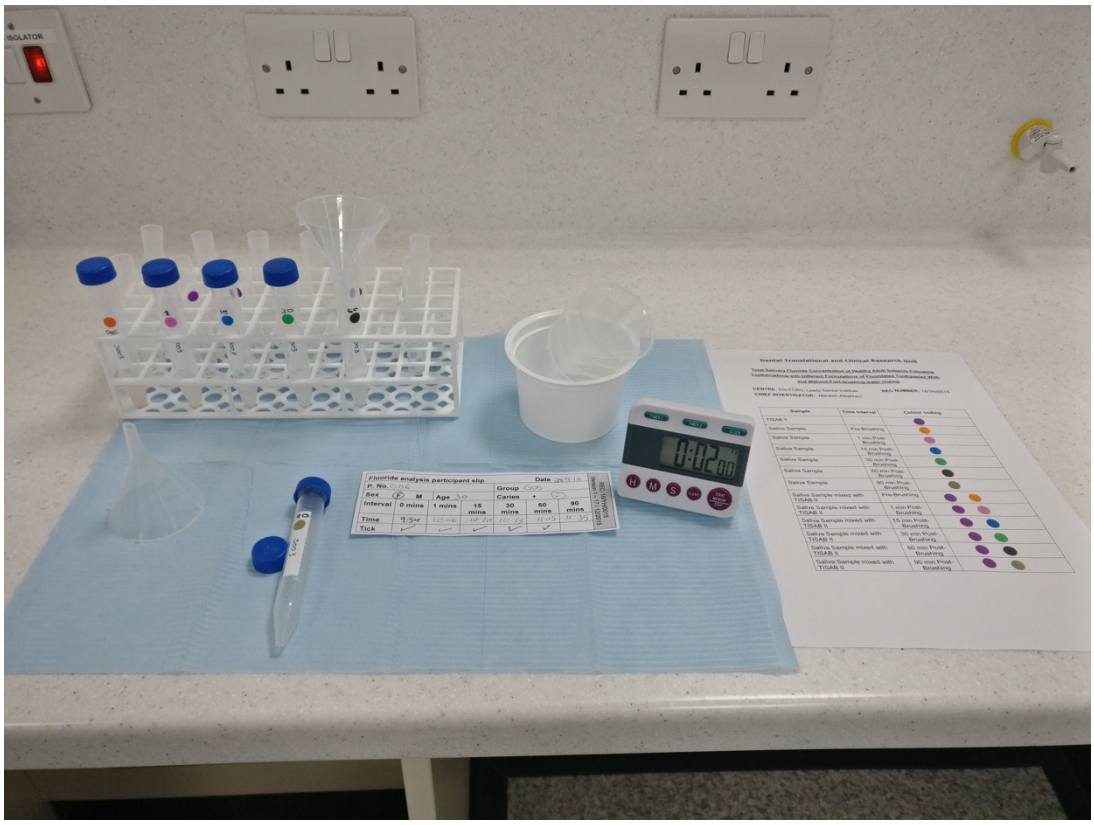


Figure 3-2 Saliva samples were collected at baseline and at five time points post-brushing (1, 15, 30, 60 and 90 minutes).

Following completion of toothbrushing, five chair-side timers were started and had the following time intervals 1, 14, 29, 59 and 89 minutes. The four latter time intervals were set 1 minute short of the intended time to allow the research staff to arrive at the participant station and prepare for saliva samples to be collected. Another one minute timer was used to time the last minute.

The expected time of collection for each saliva sample was also calculated and recorded in the case record form. This was planned as a double confirmation stage to ensure accuracy of the saliva collection timing process.

Figure 3-3 Chair-side timers were started simultaneously post-brushing for accurate timing.

3.23 Compensation to participants

Following completion of the study participants were given an envelope that contained a thank you card (Appendix C.13) and a cash amount of £10 to compensate for their time, transport and food expenses. Cash payment receipts (Appendix C.14) were signed by participants and by either the chief investigator or the research assistant. The original copy was retained in the investigator site file and a copy was provided to the participant.

Participants excluded from the study following the screening stage also received compensation to appreciate their intention to participate in the study.

A log of all the participants who received the compensation was submitted quarterly to the financial office of the Faculty of Medicine and Health of the University of Leeds.

Chapter 4 **Saliva Samples Measurement and Analysis**

4.1 Saliva sample storage

Saliva samples were preserved in the laboratory freezer (-18 degree Celsius) until analysis. The total freezing time did not exceed three months.
Each saliva sample tube was labelled with the participant's screening number, date of collection and time interval.

4.2 Preparation of 1000 ppm standard fluoride solution

The mass ratio between sodium and fluorine is 1.21:1 (Relative atomic mass of sodium = 22.990)(Relative atomic mass of fluorine = 18.998). This means that a sample of 2.21 grams of reagent-grade sodium fluoride produces one (1) gram of fluoride and 1.21 grams of sodium. When dissolved in 1.0 litre of water, an amount of 2.21 grams of sodium fluoride will produce a 1.0 gram / 1.0 litre fluoride solution. When converted to parts per million, this equals 1,000 parts per millions of fluoride solution.

$$\frac{1.0\ g}{1.0\ litre} = \frac{1{,}000\ mg}{1{,}000{,}000\ mL} = 1{,}000\ parts\ per\ million$$

To produce 1,000 ppm fluoride solution, an amount of 2.21 grams of reagent-grade sodium fluoride was dissolved in water using a 1.0 litre volumetric flask. This was stored in the fridge and used within a month. A fresh 1,000 ppmF was prepared every month.
This has also been used to prepare other fluoride standard solutions using the following formula:

$$C1\ x\ V1 = C2\ x\ V2$$

Where:

C1 = Concentration of original standard (ppm)
V1 = Volume of original standard (mL)
C2 = Concentration of standard after dilution (ppm)

V2 = Volume of standard after dilution (mL)

4.3 Preparation of 100 ppm standard fluoride solution

To prepare 100 ppm standard fluoride solution using the previous formula was followed:

C1 = 1,000

V1 = unknown

C2 = 100

V2 = 10

Applying the previous formula:

$$1{,}000\ x\ V1 = 100\ x\ 10$$

$$V1 = \ 1.0\ mL$$

This means that 1.0 mL of 1,000 ppmF diluted in 9.0 mL of water will produce 100 ppm standard fluoride solution.

A pipette was used to measure a volume of 9.0 mL of water and 1.0 mL of 1,000 ppmF.

4.4 Preparation of 10 ppm standard fluoride solution

To prepare 10 ppm standard fluoride solution the previous formula was followed:

C1 = 1,000

V1 = unknown

C2 = 10

V2 = 10

Applying the previous formula:

$$1{,}000\ x\ V1 = 10\ x\ 10$$

$$V1 = \ 0.1\ mL$$

This means that 0.1 mL of 1000 ppmF diluted in 9.9 ml of water will produce 10 ppm standard fluoride solution.

A pipette was used to measure a volume of 9.9 mL of water and 0.1 mL of 1,000 ppmF.

4.5 Preparation of 1.0 ppm standard fluoride solution

To prepare 1.0 ppm standard fluoride solution the previous formula was followed:

C1 = 100

V1 = unknown

C2 = 1.0

V2 = 10

Applying the previous formula:

$$100\ x\ V1 = 1.0\ x\ 10$$

$$V1 = \ 0.1\ mL$$

This means that 0.1 mL of 100 ppmF diluted in 9.9 ml of water will produce 1.0 ppm standard fluoride solution.

A pipette was used to measure a volume of 9.9 mL of water and 0.1 mL of 100 ppmF.

4.6 Preparation of 0.1 ppm standard fluoride solution

To prepare 0.1 ppm standard fluoride solution the previous formula was followed:

C1 = 10

V1 = unknown

C2 = 0.1

V2 = 10

Applying the previous formula:

$$10\ x\ V1 = 0.1\ x\ 10$$

$$V1 = \ 0.1\ mL$$

This means that 0.1 mL of 10 ppmF diluted in 9.9 ml of water will produce 0.1 ppm standard fluoride solution.

A pipette was used to measure a volume of 9.9 mL of water and 0.1 mL of 10 ppmF.

4.7 Preparation of 0.01 ppm standard fluoride solution

To prepare 0.01 ppm standard fluoride solution the previous formula was followed:

C1 = 1.0

V1 = unknown

C2 = 0.01

V2 = 10

Applying the previous formula:

$$1.0\ x\ V1 = 0.01\ x\ 10$$

$$V1 = \ 0.1\ mL$$

This means that 0.1 mL of 1.0 ppmF diluted in 9.9 ml of water will produce 0.01 ppm standard fluoride solution.

A pipette was used to measure a volume of 9.9 mL of water and 0.1 mL of 1.0 ppmF.

4.8 Calibration of the fluoride ion selective combination electrode

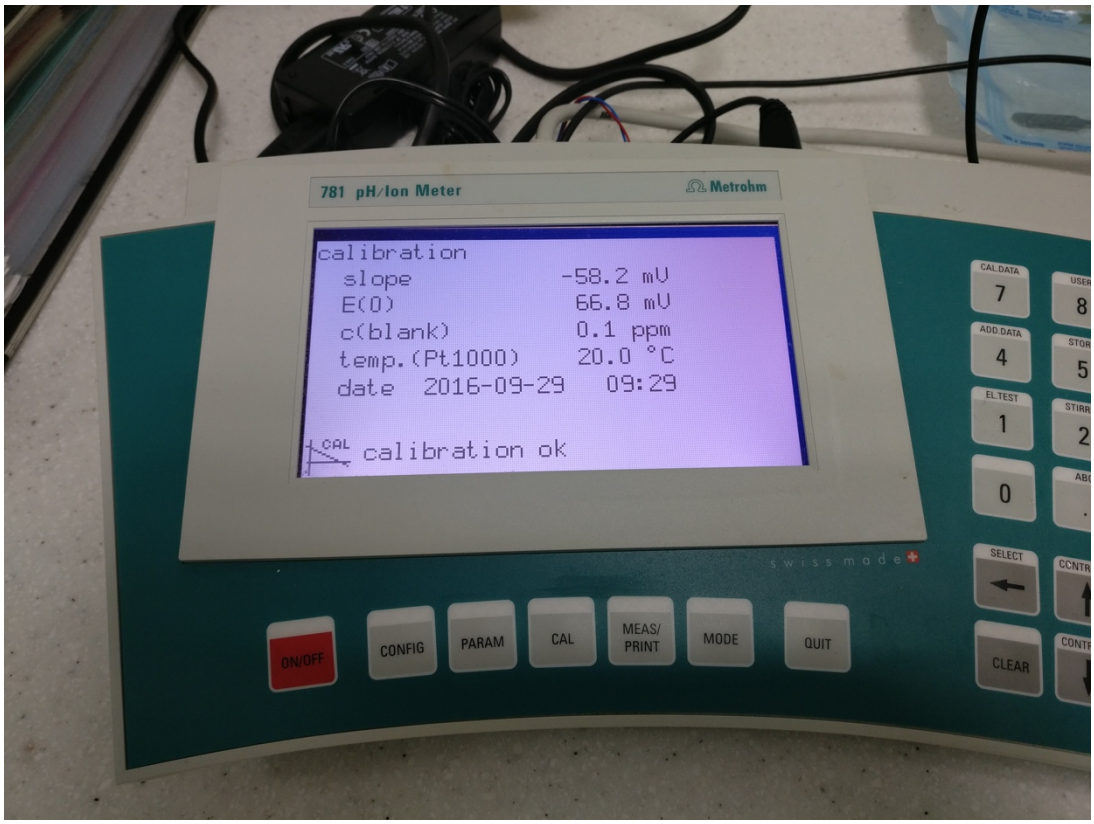


Figure 4-1 As per the manufacturer instructions, the resulting slope value for the calibration process should be between -54.0 and -60 mV when the standards are between 20-25 ˚C

Calibration of the fluoride ion selective combination electrode was performed prior to sample measurement. Manufacturer instructions were followed to perform direct calibration using 0.01, 0.1, 1.0, 10, 100 and 1000 ppm fresh standard fluoride solutions mixed with equal parts of low-level TISAB with CDTA. As per manufacturer instructions, the resulting slope value for the calibration process should be between -54.0 and -60 mV when the standards are between 20-25 ˚C.

Calibration was verified every two hours as per the manufacturer instructions. Recalibration of the electrode was performed when reading of the values of the fluoride standards had changed by 2.0 %.

4.9 Low-level total ionic strength adjustment buffer (TISAB)

Low-level TISAB was used for measurement of low levels of fluoride ion in solutions of less than 0.4 ppmF. This can be prepared by dissolving 4.0 grams of cyclohexylenedinitrilotetraacetate (CDTA), 57.0 mL of glacial acetic acid and 58.0 grams of sodium chloride (NaCl) in about 500 mL of distilled water. The pH of the solution is then adjusted to be between 5.0 and 5.5 by adding 5.0 moles of sodium hydroxide (NaOH). The solution is then diluted with 1.0 litre of distilled water.

For the present research study, a pre-prepared rather than an in-house prepared TISAB-II with CDTA solution was used (Thermo Scientific TISAB II Low level with CDTA).

4.10 Sample preparation and measurement

Saliva sample tubes were taken out of the freezer two hours prior to analysis.

Equal parts of saliva samples and TISAB II with CDTA were mixed in a sterile test tube. The test tubes were labelled with each participant’s screening number and time interval. The test tubes were kept in a tube rack to decrease the risk of accidental sample loss.

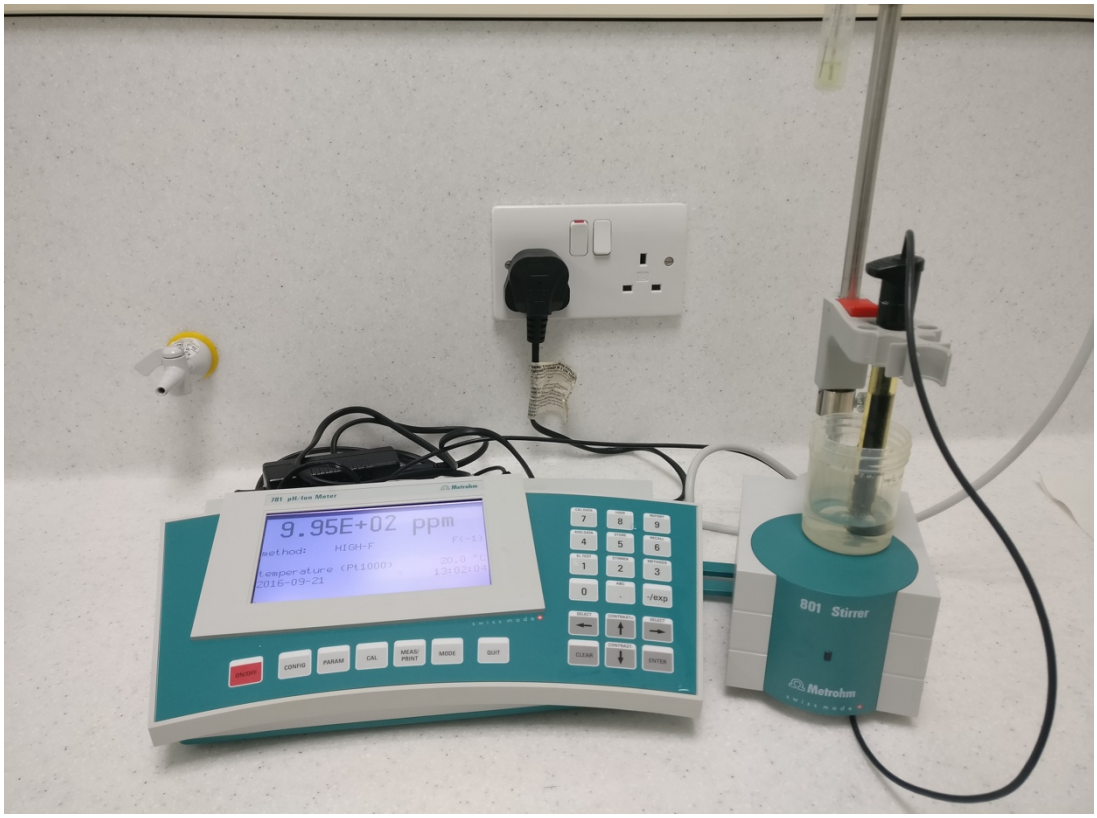


Figure 4-2 Fluoride analysis was performed using a fluoride ion-specific sensitive electrode connected to an ion analyser.

Fluoride concentrations were measured using an ion-specific sensitive electrode connected to an ion analyser. Measurements were then recorded in the relevant section of the participant's case report form (CRF).

After each measurement session, samples were disposed of safely as per local protocols of University of Leeds laboratories.

4.11 Reproducibility of measurements

Reproducibility of the results were checked by using fluoride ion standard solutions every two hours following electrode calibration. As per the manufacturer instructions, when meter values differed by 2%, recalibration of the electrode was performed.

Chapter 5 **Data Analysis and Results I**

Demographic characteristics

5.1 Introduction

The study lasted for eight months from the 21st September 2016 until the last participant was recruited on the 31st May 2017. We were contacted by 230 participants, 124 attended screening visits while four were excluded for not meeting the inclusion criteria. In total, 120 participants completed the study; 10 in each of the 12 groups.

This chapter presents the descriptive analysis of the demographic characteristics and their interaction with the salivary fluoride concentration.

The IBM SPSS 23 software was used to perform data analysis.

The predictor effects were considered to be statistically significant at the 5 % level. Statistical analysis was performed by the Chief investigator. Statistical advice was sought from Mrs. J. Kang (Department of Oral Biology, School of Densitry, University of Leeds, UK) prior to and after the performance of the statistical analysis.

5.2 Gender

Out of a total number of 120 participants, 79 (65.8%) were females. This corresponds to a M:F ratio of 1:1.9.

Salivary fluoride concentration in females was not significantly higher than in males (M=0.196, SE=0.523 ppmF, P=0.709).

Table 5.2-1 Descriptive statistics of gender across individual groups

Group	Males	Females	Group	Males	Females
Group 01	3.0	7.0	**Group 07**	3.0	7.0
Group 02	5.0	5.0	**Group 08**	2.0	8.0
Group 03	1.0	9.0	**Group 09**	3.0	7.0
Group 04	2.0	8.0	**Group 10**	2.0	8.0
Group 05	6.0	4.0	**Group 11**	4.0	6.0
Group 06	5.0	5.0	**Group 12**	5.0	5.0

5.3 Age

5.3.1.1 Distribution of age amongst all the groups

No significant interaction was found between the age and the salivary fluoride concentration (F(26.213-93.760)=0.970, P=0.515, partial Eta squared=0.213).

Table 5.3-1 Summary descriptive statistics of age (years).

Mean ± SD	Minimum value	Maximum value
27.25 ± 7.638	18	60

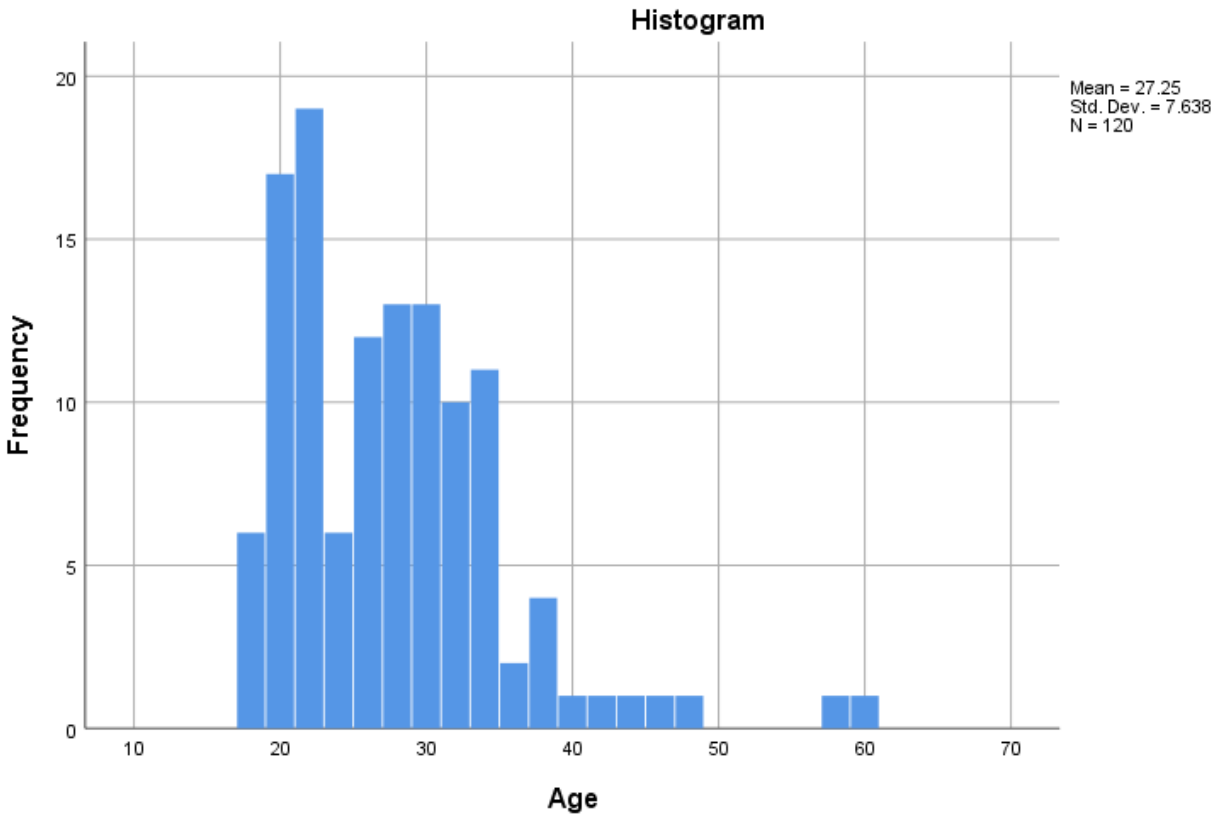


Figure 5-1 Histogram showing descriptive statistics of age (years).

5.3.1.2 Descriptive statistics of age for individual groups

Table 5.3-2 Descriptive statistics of age across individual groups.

Group Number	Mean ± SD	Minimum value	Maximum value
G01	26.90 ± 6.67	19	38
G02	27.50 ± 8.11	18	46
G03	30.30 ± 11.62	19	60
G04	26.40 ± 6.77	18	35
G05	24.50 ± 4.20	20	30
G06	24.70 ± 6.20	19	39
G07	26.10 ± 7.40	19	44
G08	26.70 ± 4.88	19	33
G09	29.50 ± 7.84	20	48
G10	29.70 ± 12.25	18	58
G11	30.00 ± 7.257	18	42
G12	24.70 ± 4.968	18	34

5.4 Caries

Out of a total number of 120 participants, 85 (70.8%) did not have clinically visible signs of caries. This corresponds to a caries-prone : caries-free ratio of 1:2.4.

Salivary fluoride concentrations in caries-free participants were not significantly higher than in caries-prone participants (M=0.393, SE=0.544 ppmF, P=0.471).

Table 5.4-1 Descriptive statistics of caries in individual groups.

Group	Positive caries	Negative caries	Group	Positive caries	Negative caries
Group 01	1.0	9.0	**Group 07**	2.0	8.0
Group 02	4.0	6.0	**Group 08**	2.0	8.0
Group 03	6.0	4.0	**Group 09**	2.0	8.0
Group 04	2.0	8.0	**Group 10**	4.0	6.0
Group 05	1.0	9.0	**Group 11**	6.0	4.0
Group 06	1.0	9.0	**Group 12**	4.0	6.0

5.5 Calculus

Out of a total number of 120 participants, 87 (72.5%) did not have clinically visible signs of calculus. This corresponds to a positive-calculus : negative-calculus ratio of 1:2.6.

Participants with calculus had higher salivary fluoride concentrations. This difference however, was not significant (M=0.079, SE=0.555, P = 0.887).

Table 5.5-1 Descriptive statistics of calculus in individual groups.

Group	Positive calculus	Negative calculus	Group	Positive calculus	Negative calculus
Group 01	2.0	8.0	**Group 07**	3.0	7.0
Group 02	1.0	9.0	**Group 08**	4.0	6.0
Group 03	2.0	8.0	**Group 09**	4.0	6.0
Group 04	2.0	8.0	**Group 10**	2.0	8.0
Group 05	3.0	7.0	**Group 11**	3.0	7.0
Group 06	4.0	6.0	**Group 12**	3.0	7.0

5.6 DMFT score

5.6.1 Descriptive statistics of DT, MT, FT and DMFT scores amongst all groups

No significant interaction was found between DT scores and salivary fluoride concentrations over (F(6.051-113.957)=1.014, P=0.420, partial Eta squared=0.051).

No significant interaction was found between MT scores and salivary fluoride concentrations (F(3.024-116.923)=0.424, P=0.737, partial Eta squared=0.011).

No significant interaction was found between FT scores and salivary fluoride concentrations (F(14.108-105.813)=0.424, P=0.438, partial Eta squared=0.055).

No significant interaction was found between DMFT scores and salivary fluoride concentrations (F(18.126-101.706)=0.424, P=0.416, partial Eta squared=0.069).

Table 5.6-1 Summary descriptive statistics of DT, MT, FT and DMFT scores.

Score	Mean ± SD	Min. value	Max. value
DT	0.77 ± 2.03	0	17
MT	0.17 ± 0.87	0	9
FT	3.81 ± 0.87	0	20
DMFT	4.74 ± 5.18	0	30

5.6.2 Descriptive statistics of DT, MT, FT and DMFT scores for individual groups

Table 5.6-2 Descriptive statistics of DT, MT, FT and DMFT for individual groups.

Group Number.	DT Mean ± SD	MT Mean ± SD	FT Mean ± SD	DMFT Mean ± SD
G01	0.10 ± 0.32	0.10 ± 0.32	3.20 ± 4.54	3.40 ± 4.62
G02	1.30 ± 1.77	0.10 ± 0.32	5.60 ± 4.25	7.00 ± 5.66
G03	1.90 ± 3.03	0.10 ± 0.32	3.70 ± 5.08	5.70 ± 7.72
G04	0.30 ± 0.68	0.00 ± 0.00	4.70 ± 3.43	5.00 ± 3.37
G05	0.40 ± 1.27	0.10 ± 0.32	2.80 ± 2.97	3.30 ± 3.86
G06	0.10 ± 0.32	0.10 ± 0.32	2.60 ± 3.20	2.80 ± 3.23
G07	2.10 ± 5.38	0.00 ± 0.00	4.90 ± 3.60	7.00 ± 6.45
G08	0.30 ± 0.68	0.00 ± 0.00	2.80 ± 8.62	3.10 ± 3.35
G09	0.30 ± 0.68	0.90 ± 2.85	5.80 ± 6.22	7.00 ± 8.86
G10	0.70 ± 1.06	0.20 ± 0.42	2.60 ± 3.31	3.50 ± 3.66
G11	1.30 ± 1.57	0.30 ± 0.68	3.80 ± 4.42	5.40 ± 4.40
G12	0.40 ± 0.52	0.10 ± 0.32	3.20 ± 2.35	3.70 ± 2.75

Table 5.6-3 Minimum and maximum values of DT, MT, FT and DMFT for individual groups.

Group Number.	DT		MT		FT		DMFT	
	Min. value	Max. value	Min. value	Max. value	Min. value	Max. value	Min. value	Max. value
G01	0	1	0	1	0	15	0	15
G02	0	4	0	1	0	12	0	15
G03	0	10	0	1	0	14	0	24
G04	0	2	0	0	0	9	0	9
G05	0	4	0	1	0	8	0	11
G06	0	1	0	1	0	9	0	9
G07	0	17	0	0	0	12	0	22

Group Number.	DT		MT		FT		DMFT	
	Min. value	Max. value	Min. value	Max. value	Min. value	Max. value	Min. value	Max. value
G08	0	2	0	0	0	9	0	11
G09	0	2	0	9	0	20	0	30
G10	0	3	0	1	0	10	0	11
G11	0	4	0	2	0	14	0	14
G12	0	1	0	1	0	8	0	10

5.7 DMFS score

5.7.1 Descriptive statistics of DS, MS, FS and DMFS scores amongst all groups

No significant interaction was found between DS scores and salivary fluoride concentrations (F(8.067-111.924)=0.719, P=0.675, partial Eta squared=0.049).

No significant interaction was found between MS scores and salivary fluoride concentrations (F(14.108-115.914)=0.325, P=0.861, partial Eta squared=0.011).

No significant interaction was found between FS scores and salivary fluoride concentrations (F(23.155-96.647)=0.763, P=0.767, partial Eta squared=0.155).

No significant interaction was found between DMFS scores and salivary fluoride concentrations (F(27.207-92.706)=0.553, P=0.959, partial Eta squared=0.140).

Table 5.7-1 Summary descriptive statistics of DS, MS, FS and DMFS scores.

Score	Mean ± SD	Min. value	Max. value
DS	0.91 ± 2.74	0	24
MS	0.81 ± 4.19	0	43
FS	6.33 ± 9.58	0	79
DMFS	8.05 ± 13.66	0	123

5.7.2 Descriptive statistics of DS, MS, FS and DMFS scores for individual groups

Table 5.7-2 Descriptive statistics of DS, MS, FS and DMFS for individual groups.

Group Number	DS Mean ± SD	MS Mean ± SD	FS Mean ± SD	DMFS Mean ± SD
G01	0.10 ± 0.32	0.50 ± 1.58	4.10 ± 6.14	4.70 ±6.50
G02	1.50 ± 2.17	0.50 ± 1.58	12.70 ± 10.90	14.70 ± 12.60
G03	2.20 ± 3.94	0.50 ± 1.58	6.30 ± 9.83	9.00 ± 13.98
G04	0.30 ± 0.68	0.00 ± 0.00	7.40 ± 5.48	7.70 ± 5.38
G05	0.40 ± 1.27	0.50 ± 1.58	3.50 ± 3.95	4.40 ± 5.54
G06	0.10 ± 0.32	0.50 ± 1.58	3.80 ± 5.20	4.40 ± 5.32
G07	2.80 ± 7.55	0.00 ± 0.00	8.90 ± 9.01	11.70 ± 13.51
G08	0.30 ± 0.68	0.00 ± 0.00	3.30 ± 3.23	3.60 ± 3.57
G09	0.40 ± 0.97	4.30 ± 13.60	12.40 ± 23.91	17.10 ± 37.52
G10	0.70 ± 1.06	1.00 ± 2.11	3.60 ± 4.58	5.30 ± 6.27
G11	1.70 ± 2.54	1.40 ± 3.27	5.20 ± 6.51	8.30 ± 8.04
G12	0.40 ± 0.52	0.50 ± 1.58	4.80 ± 4.76	5.70 ± 6.33

Table 5.7-3 Minimum and maximum values of DS, MS, FS and DMFS for individual groups.

Group Number.	DS		MS		FS		DMFS	
	Min. value	Max. value	Min. value	Max. value	Min. value	Max. value	Min. value	Max. value
G01	0	1	0	5	0	20	0	20
G02	0	6	0	5	0	33	0	33
G03	0	13	0	5	0	30	0	43
G04	0	2	0	0	0	14	0	14
G05	0	4	0	5	0	12	0	16
G06	0	1	0	5	0	15	0	15
G07	0	24	0	0	0	30	0	41

Group Number.	DS		MS		FS		DMFS	
	Min. value	**Max. value**	**Min. value**	**Max. value**	**Min. value**	**Max. value**	**Min. value**	**Max. value**
G08	0	2	0	0	0	9	0	11
G09	0	3	0	43	0	79	0	123
G10	0	3	0	5	0	14	0	19
G11	0	8	0	10	0	20	0	24
G12	0	1	0	5	0	16	0	22

Chapter 6 **Data Analysis and Results II**

Missing data analysis

6.1 Introduction

Before data analysis of salivary fluoride concentrations was carried out, missing data were replaced by multiple imputations. IBM SPSS 23 software was used to perform this analysis.

6.2 Missing values analysis

There were 4 missing values of salivary fluoride concentration from two participants due to technical and / or human error:

Table 6.2-1 Missing salivary fluoride concentration values. Missing values distribution

Participation number	Number of missing samples	Group	Time intervals missing					
			Pre-brushing	1 min	15 min	30 min	60 min	90 min
Participant A ID 27	3	G12	√				√	√
Participant B ID 66	1	G11				√		

Before replacement of missing data, pattern analysis was performed to investigate whether the missing data followed a certain pattern or a random arrangement.

The percentage of missing values throughout the entire samples was 0.556%.

The following bar chart (Figure 6-1) is called the patterns frequencies graph. This graph shows that the first pattern, the one in which no missing values present across all variables, is the most prevalent. The other patterns are much less prevalent but are roughly equal.

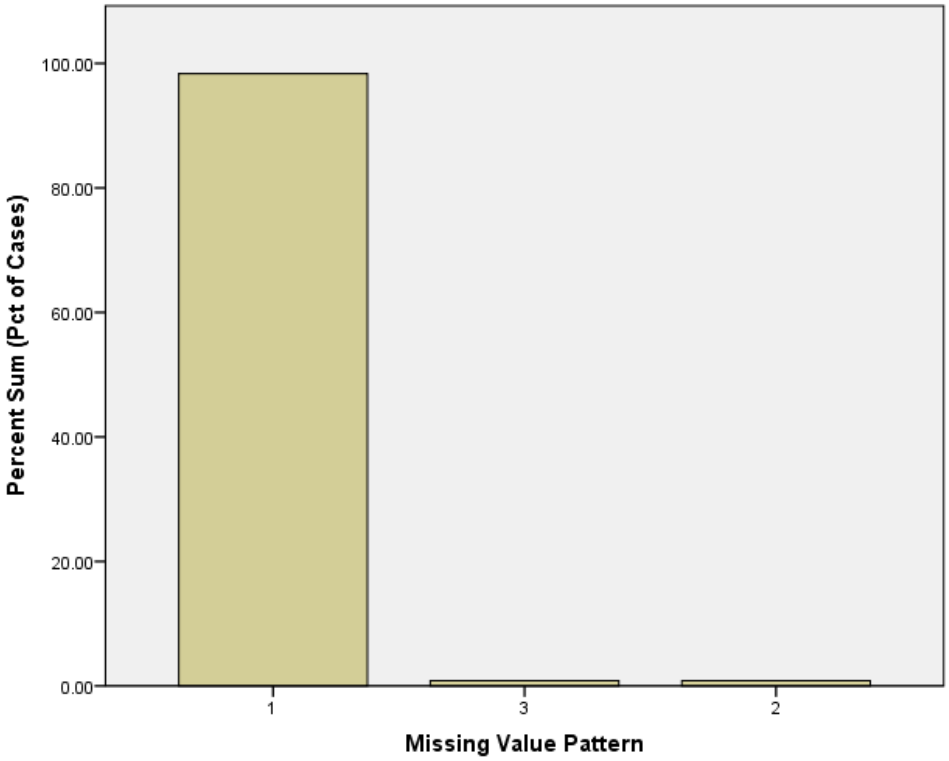


Figure 6-1 Patterns of frequencies graph.

In light of the above, it is concluded that the missing values in this research study followed a random arrangement.

Replacement of missing data was performed by multiple imputations of missing data.

Chapter 7 **Data Analysis and Results III**

Difference between fluoride formulations analysis

7.1 Introduction

Two-way mixed ANOVA with Tukey's post-hoc test and Bonferroni correction were used for the data analysis of fluoride concentrations within the different groups at the different time intervals.

Data analysis of samples was performed using IBM SPSS 23 software.

Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, approximate chi-squared value =2635.751, p <0.0005. Therefore, estimates from Greenhouse-Geisser corrections were used to assess the interaction between the time and the group number.

There was a statistically significant two-way interaction between the group number and the time on the salivary fluoride concentration, F(11.157-109.541)=11.700, P<0.0005, partial Eta squared= 0.544. This means that the salivary fluoride concentration changed significantly over time depending on which group number the participants were in.

Pairwise comparisons test with Bonferroni adjustment was performed to test where the difference of the salivary fluoride concentrations between the time intervals lies. Salivary fluoride concentrations at baseline were statistically significantly different than at 1, 15, 30 and 60 time intervals (P < 0.0005). No statistically significant difference was found between baseline salivary fluoride concentrations and at 90 minutes time interval (P = 1.000). Salivary fluoride concentrations were significantly different between 1, 15, 30, 60 and 90 minutes time intervals.

For full descriptive statistics of means (M), standard deviations (SD) and confidence intervals (CI) of the salivary fluoride concentrations, please refer to Appendix D.1.

The codes were kept safe in a locked cabinet with the research assistant and were revealed after the data analysis stage and write-up stage (Appendix C.15).

Code breakers will be presented at the beginning of this chapter to aid in data analysis.

7.2 Code breaker

Table 7.2-1 Groups code breaker.

Rinsing method	Group	Toothpaste formulation
Rinsing groups post-brushing	G02	Amine fluoride
	G03	Sodium fluoride and sodium monofluorophosphate combined
	G05	Sodium monofluorophosphate
	G08	Sodium fluoride
	G11	Stannous fluoride and sodium fluoride combined
	G12	No fluoride
Non-rinsing groups post-brushing	G01	No fluoride
	G04	Amine fluoride
	G06	Sodium monofluorophosphate
	G07	Sodium fluoride and sodium monofluorophosphate combined
	G09	Sodium fluoride
	G10	Stannous fluoride and sodium fluoride combined

7.3 Two-way mixed ANOVA with Tukey's post-hoc test Non-rinsing groups

There was a statistically significant effect of time on salivary fluoride concentrations for all non-rinsing groups ($P < 0.0005$) except in G01 ($P=0.119$).

Please refer to Appendix D.2 for p-values tables of multiple pairwise comparisons between non-rinsing groups.

7.3.1 Comparison of salivary fluoride concentration at baseline between non-rinsing groups

There was no statistically significant difference in salivary fluoride concentrations between the non-rinsing groups, F(5,54)=2.066, P= 0.084, partial Eta squared=0.161.

7.3.2 Multiple comparisons of salivary fluoride concentration at 1 minute time interval between non-rinsing groups

Key

Highlighted cell	Denotes significant p-values
(-)	column group value < row group value
(+)	column group value > row group value

Table 7.3-1 Mean difference (column - row) between non-rinsing groups at 1 minute time interval.

	G01	G04	G06	G07	G09
G04	**-33.73**				
G06	**-12.74**	**+20.99**			
G07	**-18.09**	**+15.64**	**-5.34**		
G09	**-35.47**	**-1.74**	**-22.73**	**-17.38**	
G10	**-21.89**	**+11.84**	**-9.14**	**-3.80**	**+13.58**
Standard error = 5.49 ppmF					

7.3.3 Multiple comparisons of salivary fluoride concentration at 15 minutes time interval between non-rinsing groups

Table 7.3-2 Mean difference (column - row) between non-rinsing groups at 15 minutes time interval.

	G01	G04	G06	G07	G09
G04	**-2.73**				
G06	**-1.85**	**+0.88**			
G07	**-1.46**	**+1.27**	**+0.39**		
G09	**-3.27**	**-0.54**	**-1.42**	**-1.81**	
G10	**-1.00**	**+1.73**	**+0.85**	**+0.46**	**+2.27**
Standard error = 0.72 ppmF					

7.3.4 Multiple comparisons of salivary fluoride concentration at 30 minutes time interval between non-rinsing groups

Table 7.3-3 Mean difference (column - row) between non-rinsing groups at 30 minutes time interval.

	G01	G04	G06	G07	G09
G04	**-1.18**				
G06	**-0.50**	**+0.68**			
G07	**-0.33**	**+0.85**	**+0.17**		
G09	**-0.75**	**+0.43**	**-0.25**	**-0.42**	
G10	**-0.23**	**+0.94**	**+0.27**	**+0.10**	**+0.52**
Standard error = 0.23 ppmF					

7.3.5 Multiple comparisons of salivary fluoride concentration at 60 minutes time interval between non-rinsing groups

Table 7.3-4 Mean difference (column - row) between non-rinsing groups at 60 minutes time interval.

	G01	G04	G06	G07	G09
G04	**-0.46**				
G06	**-0.14**	**+0.32**			
G07	**-0.11**	**+0.35**	**-0.03**		
G09	**-0.26**	**+0.20**	**-0.12**	**-0.15**	
G10	**-0.08**	**+0.38**	**+0.06**	**+0.03**	**+0.18**
Standard error = 0.08 ppmF					

7.3.6 Multiple comparisons of salivary fluoride concentration at 90 minutes time interval between non-rinsing groups

Table 7.3-5 Mean difference (column - row) between non-rinsing groups at 90 minutes time interval.

	G01	G04	G06	G07	G09
G04	**-0.28**				
G06	**-0.07**	**+0.21**			
G07	**-0.06**	**+0.22**	**+0.01**		
G09	**-0.12**	**+0.17**	**-0.04**	**-0.05**	
G10	**-0.03**	**+0.25**	**+0.04**	**+0.03**	**+0.09**
Standard error = 0.05 ppmF					

7.3.7 Comparison plot between non-rinsing groups

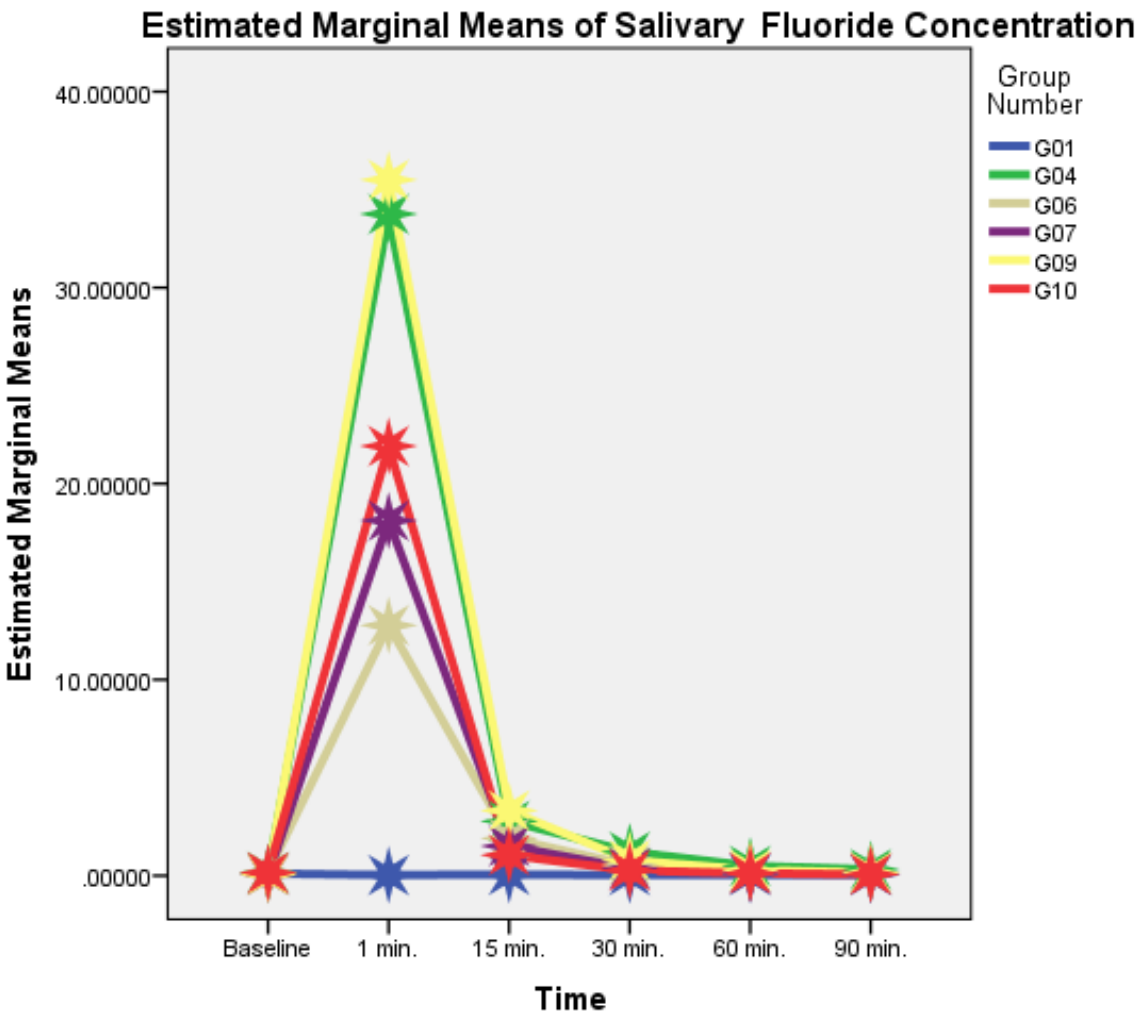


Figure 7-1 Estimated marginal means of salivary fluoride concentrations (ppmF) between non-rinsing groups.

7.4 Two-way mixed ANOVA with Tukey's post-hoc test: Rinsing groups

There was a statistically significant effect of time on salivary fluoride concentrations for all rinsing groups ($P < 0.0005$).

Please refer to appendix D.3 for p-values of multiple pairwise comparisons between rinsing groups.

7.4.1 Comparison of salivary fluoride concentration at baseline between rinsing groups

There was no statistically significant difference in salivary fluoride concentrations between the non-rinsing groups, $F(5,54)=1.589$, $P= 0.179$, partial Eta squared=0.128.

7.4.2 Multiple comparisons of salivary fluoride concentration at 1 minute time interval between rinsing groups

Key

Highlighted cell	Denotes significant p-values
(-)	column group value < row group value
(+)	column group value > row group value

Table 7.4-1 Mean difference (column - row) between rinsing groups at 1 minute time interval.

	G02	G03	G05	G08	G11
G03	**+4.58**				
G05	**+7.90**	**+3.31**			
G08	**+1.76**	**-2.82**	**-6.13**		
G11	**-0.85**	**-5.43**	**-8.73**	**-2.61**	
G12	**+16.83**	**+12.25**	**+8.94**	**+15.07**	**+17.67**
Standard error = 3.31 ppmF					

7.4.3 Multiple comparisons of salivary fluoride concentration at 15 minutes time interval between rinsing groups.

Table 7.4-2 Mean difference (column - row) between rinsing groups at 15 minutes time interval.

	G02	G03	G05	G08	G11
G03	+0.29				
G05	+0.78	+0.49			
G08	-0.05	-0.35	-0.83		
G11	-0.60	-0.89	-1.38	-0.54	
G12	+1.61	+1.32	+0.83	+1.66	+2.21
Standard error = 0.46 ppmF					

7.4.4 Multiple comparisons of salivary fluoride concentration at 30 minutes time interval between rinsing groups.

Table 7.4-3 Mean difference (column - row) between rinsing groups at 30 minutes time interval.

	G02	G03	G05	G08	G11
G03	+0.12				
G05	+0.30	+0.18			
G08	+0.1	-0.01	-0.19		
G11	+0.05	-0.07	-0.26	-0.06	
G12	+0.54	+0.42	+0.24	+0.43	+0.49
Standard error = 0.14 ppmF					

7.4.5 Multiple comparisons of salivary fluoride concentration at 60 minutes time interval between rinsing groups

Table 7.4-4 Mean difference (column - row) between rinsing groups at 60 minutes time interval.

	G02	G03	G05	G08	G11
G03	**+0.13**				
G05	**+0.21**	**+0.08**			
G08	**+0.10**	**-0.03**	**-0.12**		
G11	**+0.14**	**+0.01**	**-0.07**	**+0.04**	
G12	**+0.28**	**+0.16**	**+0.08**	**+0.18**	**+0.14**
Standard error = 0.08 ppmF					

7.4.6 Multiple comparisons of salivary fluoride concentration at 90 minutes time interval between rinsing groups

Table 7.4-5 Mean difference (column - row) between rinsing groups at 90 minutes time interval.

	G02	G03	G05	G08	G11
G03	**+0.07**				
G05	**+0.12**	**+0.04**			
G08	**+0.04**	**-0.04**	**-0.08**		
G11	**+0.10**	**+0.02**	**-0.02**	**+0.06**	
G12	**+0.15**	**+0.08**	**+0.04**	**+0.12**	**+0.06**
Standard error = 0.04 ppmF					

7.4.7 Comparison plot between rinsing groups

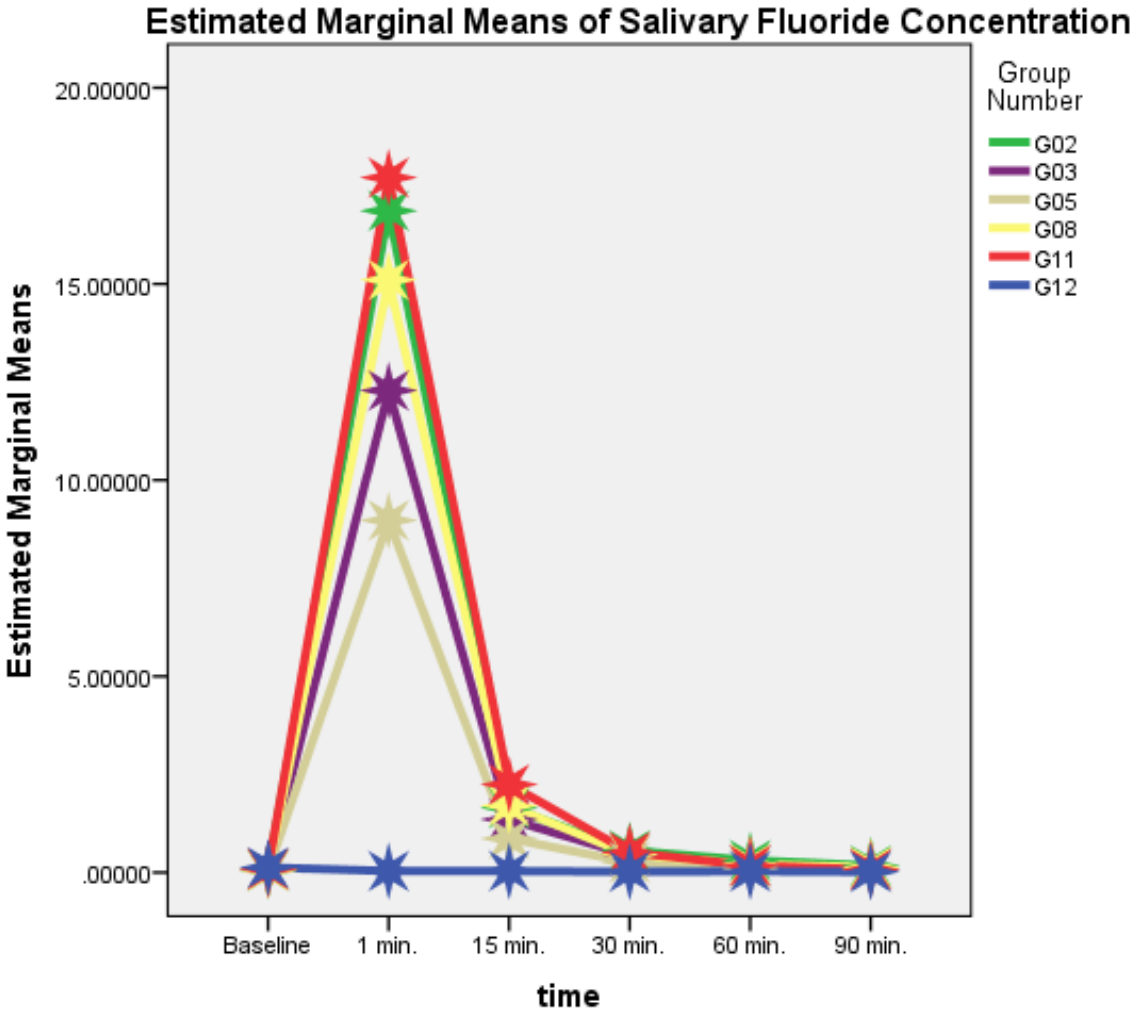


Figure 7-2 Estimated marginal means of salivary fluoride concentrations (ppmF) between rinsing groups.

7.5 Comparison plot between all groups

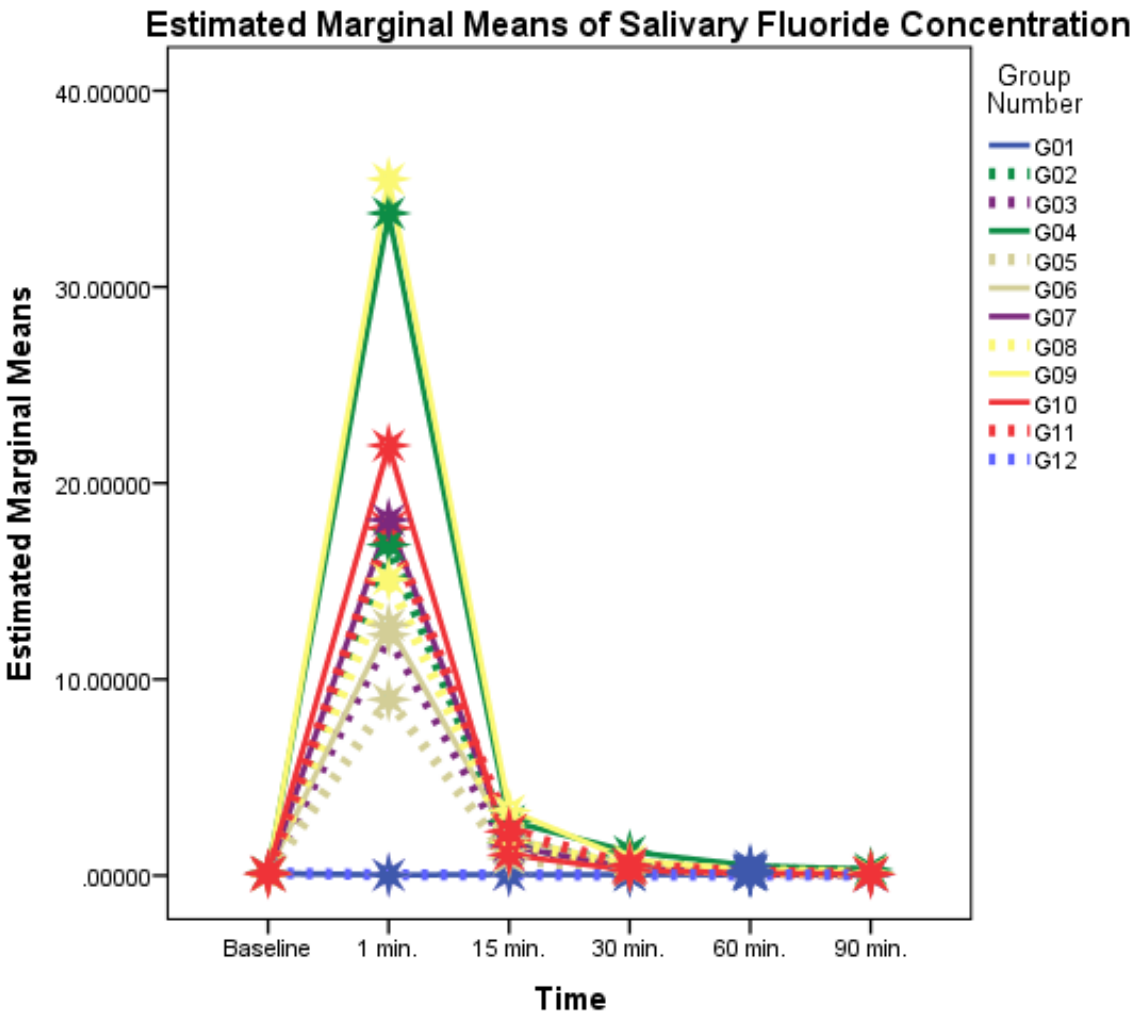


Figure 7-3 Estimated marginal means of salivary fluoride (ppmF) concentrations between all groups[1]

[1] Dotted-lines represent rinsing groups.

Solid-lines represent non-rinsing groups.

Chapter 8 **Data Analysis and Results IV**

Post-brushing rinsing method analysis

8.1 Introduction

Two-way mixed ANOVA test with Bonferroni correction was used for the data analysis of fluoride concentrations within individual groups comparing between rinsing and non-rinsing data.

Statistical analysis was perform using IBM SPSS 23 software.

Code breakers will be presented at the beginning of this chapter to aid in data analysis.

8.2 Code breaker

Table 8.2-1 Groups code breaker.

Rinsing method	Group	Toothpaste formulation
Rinsing groups post-brushing	G02	Amine fluoride
	G03	Sodium fluoride and sodium monofluorophosphate combined
	G05	Sodium monofluorophosphate
	G08	Sodium fluoride
	G11	Stannous fluoride and sodium fluoride combined
	G12	No fluoride
Non-rinsing groups post-brushing	G01	No fluoride
	G04	Amine fluoride
	G06	Sodium monofluorophosphate
	G07	Sodium fluoride and sodium monofluorophosphate combined
	G09	Sodium fluoride
	G10	Stannous fluoride and sodium fluoride combined

8.3 Control toothpaste

Salivary fluoride concentrations in non-rinsing groups were not statistically significantly higher than in rinsing groups (M=0.005, SE=0.026 ppmF, P=0.839).

8.4 Amine fluoride

Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, approximate chi-squared value =408.887, p <0.0005. Therefore, estimates from Greenhouse-Geisser corrections were used to assess the interaction between the time and the group number.

There was a statistically significant two-way interaction between the rinsing method and time on salivary fluoride concentrations, F(1.010-18.172)=7.348, P<0.0005, partial Eta squared= 0.290. This means that the salivary fluoride concentration changed significantly over time depending on the post-brushing rinsing method.

Salivary fluoride concentrations in non-rinsing groups were statistically significantly higher than in rinsing groups (3.189±1.24 ppmF, P=0.019).

Highlighted cell	Denotes significant p-values ≤ 0.005

Table 8.4-1 Comparison of salivary fluoride concentrations at all time intervals between rinsing and non-rinsing groups within AmF groups.

Time intervals	F (1,18)	P-value	Partial Eta squared
1 minute	7.268	0.015	0.288
15 minutes	3.395	0.082	0.159
30 minutes	3.133	0.169	0.102
60 minutes	1.614	0.220	0.082
90 minutes	3.040	0.098	0.144

8.5 Sodium fluoride

Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, approximate chi-squared value =470.249, p <0.0005. Therefore, estimates from Greenhouse-Geisser corrections were used to assess the interaction between the time and the group number.

There was a statistically significant two-way interaction between the rinsing method and time on salivary fluoride concentrations, F(1.010-18.185)=9.774, P=0.006, partial Eta squared= 0.352. This means that the salivary fluoride concentration changed significantly over time depending on the post-brushing rinsing method.

Salivary fluoride concentrations in non-rinsing groups were statistically significantly higher than in rinsing groups (3.74±1.24 ppmF, P=0.007).

Table 8.5-1 Comparison of salivary fluoride concentration at all time intervals between rinsing and non-rinsing groups within NaF groups.

Time intervals	F (1,8)	P-value	Partial Eta squared
1 minute	9.743	0.006	0.351
15 minutes	3.748	0.069	0.172
30 minutes	3.546	0.076	0.165
60 minutes	1.159	0.296	0.061
90 minutes	0.206	0.655	0.011

8.6 Sodium monofluorophosphate

Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, approximate chi-squared value =360.092, p <0.0005. Therefore, estimates from Greenhouse-Geisser corrections were used to assess the interaction between the time and the group number.

There was no statistically significant two-way interaction between the rinsing method and time on salivary fluoride concentrations, F(1.058-19.042)=2.996,

P=0.98, partial Eta squared= 0.143. This means that the salivary fluoride concentration did not change significantly over time depending on the post-brushing rinsing method.

Salivary fluoride concentrations in non-rinsing groups were statistically significantly higher than in rinsing groups (0.895±0.413 ppmF, P=0.044).

Table 8.6-1 Comparison of salivary fluoride concentration at all time interval between rinsing and non-rinsing groups within NaMFP groups.

Time intervals	F (1,8)	P-value	Partial Eta squared
1 minute	3.269	0.087	0.154
15 minutes	5.461	0.031	0.233
30 minutes	4.896	0.040	0.214
60 minutes	3.961	0.062	0.180
90 minutes	6.705	0.019	.271

8.7 Sodium monofluorophosphate and sodium fluoride combined

Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, approximate chi-squared value =425.447, p <0.0005. Therefore, estimates from Greenhouse-Geisser corrections were used to assess the interaction between the time and the group number.

There was no statistically significant two-way interaction between the rinsing method and time on salivary fluoride concentrations, F(1.011-18.197)=2.481, P=0.132, partial Eta squared= 0.121. This means that the salivary fluoride concentration did not change significantly over time depending on the post-brushing rinsing method.

Salivary fluoride concentrations in non-rinsing groups were not statistically significantly higher than in rinsing groups (M=0.976, SE=0.74 ppmF, P=0.201).

Table 8.7-1 Comparison of salivary fluoride concentration at all time interval between rinsing and non-rinsing groups within NaF+NaMFP groups.

Time intervals	F (1,8)	P-value	Partial Eta squared
1 minute	2.373	0.141	0.116
15 minutes	0.086	0.773	0.005
30 minutes	0.184	0.673	0.214
60 minutes	0.175	0.681	0.010
90 minutes	0.016	0.900	0.001

8.8 Stannous fluoride and sodium fluoride combined

Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way interaction, approximate chi-squared value =443.060, p <0.0005. Therefore, estimates from Greenhouse-Geisser corrections were used to assess the interaction between the time and the group number.

There was no statistically significant two-way interaction between the rinsing method and time on salivary fluoride concentrations, F(1.020-18.368)=0.970, P=0.339, partial Eta squared= 0.051. This means that the salivary fluoride concentration did not change significantly over time depending on the post-brushing rinsing method.

Salivary fluoride concentrations in non-rinsing groups were not statistically significantly higher than in rinsing groups (M=0.462, SE=0.861 ppmF, P=0.598).

Table 8.8-1 Comparison of salivary fluoride concentration at all time interval between rinsing and non-rinsing groups within NaF + SnF groups.

Time intervals	F (1,8)	P-value	Partial Eta squared
1 minute	0.786	0.387	0.042
15 minutes	3.844	0.066	0.176
30 minutes	4.122	0.057	0.186
60 minutes	1.935	0.181	0.097
90 minutes	0.195	0.664	0.011

8.9 Comparison plot between rinsing and non-rinsing groups

Overall, salivary fluoride concentrations in non-rinsing groups were statistically significantly higher than in rinsing groups (1.545±0.475 ppmF, P=0.002).

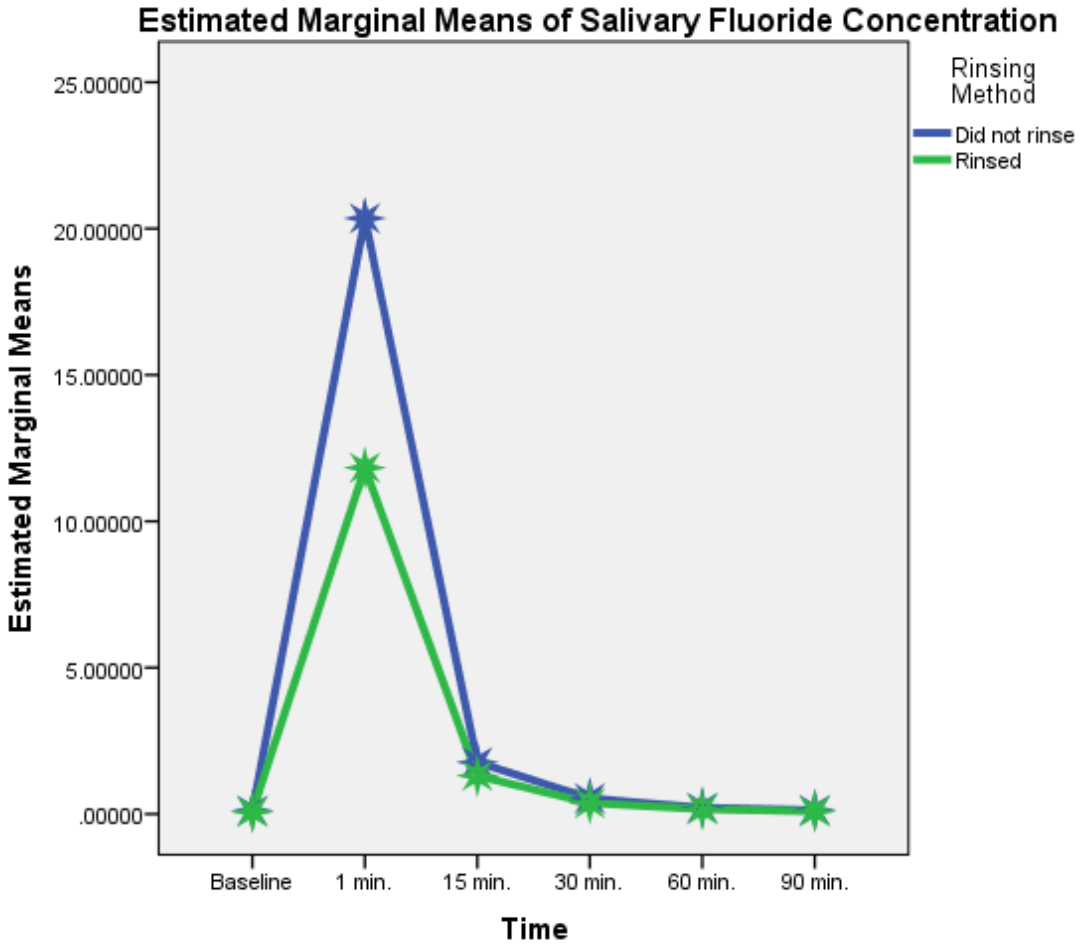


Figure 8-1 Estimated marginal mean of salivary fluoride concentration (ppmF) between rinsing and non-rinsing groups

Chapter 9 **Discussion**

9.1 Introduction

In this chapter, the present study is reviewed in a critical manner highlighting the strengths and the limitations and how improvements could be achieved. Discussion of reasons for performing specific designs and / or statistical tests are discussed.

This chapter will be concluded with a comparison between the results obtained from this trial and the published literature, and with suggested future research.

9.2 Literature review

Although the literature review in this study was performed in a systematic way, it could have been improved by conducting a systematic review. It is important that systematic reviews are performed by at least two independent assessors to increase the likelihood of error detection. First-time authors are encouraged to work with other authors who are experienced in the systematic review process. The Cochrane collaboration published a handbook to help authors to conduct good quality systematic reviews (Higgins and Green, 2011). It also provides training and online learning modules on their website (training.cochrane.org/).

9.3 Title, abstract, aims and objectives

The title of this research study was formulated according to the PICO format to summarise the aim(s) of the study, the targeted population, both the control and the comparison intervention(s) and the specific and measureable primary and secondary objectives.

The type of the study (Double-blinded randomised controlled trial) has been identified in the title.

The abstract gives a structured summary of the trial design, methods, results, and conclusions.

The aim of the study was clear. Objectives and hypothesis were specific. The study assumed null hypotheses; in that no significant differences were assumed between the studied formulations / rinsing regimens.

9.4 Randomised controlled trials versus crossover studies

A double-blinded randomised controlled trial design was conducted, as it is the most accurate way to compare between different interventions and their effects. It is an effective method that allows the establishment of a counterfactual. It might not be possible to exclude every single confounding factor in this study as remineralisation and demineralisation are both dynamic processes and the concentration of fluoride is constantly changing. Limiting the confounding factors would also limit the generalisability of the results; thus weakening the power of study. The study maintained individual-related variations but controlled both the concentration and the amount of the fluoridated toothpastes used.

Although many previous studies have adopted a crossover design this study was designed differently. Crossover design would control individual variations, which would control a greater range of known and unknown confounding factors; it would also limit the generalisation of the results as discussed above.

The number of study arms being investigated would determine the number of appointments each participant was to be seen, thus would impact on the drop-out rate. The chance of participants dropping out of the study would increase with the number of products being investigated. Limiting the number of visits minimises the amount of missing data in the clinical research due to drop-outs (Kang, 2013).

A randomised controlled trial would allow a greater sample size to be collected and reduce the drop-out rate since each participant would be seen once only.

9.5 Simple randomisation versus stratified randomisation

To achieve equal distribution of data amongst the different groups, stratified randomisation could have been considered. Simple radomisation, used in

this design, would explain why groups were not balanced in terms of the prognostic variables (i.e. age, gender, calculus and caries).

To perform stratified distribution, 120 participants would need to be recruited first and then randomly distributed to the groups. It means that participants needed to wait for at least 9 months prior to the commencement of the study. There was a chance of participants dropping out for several reasons (i.e. graduation, exams, etc.) negatively impacting on the sample size.

9.6 Blinding of the groups

The chief investigator was blinded to the toothpaste formulation and the rinsing methods used. Participants were blinded to the toothpaste formulation but were aware of the rinsing methods of their groups. The statistical analysis was performed and rechecked by both the chief investigator and the statistician who was also blinded to the toothpaste formulation and the rinsing methods used.

Therefore, by double-blinded, the author of this thesis refers to both the chief investigator and the statistician.

Participants were clearly instructed not to disclose the rinsing method to the chief investigator. Blinding of participants was also important in this research to ensure that they did not disclose the type of toothpaste to the chief investigator.

9.7 Study settings

The study was performed at the DenTCRU clinic with the presence of a research assistant present during all clinical sessions.

Participants remained in the clinic during the study under direct supervision of the research staff to ensure adherence to instructions.

Brushing time and sample collection was timed using a count-down timer by either of the research staff to ensure that the conditions were as controlled as possible.

The time intervals were calculated using two different ways to decrease chances of error. Five chair-side timers were started simultaneously as soon as brushing ended to ensure timing was calculated as accurately as possible.

The brushing start time was also recorded and the expected times of the sample collection were recorded in each participant's CRF form. Dental digital clocks for each chair were used to ensure accuracy of timing. Participants completed the study in the same chair throughout the appointment (Figure 3-3).

Each toothpaste was dispensed using a spatula for more controllability over the dispensed amounts. A scale was used to measure an amount of 1.0 g of toothpaste. Measurement of the weights of the toothbrushes prior and after toothpaste dispensing were taken to increase accuracy of measurements.

The amount of distilled water used for post-brushing rinsing was measured using a pipette rather than a graduated beaker as to reduce human error (i.e. if a beaker was used the amount of water would differ depending on the level of the beaker to the eye of the examiner).

The rinsing was also timed using a digital count-down timer and a separate rinsing log was kept which was only accessible by the research assistant. This was checked by the chief investigator following the statistical analysis stage to confirm adherence to the protocol.

Labelling of sample collection tubes and test tubes followed both a colour coded system and word labelling. A colour-coded chart was produced and was part of the dental chair set-up for each participant.

Dental chair set-up was performed by both the chief investigator and the research assistant to reduce chances of human error.

9.8 Materials under investigation

The experimental materials investigated were taken from the list of available brands in United Kingdom published by the Department of Health and British Association for the Study of Community Dentistry Toolkit (2017) - Delivering better oral health: an evidence-based toolkit for prevention. Amine fluoride toothpaste is not available in the United Kingdom in major pharmacies and stores but it is available in some department store companies such as Harrods and Harvey Nichols stores. Amine fluoride toothpastes was not included in the list published by the Department of Health and British Association for the Study of Community Dentistry (2017). Amine fluoride formula was included in this research study as previously conducted studies

suggested it resulted in significantly high salivary fluoride concentrations following toothbrushing when compared to sodium fluoride and sodium monofluorophosphate.

In the UK, R.O.C.S® toothpaste contains a combination of amine fluoride and xylitol and could be found in several department store companies.

All toothpastes, except for one, had similar colour. One of the toothpastes had a blue gel-like texture with white abrasive particles. It is important to note that the chief investigator remained blinded to the type of toothpastes, until the codes were broken.

Each participant brushed with 1.0 gram of toothpaste. The addition of large particles could potentially mean that the particles weight was part of the final toothpaste weight (1 g). Meaning that 1 g of different toothpastes contained different amounts of fluoride. The amount of fluoride in 1 gram of toothpaste can also be affected by the formulation used (i.e. 1 molecule of sodium fluoride has 1 fluoride atom while a molecule of stannous fluoride has 2 fluoride atoms) and the molecular weight of the compound (refer to section 1.7 in chapter 1).

A toothpaste tube has a combination of other ingredients such as flavorings, humectants, abrasives and detergents. The interaction between all of those factors with the fluoride formula in the toothpaste can affect the behaviour of the toothpaste formulation in the oral environment.

9.9 Sample size calculation

As recommended by the Yorkshire and The Humber – Sheffield Research Ethics Committee, power calculations were performed without using the data from the control groups. Calculations suggested that at least 3 subjects for each group were required to achieve 100% power.

The number of the participants we aimed to recruit was a multiplication of the number of the arms of the study. This was to ensure the probability of all toothpastes being used equally, thus reducing potential bias.

The sample resembled the population in terms of caries risk and oral hygiene. This study was not restricted to only those with low caries risk and good oral hygiene otherwise the results would only be applicable to the participants of the study. It has been shown previously, however, that there

were no significant differences in salivary fluoride levels between caries-free and caries-prone children (Nazzal et al., 2016).

9.10 Publicity and recruitment of participants

Circular emails were short and the subject line included a short description of the study followed by the word “circular”. Subject line: Participants needed for tooth-brushing – Circular.

The email started with:

'Circular email for use for recruitment of participants for study ref: REC 16/YH/0015, approved by Yorkshire and The Humber – Sheffield Research Ethics Committee.

This project contributes to the University's role in conducting research, and teaching research methods. You are under no obligation to reply to this email, however if you choose to, participation in this research is voluntary and you may withdraw at any time. Please see attached for more details.

A copy of the recruitment flyer, the information sheet and consent sheet were attached to the circular email.

9.11 Participant information sheet and informed consent

The information sheet could be improved by inclusion of both inclusion and exclusion criteria.

Participants were given the information sheet at least 48 hours prior to their appointments to aid in the informed decision making. A separate log was issued confirming dates of which participation sheets were given and date of informed consent. The CRF was also designed to ensure adherence to the protocol.

9.12 Salivary fluoride analysis

The majority of the previously conducted salivary clearance studies analysed fluoride ion concentration in the saliva samples with addition of a low-level TISAB II and the use of ion-specific electrode (Hirose et al., 2015; Nazzal et al., 2016; Issa and Toumba, 2004; Campus, et al., 2003; Duckworth and Morgan, 1991). Campus et al. (2003) added to the samples, additional to

low-level TISAB, 5 ml of distilled water prior to measurement. No explanation was provided on why this method was followed.

The only study, in the literature, that used gas chromatography was Bruun et al. (1984). Samples were gently centrifuged (7g / 5 minutes) to remove abrasive materials prior to sample freezing (-18 degree Celsius).

There is no standardised protocol for fluoride analysis. Methods of fluoride analysis include mass spectrometry, gas chromatography, ion chromatography, electrolysis, catalytic-enzymatic and radioanalytical methods (Venkateswarlu, 1994). According to Martinez-Mier et al. (2011), the most commonly used methods for fluoride analysis are gas chromatography, ion chromatography and fluoride ion-selective electrode.

In the present research study, gas and ion chromatography machines were not available for use; a fluoride ion-specific electrode was therefore used for sample analysis.

Colourimetric methods are more time consuming and less accurate compared to the other methods (Agency of Toxic Substances and Disease Registry, 2015).

The gas chromatography method is more sensitive as it is able to detect nanogram quantities of fluoride. Unlike the fluoride ion-selective electrode method, gas chromatography can detect both free and bound fluoride ions (Agency of Toxic Substances and Disease Registry, 2015).

The electrode measures the potential that corresponds to the fluoride ion activity in the solution (Martinez-Mier et al., 2011). This method is simple, sensitive and rapid. Hydroxyl ions could cause significant interference with the electrode fluoride analysis, the pH of the solution analysed is therefore adjusted to approximately 5 to eliminate interference. The addition of TISAB is used to adjust samples and standards to the same ionic strength and pH, allowing the concentration rather than the activity to be measured (Agency of Toxic Substances and Disease Registry, 2015).

One of the most accurate methods for sample preparation is acid-hexamethyldisiloxane (HMDS) diffusion by Taves (1986). This method aims to free the fluoride from its organic and inorganic matrices (Agency of Toxic Substances and Disease Registry, 2015).

Only one reading per sample was taken and the reliability and the reproducibility of the measurements were performed by measuring freshly prepared standard fluoride solutions (0.01, 0.1, 1.0, 10, 100 and 1,000 ppmF). This method was time consuming as calibration was performed every two hours and when the measurements were different than the standard by 2%, recalibration was performed.
It would have been more preferable however, to take multiple readings per sample and use the averages in the statistical analysis.

9.13 Missing data

There are several ways to handle missing values during statistical analysis. One way is by dropping or omitting the data from being analysed. This applies when the number of missing values is less than 5% (Kang, 2013). This means there is a decrease in the overal sample size.
The other way is imputation and replacement of missing data by different methods such as series mean, mean of nearby points, median of nearby points, linear interpolation or linear trend at point (Kang, 2013).
Sensitivity analysis with the replaced missing values and without the replaced missing values remained unchanged for every outcome.

9.14 Demographic characteristics

The ratio of males to females in the population recruited in this study was 1: 1.9. The majority of the participants were students at the university of Leeds studying at different schools. According to the Complete University guide website (https://www.thecompleteuniversityguide.co.uk/leeds/), the ratio of males to females registered at the University of Leeds is 1:1.6. This ratio is comparable to the ratio of the participants in this study. This can potentially reflect on the representation of the participants recruited in the study in relation to the total population.
Another point worth noting, is confounding of lip sticks / lip balms of the results of the study. Many female participants attended wearing lipsticks and the saliva samples were coloured which could mean that traces of the lip sticks were mixed with the collected saliva samples. No evidence was found in the literature to reflect further on this.

Caries and calculus were not evenly distributed amongst the groups. One reason could have been that visible levels of caries and calculus were only recorded. There was no ethical nor clinical justification for radiographic examination for caries detection.

The distribution of caries and calculus in the sample of this research could also reflect the distribution of caries and calculus within the entire population. According to the latest Adult Health Survey (Steele and O'Sullivan, 2011), the prevalence of caries (using the natural tooth crowns as the measure) in England was 28%. This figure is comparable to the percentage of participants with clinically visible caries (29.2%) within this research study.

The Adult Health Survey (Steele and O'Sullivan, 2011) also states that about 68% of dentate adults had calculus in at least one sextant. This does not reflect the percentage of participants with clinically visible calculus (27.5%) within this research study. A possible reason for this could be under-diagnosis as the examination was visual only. No Basic Periodontal Probe was used to diagnose the presence of sub-gingival or minimal levels of calculus.

Participants with clinically visible caries and calculus were advised to see their general dental practitioners for further assessment.

9.15 Statistical analysis

The data did not follow a normal distribution and significant outliers were noticed across several time intervals. This could be simply explained by the normal individual variations.

There is no equivalent test to two-ways mixed ANOVA for non-parametric data. There are two ways on how we could have handled the data. The first, is to accept that the data are non-parametric and to proceed with statistical tests.

The other way is data transformation, bearing in mind that it does not always work. Data transformation means that every single value across all the variables would be transformed; meaning that statistical tests are not going to be performed on original data.

We have accepted individual variations of salivary fluoride concentrations and therefore no data transformation was conducted.

Two-way mixed-design analysis of variance test model (two-way mixed ANOVA) is used to test differences between two or more independent groups while subjecting participants to repeated measures. The aim of this test is to measure the interaction between the within-subjects factor and between-subjects factor on the dependent variable.

Applying this to the scenario of the design of this research, mixed ANOVA is used in studies were a dependent variable has been measured (i.e., salivary fluoride concentration) over two or more time points or all subjects have undergone two or more conditions (e.g., time), but also when the subjects have been assigned to two or more different groups (groups 01 – 12 have undergone different interventions). Explaining this further, the aim is to measure the interaction between the time (within-subjects factor) and the different groups conditions (between-subjects factor) on the salivary fluoride concentration (dependent variable).

Two-ways mixed ANOVA was also used to compare between rinsing and non-rinsing results within the individual formulations.

Performing multiple pair-wise comparisons would lead to a higher probability of making a type-I error. That is, increasing the likelihood of reporting significant difference between some of the pairs that have no real differences. This is relevant in the case of this research because the between-subject variable (group number) had more than two categories (12 groups).

These limitations could be overcome by multiple comparison analysis. Tukey's multiple comparison analysis method tests each experimental group(s) and control groups against each other. This method reduces probability of type-1 errors. Other tests for comparing multiple pairs include Newman-Keuls method. This method, however, is more liable for making type-I errors (McHugh, 2011).

Performing multiple comparisons (comparisons between the groups, time points and the interaction between the time and the group) will lead to wider confidence intervals. Bonferroni correction test is used to adjust the significance level in relation to the number of pairwise comparisons (0.05 is divided by (n); where n is the number of comparisons). The software IBM SPSS, however, applies a mathematically equivalent adjustments. The P-

value is adjusted in relation to the number of comparisons (i.e. P-value is multiplied by the number of comparisons, while significant level remains at 0.05).

9.16 Limitations of present research study

- Randomisation of the participant numbers (120 participants) to the groups prior to recruitment only limits the final recruited number to be exactly 120. The probability of randomly dividing 120 participants into 12 groups of size 10 was calculated using an online combination calculator (using the following mathematical equation):

$$\frac{(n!)}{m!\,(n-m)!}$$

Where:
n is the total number of the population.
m is number of subjects in each group.
To divide 120 participants into 12 groups of 10 the are 116068178638776 different combinations. So the probability of each participant being randomly assigned into one of the different groups would be 1 / 116068178638776.
The probability of one participant being randomly assigned to one of the 12 groups would be 1 / 12.
This method however, had the advantage of producing equal group sizes.
Stratified sample randomisation has the advantage of dividing the total sample into strata of similar statistical properties.
Randomisation of the toothpastes to the participants on the day of the experiment (i.e. manual or computer draw software) would allow recruitment of more than 120 participants but the groups might end up with unequal sizes.

- Ion selective electrode method was used for fluoride ion analysis. Although, this method is widely used and is considered acceptable for fluoride analysis, other methods could have been used such as gas or ion chromatography. These methods have higher sensitivity and the ability to detect both bound and free fluoride ions. This could

potentially aid in the detection of low salivary fluoride concentrations following brushing with NaMFP toothpastes.

- Taking only one measurement for each saliva sample rather than multiple (i.e. 3) could have been responsible for the non-parametric distribution of the data and the presence of multiple outliers. Although, this could also be explained by individual variations.

9.17 Comparison of the results with literature review

The results of this research study were comparable to the results of the previously conducted studies in that rinsing post-brushing resulted in significantly lower salivary fluoride concentrations when compared to the non-rinsing groups (Nazzal et al., 2016; Issa and Toumba, 2004; Campus et al., 2003). This supports the recommendation of no rinsing post-brushing (Department of Health and British Association for the Study of Community Dentistry, 2017; Scottish Intercollegiate Guidelines Network, 2014).

Previously conducted studies concluded that AmF toothpaste resulted in significantly higher levels of salivary fluoride when compared to NaF and NaMFP. The present research agrees that AmF resulted in significantly higher salivary fluoride concentration for the longest period of time (90 minutes), for both rinsing and non-rinsing groups when compared to control groups (Issa and Toumba, 2004; Campus et al, 2003; Attin and Hellwig, 1996). This could be explained by the alignment of AmF as the hydrophilic part is arranged closely to the enamel of the tooth, while the hydrophopic part is arranged on the outside (Priyadarshini et al., 2013).

All fluoridated toothpastes were associated with higher salivary fluoride concentrations when compared to control groups at the one minute time interval, except for NaMFP toothpaste (Issa and Toumba, 2004; Attin and Hellwig, 1996).

Hirose et al. (2015) suggested that significant difference was found between NaMFP and NaF in terms of salivary fluoride concentration following rinsing post-brushing. The results of this study agree that a significant difference was only found at the one minute time interval for the non-rinsing groups. No significant difference was found between the two formulations within the

rinsing groups. This could be due to differences in sample size and study design between the present study and that of Hirose et al. (2015).

The results of this study are in agreement with Nazzal et al. (2016), in that no significant differences in the salivary fluoride concentrations were found between caries-free and caries-prone participants.

Issa and Toumba reported that salivary fluoride concentrations with NaF toothpaste remained high at 120 minutes. However, results were not reported in terms of significant difference.

9.18 Future research

Salivary clearance studies test the performance of a single topical application of a fluoridated product (i.e. toothpastes) over a limited period of time (usually hours). On the other hand, equilibrium studies, as discussed by Duckworth and Morgan (1991), test the performance of toothpastes over a repeated and regular use.

Previously conducted studies reports that salivary fluoride concentrations return to baseline by 180 minutes. Conducting similar salivary fluoride clearance studies is less likely to provide new information unless further fluoride compounds are incorporated into toothpastes (i.e. calcium fluoride). Equilibrium studies are advised to be considered in future research, but it is unknown if they are likely to provide new information or evidence in this field.

Based on the results of this research study and the literature review, to maintain high constant fluoride levels in the oral environment, it would be advised that people brush three-hourly. This is of course unrealistic and would be considered by a lot of individuals as not practical. Further prospective-type research needs to be conducted to assess the frequency of toothbrushing and its clinical significance in term of caries prevention. Ethical issues and confounding factors should be considered while planning such research designs.

An alternative method for frequent brushing would be the use of slow-releasing fluoride devices (SRFD). One of the major limitations of this technology was the low retention rate but this has now been resolved with the latest devices (Pessan et al, 2008). SRFD's are a promising technology

but the clinical effectiveness in relation to caries prevention needs to be supported with research of high quality.

Perhaps the concept of a toothpaste needs to be updated. Several dermatological companies manufacture night-time creams which are heavier in texture compared to day-time creams. A toothpaste that is high in viscosity (i.e. varnish) but have the licensed amount of fluoride concentration can potentially coat the teeth for a longer period of time when compared to a regular toothpaste. This would also reflect on the salivary fluoride clearance. Having a layer of a heavy toothpaste adhered to teeth would not be considered as aesthetic, which could be a down-side for such a suggestion. A night-time toothpaste could potentially be manufactured but this means people would have more than one toothpaste which can be confusing to some and maybe less affordable to others. In theory a night-time toothpaste would also redefine the practice of brushing. Toothbrushing aids in disturbing the plaque film, but with a night-time toothpaste, the plaque film would possibly be replaced by a toothpaste film for a limited period of time.

A higher viscosity toothpaste, when compared to a regular toothpaste, could possibly be less efficient at penetrating interproximal tooth surfaces (i.e below contact points).

Table 10-1 shows the salivary fluoride concentrations at 1 minute time interval for non-rinsing groups compared to the original toothpaste concentration.

Table 9.18-1 Percentage of salivary fluoride concentration in relation to the toothpaste concentration at 1 minute interval for non-rinsing groups.

Group No.	Toothpaste formulation	Toothpaste concentration (ppmF)	Mean salivary fluoride concentration (ppmF)	Percentage of mean salivary fluoride concentration in relation to toothpaste concentration (%)
G04	AmF	1,400	33.760	2.41
G06	NaMFP	1,450	12.775	0.88
G07	SnF + NaF	1,450	18.118	1.2
G09	NaF	1,450	35.500	2.44
G10	NaF + MFP	1,450	21.919	1.5

Hydrolysis is the process of water breaking down the substance into its ionic form. Table (10-1) shows that 1.0 gram weight of AmF or NaF toothpastes would result in 2.4% of their initial toothpaste concentration at 1 minutes time interval. For other NaF+SnF and NaF+NaMFP combined formulations, 1.0 gram of the toothpaste resulted in 1.2 and 1.5% of toothpastes concentrations respectively. The sodium fluoride concentration (NaF) in SnF + NaF toothpaste and NaMFP + NaF is 450 ppmF and 350 ppmF respectively.

It is interesting that 1.0 g of NaMFP (1,450 ppmF) released less fluoride ions at the one minute time interval compared to the same weight of combined NaF+NaMFP toothpaste (NaMFP concentration is 1,100). This means that the initial release of fluoride is more likely to be due to the rapid hydrolysis of sodium fluoride compound in the combined NaF+NaMFP.

It could be concluded that AmF and NaF perform faster hydrolysis when compared to SnF and NaMFP compounds. This does not support Bruun et al. (1984) who concluded that within the first 10 minutes NaMFP toothpaste resulted in a gradual increase in fluoride ions and explained this by the rapid hydrolysis of the compound. On the contrary, Hirose et al. 2015 showed that NaF toothpaste resulted in higher salivary fluoride concentrations than NaMFP. It is important to highlight that Bruun et al. (1984) used gas chromatography for ion analysis while Hirose et al. (2015) used a fluoride ion-selective electrode. So differences could be due to different study designs, saliva sample preparation and fluoride analysis techniques.

This would raise several argument points with regard to toothbrushing practices:

- Does the limited release of fluoride ions from highly concentred toothpastes reflect on the limited contact with saliva?
- How does the hydrolysis process of the toothpaste in saliva compare to water?
- Would water-wetting the toothpaste prior to brushing initiate the hydrolysis process and results in higher fluoride ions being released?
- Would the shape of the dispensed toothpaste affect the hydrolysis process; thus reflecting on the salivary fluoride concentrations (i.e.

smear layer provides a larger surface area for interaction with water / saliva)?

- Would the duration of the brushing affect the amount of saliva breaking down toothpaste compounds releasing more fluoride ions?

And the most important argument is, whether any of the previous practices are likely to have a clinically significant effect in term of caries prevention.

Chapter 10 **Conclusions**

10.1 Rejected hypotheses

H_{01}[2]**:** All of the following toothpaste formulations of similar total fluoride concentrations have no significant differences in term of salivary clearance concentrations of fluoride when measured at different time intervals:

- Non-fluoridated toothpaste
- Sodium fluoride toothpaste
- Sodium fluoride and sodium monofluorophsophate combined toothpaste
- Stannous fluoride and sodium fluoride combined toothpaste
- Amine fluoride toothpaste
- Sodium monofluorophosphate toothpaste

H_{02}[3]: No significant difference exists between rinsing and no-rinsing post-brushing with regard to salivary fluoride concentrations amongst all the studied formulations.

10.2 Accepted hypotheses[4]

H_{03}: No significant interaction exists between the gender of the participants and the salivary fluoride concentrations.

H_{04}: No significant interaction exists between caries status of the participants and the salivary fluoride concentrations.

H_{05}: No significant interaction exists between the presence of calculus and the salivary fluoride concentrations.

H_{06}: No significant interaction exists between the age of the participants and the salivary fluoride concentrations.

---

[2] Please refer to chapter 7 for statistical analysis.

[3] Please refer to chapter 8 for statistical analysis.

[4] Please refer to chapter 5 for statistical analysis.

H_{07}: No significant interaction exists between the DT, FT, MT and DMFT scores and the salivary fluoride concentrations.

H_{08}: No significant interaction exists between the DS, MS, FS and DMFS scores and the salivary fluoride concentrations.

10.3 Summary

- Salivary fluoride concentration was statistically significantly higher in non-rinsing groups compared to rinsing groups.
- Salivary fluoride concentration post-brushing with the following toothpastes were significantly higher than the control toothpaste at the one minute time interval for both rinsing and non-rinsing groups (NaF, NaF+NaMFP, AmF and SnF+NaF).
- Salivary fluoride concentration post-brushing with NaMFP toothpaste was not significantly higher than the control toothpaste for both rinsing and non-rinsing groups.
- Salivary fluoride concentration post-brushing with NaF toothpaste remained significantly higher than the control toothpaste at 1, 15, 30 and 60 minutes time intervals for the non-rinsing group. It was significantly different at 1, 15 and 30 minutes for the rinsing groups.
- Salivary fluoride concentration post-brushing with AmF toothpaste remained significantly higher than the control toothpaste even at the 90 minutes time interval with and without water rinsing post-brushing.
- For non-rinsing groups, salivary fluoride concentrations post-brushing with NaF+NaMFP toothpaste remained significantly higher than the control toothpaste at the one and 15 minute time intervals. It was only significant at the one minute time interval for the rinsing groups.
- Salivary fluoride concentrations post-brushing with SnF+NaF toothpaste remained significantly higher than the control toothpaste at the 1, 15 and 30 minutes time intervals for both rinsing and non-rinsing groups.
- For non-rinsing groups, salivary fluoride concentrations for AmF toothpaste were significantly higher than the other toothpaste formulations at the 90 minutes time interval.

- There were no significant differences in salivary fluoride concentrations at the 90 minutes intervals between NaF, NaF+MFP, SnF+NaF and NaMFP, for both rinsing and non-rinsing groups.
- For rinsing groups, salivary fluoride concentrations for AmF toothpaste were significantly higher than any of the other toothpaste formulations at all of the time points.
- For non-rinsing groups, salivary fluoride concentrations for NaF toothpaste were not significantly different from any of the other toothpastes at 60 minutes time interval.
- There was no significant difference in salivary fluoride concentrations between rinsing and non-rinsing groups for NaMFP toothpaste at the one minute time interval. However, there was a significant difference at 15, 60 and 90 minutes.
- There was a significant difference in salivary fluoride concentrations between rinsing and non-rinsing groups for both NaF and AmF toothpaste at the one minute time interval. However, there was no significant difference at 15, 30, 60 and 90 minutes.

10.4 Summary of Key outcomes

- This present study supports the current guidelines in discouraging rinsing post-brushing as it significantly reduces the concentration of the salivary fluoride ions post-brushing.
- Salivary fluoride ion concentrations following brushing with sodium monofluorophosphate toothpastes were not statistically significantly higher than the non-fluoridated toothpaste (0 ppmF). It is not known whether this is likely to produce a clinically significant effect with regard to the effectiveness of NaMFP toothpastes in caries prevention.

  Amine fluoride toothpaste performed the best in terms of having significantly higher salivary fluoride ion concentrations for a prolonged period of time. High salivary fluoride concentrations in the saliva for prolonged periods of time are associated with decreased caries experience.

Chapter 11 **Ethical and legal considerations**

11.1 Risks and benefits

As stated in the aims section, this research aimed to measure salivary fluoride over a 90 minute period following the use of different toothpaste formulations and rinsing techniques. Physical risks might involve fainting since participants had to come starved (no food or water was allowed) for the research. All appointments were arranged as early morning appointments to reduce risk of fainting. In case of fainting, it was planned for participants to be withdrawn from the study, to be provided with compensation and any data collected up to that point would still be analysed (intention to treat analysis). The research was conducted in the Leeds Dental Institute where the clinic is equipped with a crash trolley to deal with medical emergencies such as fainting. The Leeds Dental Institute has quick access to Leeds General Infirmary. Furthermore, the chief investigator and the research assistant are both appropriately trained in managing medical emergencies.

Participants were seen only once, minimising disadvantages related to time-off work.

Only ASA I and II participants were included in the study which means this did not include participants who had potential infectious diseases such as hepatitis B. However, undeclared infectious disease was not to be excluded; therefore, all samples were dealt with according to the Leeds Dental Institute cross infection control protocols. This included treating every salivary sample as infectious and cross infection control measures were taken. During the analysing stage and sample storage, this study complied with the local safety protocols in the university laboratory.

With every research that includes human participants, every participant is always treated as being potentially infectious for blood-borne pathogens and the risk of undeclared infectious diseases remain as a possibility. Cross-infection control measures that were applied included: wiping dental chairs and units before and after every participant, single-use isolation barriers used on light and dental unit handles and clinical waste and participant-contaminated waste (including saliva) were disposed of in the appropriate

bins according to the local safety protocols of the University of Leeds laboratory and the Leeds Dental Institute clinics.
There was no potential for reputational risk to the University related to this research.

11.2 Informed consent

The study aimed to recruit adults over 18 years, who by the Mental Capacity Act 2005 are capable of giving consent. No vulnerable participants were included in the study.
Written informed consent was obtained and recorded from all participants wishing to take part in this research. An information sheet and consent form were provided to all participants at least 48 hours before informed consent was obtained, to give them sufficient time to consider participation. The information sheet was written in English lay language explaining the correct and pertinent information for the participants to make an informed choice.
For those whose first language was not English with communication difficulty, it was planned for an interpreter to be provided who would help with the translation of information and consent sheets.
All participants had the freedom to withdraw from the research at any point without giving a reason. This was clearly explained on the consent sheet.

11.3 Inducement and coercion

Even though participants were provided compensation for their time, the compensation was reasonable and less likely had an effect on their informed judgment to participate. For this research the compensation covered transportation fees or post-research meals since they had to come starved for research. This was in the form of financial compensation (10.00 GBP).

11.4 Confidentiality, anonymity and illegal activity

All participant information and research data were stored in a password-protected folder on the University server. Consent forms and any hard copies were stored in a locked filing cabinet inside the University premises. Participants were informed that data will be anonymised so that they cannot

be identified even in case of results dissemination (i.e peer-reviewed journals). Data will be kept for two years after submission of this thesis for publication or three years following the completion of data collection, whichever is longest. Data will then be destroyed through the University's policy of destroying research participants' data.
It is not likely that this research would have included the possibility of uncovering illegal activity that might require breach of confidentiality.

11.5 Data protection

Collection of personal data will follow the eight principals of the Data Protection Act 1998. Any hard copies are stored in a locked filing cabinet inside the University of Leeds and were never taken out of the premises at any time. Electronic data on the other hand were stored in a password-protected folder on the secure University of Leeds server to ensure data were protected and for the back-up of data.

11.6 Conflicts of interest

There was no potential for conflicts of interests in this research. This project was part of the Professional Doctorate Degree in Paediatric Dentistry. The chief investigator is Mrs. M. Albahrani under the supervision of Professor J. Toumba (main supervisor).

11.7 Environmental Impact

There was no significant environmental impact involved in this project. Saliva was disposed safely according to the Human Tissue Authority's Code of Practice.

11.8 Ethical approvals obtained

All the required details and information regarding the study were provided to the ethical committee of the following bodies and the following ethical approvals were granted prior to the commencement of the study:

1. Sponsorship approval: (Appendix B.1 and B.2)
   Ms Claire Skinner on behalf of the University of Leeds

Faculty research manager, Head of Research Support for the Faculty of Medicine and Health.

2. NHS Research Ethics Committees (RECs) approval: (Appendix B.3)
   The REC form, along with all relevant documents were authorised and submitted through the Integrated Research Application System (IRAS ID: 19095). The application was reviewed and validated and an appointment for a full ethical review meeting had been booked to take place on the 1st February 2016 at 2:20 pm by the Yorkshire and The Humber – Sheffield Research Ethics Committee (REC reference: 16/YH/0015).
   The members of the committee present gave a favourable ethical opinion of this research study subject to the conditions below:

1. Management permission to be obtained from each host organisation prior to the start of the study at the site concerned. For this research study this would be obtaining both the R&D and CSU approvals.
2. Registration of the clinical trial on a publicity accessible database:
   The protocol of the study has been submitted for publication on the following data accessible website and the protocol was made accessible to the public.
   Unique ID: 16/YH/0015
   ClinicalTrials.gov Identifier: NCT02740803
   Details could be found on the following link:
   https://clinicaltrials.gov/ct2/show/NCT02740803

3. Clinical Service Unit (CSU) approval: (Appendix B.4)
   The protocol of this research study was sent to Mr. Alastair Speirs (Clinical Director in the University of Leeds), who advised that Leeds Dental Institute CSU approval subject to obtained the REC ethical approval.
4. NHS Research and Development (R&D) approval: (Appendix B.5)
   NHS permission for research has been granted for this project at the Leeds Teaching Hospitals Trust (LTST).
   LTHT R&I Number DT16/003

A substantial amendment request was made on the 25th February 2016 and the following approvals were also obtained.

- Sponsorship approval
- NHS Research Ethics Committees (RECs) approval (Appendix B.6)
- Clinical Service Unit (CSU) approval (Appendix B.7)
- NHS Research and Development (R&D) approval (Appendix B.8)

11.9 Declaration of the end of study

After recruitment of participant number 120, a notice of declaration of the end of study was sent to all the bodies who provided ethical approvals to this research project. This was sent on the 15th June 2017 and an acknowledgment was received on the 16th June 2017 (Appendix B.9).

The research summary was sent to the Yorkshire and The Humber – Sheffield Research Ethics Committee on the 23rd October 2017 to the following e-mail address:

[nrescommittee.yorkandhumber-sheffield@nhs.net].

11.10 Results Dissemination

Results were disseminated to all participants using their preferred method specified in the consent sheet form. (i.e. e-mail or home address). A letter of appreciation was also attached (Appendix C.16).

List of References/ Bibliography

Agency of Toxic Substances and Disease Registry. 2015. Fluorides, Hydrogen Fluoride, and Fluoride: Analytic methods. [Online] Atlanta: Agency for Toxic Substances and Disease Registry. [Accessed 25 August 2017] Available from:https://www.atsdr.cdc.gov/ToxProfiles/tp11-c7.pdf

Ammari J., Baqain Z. and Ashley P. 2007. Effects of programs for prevention of early childhood caries. A systematic review. Medical Principles and Practice. 16(6), pp.437-42.

Arnold, W., Dorow, A., Langenhorst, S., Gintner, Z., Banoczy, J., and Gaengler, P. 2006. Effect of fluoride toothpastes on enamel demineralization. BMC Oral Health. [Online]. 6(8), pp.1-6. [Accessed 16 August 2017] Available from: https://bmcoralhealth.biomedcentral.com/articles/10.1186/1472-6831-6-8

Attin, T. and Hellwig, E. 1996. Salivary fluoride content after toothbrushing with a sodium fluoride and an amine fluoride dentifrice followed by different mouthrinsing procedures. Journal of Clinical Dentistry. 7(1), pp.6-8.

Benson P., Parkin N., Millett D., Dyer F., Millett, D. Furness, S. and Germain, P. 2013. Fluorides for the prevention of white spots on teeth during fixed brace treatment. Cochrane Database of Systematic Reviews. [Online]. Issue 12. Art. No. CD003809. [Accessed 18 December 2016] Available from: DOI: 10.1002/14651858.CD003809.pub3.

Bentley E., Ellwood R. and Davies R. 1999. Fluoride ingestion from toothpaste by young children. British Dental Journal. [Online]. 186(9), pp.460-462. [Accessed 18 December 2016] Available from: http://www.nature.com/bdj/journal/v186/n9/full/4800140a.html

Bowden, J. 1990. Effects of fluoride on microbial ecology of dental plaque. Journal of Dental Research. [Online]. 69 Spec No:653-659; discussion 682-683. [Accessed 2 July 2015] Available from: http://journals.sagepub.com/doi/pdf/10.1177/00220345900690S127

Brady, J. and Senese F. 2009. Chemistry matter and its changes. 5th ed. United States of America: John Wiley & Sons publications.

Brown, W. E., Gregory, T. M. and Chow, L. C. 1977. Effects of fluoride on enamel solubility and cariostasis. Caries Research. [Online] 11(Suppl. 1), pp.118–124. [Accessed 15 July 2015] Available from: https://0-doi-org.wam.leeds.ac.uk/10.1159/000260298

Brudevold, F. and McCann, H. 1968. Enamel solubility tests and their significance in regard to dental caries. Annals of the New York Academy of Science. [Online] 153, pp.20–51. [Accessed 2 July 2015] Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.1968.tb11725.x/full

Bruun, C., Givskov, H. and Thylstrup, A. 1984. Whole saliva fluoride after toothbrushing with NaF and MFP dentifrices with difference F concentrations. Caries Research. [Online] 18(3). pp.282-288. [Accessed 15 July 2015] Available from: https://0-doi-org.wam.leeds.ac.uk/10.1159/000260778

Burrows, A., Holman, J., Parsons, A. and Pilling, G. 2009. Chemistry: Introducing inorganic, organic and physical chemistry. Edition 2. Oxford: Oxford University Press.

Campus, G., Lallai M., Carboni R. 2003. Fluoride concentration in saliva after use of oral hygiene products. Caries Research. [Online] 37(1), pp.66-70. [Accessed 2 July 2015] available from: https://doi.org/10.1159/000068220

Carey, C. 2014. Focus on Fluorides: Update on the Use of Fluoride for the Prevention of Dental Caries. The Journal of Evidence-Based Dental Practice. [Online] 14(Suppl.), pp.95–102. [Accessed 8 January 2016] Available from:
http://doi.org/10.1016/j.jebdp.2014.02.004

Center of Scientific Information .2017. Oral Health Topics: Toothpastes. Updated 13 April 2017. ADA Science Institute [Online] [Accessed 28 June 2017]. Available from:
http://www.ada.org/en/member-center/oral-health-topics/toothpastes

Chestnutt I., Schafer F., Jacobson A., Stephen K. 1998. The influence of toothbrushing frequency and post-brushing rinsing on caries experience in a caries clinical trial. Community Dentistry Oral Epidemiology. [Online] 26(6), pp.406-411. [Accessed 29 June 2017]. Available from:
http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0528.1998.tb01979.x/abstract

Chong, L., Clarkson, J., Dobbyn-Ross, L. and Bhakta, S. 2014. Slow-release fluoride devices for the control of dental decay. Cochrane Database of Systematic Reviews. [Online]. Issue 11. Art. No. CD005101. [Accessed 10 January 2017] Available from:
DOI: 10.1002/14651858.CD005101.pub3.

Crommelin D., Higuchi W., Fox J., Spooner P., Katdare A. 1983. Dissolution rate behavior of hydroxyapatite-fluorapatite mixtures. Caries Research. 17(4), pp.289-96.

DenBesten P. and Ko H. 1996. Fluoride levels in whole saliva of preschool children after brushing with 0.25 g (pea-sized) as compared to 1.0 g (full-brush) of a fluoride dentifrice. Pediatric Dentistry. [Online]18(4), pp.277-280. [Accessed 5 July 2016] Available from:
http://www.aapd.org/assets/1/25/DenBesten-18-04.pdf

DePaola P., Soparkar P., Triol C., Volpe A., Garcia L., Duffy J., Vaughan B. 1993.The relative anticaries effectiveness of sodium monofluorophosphate and sodium fluoride as contained in currently available dentifrice formulations. American Journal of Dentistry. 6 Spec No. S7-12.

Department of Health and British Association for the study of Community Dentistry. 2017. Delivering Better Oral Health. An evidence-based toolkit for prevention. 3rd ed. London; Public Health England.

Drummond B., Curzon M. and Strong M. 1990. Estimation of fluoride absorption from swallowed fluoride toothpastes. Caries Research. 24(3), pp.211-215.

Duckworth, R. and Morgan, S. 1991. Oral fluoride retention after use of fluoride dentifrices. Caries Research. 25(2), pp.123-129.

Ekstrand J. and Ehrnebo M. 1980. Absorption of fluoride from fluoride dentifrices. Caries Research 14(2), pp.96-102.

Ellingsen J., Eriksen H. and Rölla G. 1982. Extrinsic dental stain caused by stannous fluoride. Scandinavian Journal of Dental Research. 90(1), pp.9-13.

Elliot, J. 1964. The Crystallographic Structure of Dental Enamel and Related Apatites. Ph. D. thesis, University of London.

Ericsson, Y. 1967. Biologic splitting of PO_3F ions. Caries Research. 1, pp.144-152.

European Commission. 2003. Opinion of the Scientific Committee on Cosmetic Products and Non-food products Intended for Consumers. European Commission [Online] [Accessed 16 August 2017] Available from: http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out219_en.pdf

European commission. 2016. Fluoride compounds approved by the European Commission to be used in oral health products. European Commission. [Online] [Accessed 16 August 2017] Available from: http://ec.europa.eu/growth/toolsdatabases/cosing/pdf/COSING_Annex%20III_v2.pdf

Evans, R. and Stamm, J. 1991. An Epidemiologic estimate of the critical period during which human maxillary central incisors are most susceptible to fluorosis. Journal of Public Health Dentistry. 51, pp.251–259.

Facer, G. 2013. AS Chemistry. 2nd ed. Italy: Phillip Allan Updates.

Featherstone, J. 1999. Prevention and reversal of dental caries. Community Dentistry and Oral Epidemiology. 27(1), pp.31-40.

Gron, P., Brudevold, F., and Aasenden, R. 1971. Monofluorophosphate interactions with hydroxyapatite and intact enamel. Caries Research. 5, pp.202-214.

Gron P. 1973. Remineralization of enamel lesions in vivo. Oral Science Reviews Journal. 3, pp.84-99.

Hattab, F. 1989. The state of fluorides in toothpastes. Journal of Dentistry. 17(2), pp.47-54.

Heifetz S. and Horowitz H. 1984. The amounts of fluoride in current fluoride therapies: safety considerations for children. Journal of Dentistry for Children. 51(4), pp.257-269.

Higgins J., Green S. 2011. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration. [Online] [Accessed 1 October 2017] Available from: www.handbook.cochrane.org.

Hirose, M., Murata, Y., Fukuda, A., Fujita, Y., Otomo, M., Yahata, S., Saitoh, M. 2015. Fluoride retention in saliva following toothbrushing using different types of fluoridated dentifrices containing 1500 ppmF of NaF and MFP. Pediatric Dental Journal. 25(2), pp.45-49.

Holloway, P., Worthington H. 1993. Sodium fluoride or sodium monofluorophosphate? A critical view of a meta-analysis on their relative effectiveness in dentifrices. American Journal of Dentistry. 6, Spec No:S55-8.

Ismail A. and Hasson H. 2008. Fluoride supplements, dental caries and fluorosis: a systematic review. Journal of American Dental Association. 139(11), pp.1457-1468.

Issa, A. and Toumba, K. 2004. Oral fluoride retention in saliva following toothbrushing with child and adult dentifrices with and without water rinsing. Caries Research. 38(1), pp.15-19.

Jacobsen, S., Goldberg, J., Miles, T., Brody, J., Stiers, W. and Rimm, A. 1990. Regional variation in the incidence of hip fracture US white women aged 65 years and older. Journal of American Medical Association. 264(4), 500-502.

Kang, H. 2013. The prevention and handling of the missing data. Korean Journal of Anesthesiology. 64(5), pp.402–406.

Shourie, K., John, H. and Hodge, H. 1950. Preliminary studies of the caries inhibiting potential and acute toxicity of sodium monofluorophosphate. 29(4), pp.529-533.

Koo H. 2008. Strategies to enhance the biological effects of fluoride on dental biofilms. Advance Dental Research. 20(1), pp.17-21.

Koulourides T. 1982. Increasing tooth resistance to caries through remineralisation. Foods and Nutrition Dental Health. 2:193-207.

Kumar S., Tadakamadla, J. and Johnson, N. 2016. Effect of toothbrushing frequency on incidence and increment of dental caries. 95(11), pp.1230 – 1236.

Li, P., Stuart, A., and Allison, D. 2015. Multiple Imputation: A Flexible Tool for Handling Missing Data. Journal of American Medical Association. 314(18), pp.1966–1967.

Lovius, B. and Goose, D. 1969. The effect of fluoridated water on tooth morphology. British Dental Journal. 127(7), pp.322–324.

Lynch R., Navada, R. and Walia, R. 2004. Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. International Dental Journal. 54(5 Suppl 1), pp.304-309.

Machiulskiene V., Richards, A., Nyvad, B. and Baelum, V. 2002. Prospective study of the effect of post-brushing rinsing behaviour on dental caries. Caries Research. 36(5), pp.301-307.

Marinho V., Higgins J., Logan S., Sheiham (deceased), A. 2003. Fluoride toothpastes for preventing dental caries in children and adolescents. Cochrane Database of Systematic Reviews. Issue 1. Art. No. CD002278. DOI: 10.1002/14651858.CD002278.

Marinho V., Worthington H., Walsh T. and Clarkson, J. 2013. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database of Systematic Reviews. Issue 7. Art. No. CD002279. DOI: 10.1002/14651858.CD002279.pub2.

Marinho V., Worthington H., Walsh T., and Chong L. 2015. Fluoride gels for preventing dental caries in children and adolescents. Cochrane Database of Systematic Reviews. Issue 6. Art. No. CD002280.
DOI: 10.1002/14651858.CD002280.pub2.

Marinho V., Chong L., Worthington H. and Walsh, T. 2016. Fluoride mouthrinses for preventing dental caries in children and adolescents. Cochrane Database of Systematic Reviews. Issue 7. Art. No. CD002284.
DOI: 10.1002/14651858.CD002284.pub2

Martinez-Mier, E., Cury, J., Heilman, J., Katz, B., Levy, S., Li, Y., Maguire, A., Margineda, J., O'Mullane, D., Phantumvanit, P., Soto-Rojas, A., Stookey G., Villa, A., Wefel, J., Whelton, H., Whitford, G., Zero, D., Zhang, W. and Zohouri, V. 2011. Development of gold standard ion-selective electrode-based methods for fluoride analysis. Caries Research. 45(1), pp.3-12.

McHugh, M. 2011. Multiple comparison analysis testing in ANOVA. Biochemia Medica. 21(3), pp.203-209.

Mercer, V and Muhler, J. 1972. Comparison of single topical applications of sodium fluoride and stannous fluoride. Journal of Dental Research. 51(5), pp. 1325 – 1330.

Nazzal, H., Duggal, M, Kowash, M., Kang, J., and Toumba, K. 2016. Comparison of residual salivary fluoride retention using amine fluoride toothpastes in caries-free and caries-prone children. European Archives of Paediatric Dentistry, 17(3), pp.165–169.

Nevitt, G., Witter, D., and Bowman, W. 1958. Topical applications of sodium fluoride and stannous fluoride. Public Health Reports, 73(9), pp.847–850.

Paul, S., Tandon S. and Murthy, K. 1993. Effect of fluoride dentifrices on salivary fluoride levels in children. Indian Journal of Dental Research. 4, pp.95-101.

Pessan, J., Al-Ibrahim, N., Buzalaf, M., & Toumba, K. 2008. Slow-release fluoride devices: a literature review. Journal of Applied Oral Science, 16(4), pp.238–244.

Poulsen, S., Errboe, M., Lescay Mevil, Y. and Glenny, A. 2006. Potassium containing toothpastes for dentine hypersensitivity. Cochrane Database of Systematic Reviews 2006, Issue 3. Art. No. CD001476.
DOI: 10.1002/14651858.CD001476.pub2.

Poulsen, S. 2009. Fluoride-containing gels, mouth rinses and varnishes: an update of evidence of efficacy. European Archives of Paediatric Dentistry. 10(3), pp.157-161.

Priyadarshini, S., Raghu, R., Shetty, A., Gautham, P., Reddy, S., and Srinivasan, R. 2013. Effect of organic versus inorganic fluoride on enamel microhardness: An in vitro study. Journal of Conservative Dentistry : Journal of Consecutive Dentistry. 16(3), pp.203–207.

Public Health England .2012. National Dental Epidemiology Programme for England: Oral Health Survey of Five-year-old Children 2012. A Report on the Prevalence and Severity of Dental Decay. London: PHE.

Rennie, R. 2016. A Dictionary of Chemistry. 7th ed. Oxford: Oxford University Press.

Robinson, C. 2009. Fluoride and caries lesions: interaction and mechanism of action. European Archives of Paediatric Dentistry. 10(3), pp.136-140.

Rosin-Grget, K., Peros, K., Sutej, I. and Basic, K. 2013. The cariostatic mechanisms of fluoride. Acta Medica Academica. 42(2), pp.179-188.

Rumble, R. 2017. CRC Handbook of Chemistry and Physics. 98th Edition. Florida: CRC Press.

Saporito R., Boneta A., Feldman C., Cinotti W., Sintes J., Stewart B., Volpe A. and Proskin H. 2002. Comparative anticaries efficacy of sodium fluoride and sodium monofluorophosphate dentifrices. A two-year caries clinical trial on children in New Jersey and Puerto Rico. American Journal of Dentistry. 13(4), pp.221-6.

Scharfstein, D., Hogan, J., and Herman, A. 2012. On the prevention and analysis of missing data in randomized clinical trials: The state of the art. The Journal of Bone and Joint Surgery. American Volume. 94(Suppl 1), pp.80–84.

Scottish Intercollegiate Guidelines Network. 2014. Dental Interventions to Prevent Caries in Children: A national clinical guideline. [SIGN 138]. [Online] Edinburgh: Scottish Intercollegiate Guidelines Network. [Accessed 25 June 2016]. Available from: http://www.sign.ac.uk/assets/sign138.pdf

Sensabaugh, C. and Sagel, M. 2015. Stannous fluoride dentifrice with sodium hexametaphosphate: Review of Laboratory, Clinical and Practice-Based Data. Hygiene Tribute. March April; 1B-10B.

Shulman J. and Wells L. 1997. Acute fluoride toxicity from ingesting home-use dental products in children, birth to 6 years of age. Journal of Public Health Dentistry. 57(3), pp.150-158.

Sjögren, K., Birkhed, D. and Rangmar B. 1995. Effect of a modified toothpaste technique on approximal caries in preschool children. Caries Research. 29(6), pp.435-441.

Soares, C., Amaral, F., Mesquita, M., Franca, F., Basting, R. and Turssi, C. 2015. Toothpastes containing abrasive and chemical whitening agents: efficacy in reducing extrinsic dental staining. General Dentistry. 63(6), pp.24-28.

Steele, J. and O'Sullivan, I. 2011. Executive Summary Adult Dental Survey. [Online] London: The Health and Social Care Information Centre. [Accessed 25 August 2017]
Available from: http://digital.nhs.uk/media/16625/AdultDental-Health-Survey-England-Wales-Northern-Ireland-2009Summary/Any/adul-dent-heal-surv-summ-them-exec-2009-rep2

Taves, D. 1968. Determination of submicromolar concentrations of fluoride in biological samples. Talanta. 15, pp.1015-1023.

Toumba, K. and Curzon, M. 2001. Fluoride concentrations in saliva related to dental caries and prevalence in primary teeth. European Journal of Paediatric Dentistry. 1, pp.15-19.

Tubert-Jeannin, S., Auclair, C., Amsallem, E., Tramini, P., Gerbaud, L., Ruffieux, C., Schulte, A., Koch, M., Rège-Walther, M. and Ismail, A. 2011. Fluoride supplements (tablets, drops, lozenges or chewing gums) for preventing dental caries in children. Cochrane Database of Systematic Reviews. Issue 12. Art. No. CD007592.
DOI: 10.1002/14651858.CD007592.pub2

Twetman, S., Petersson, L., Axelsson, S., Dahlgren, H., Holm A,. Kallestal, C. 2004. Caries-preventive effect of sodium fluoride mouthrinses: a systematic review of controlled clinical trials. Acta Odontologica Scandcandinavica. 62(4), pp.223-230

Twetman, S. 2009. caries prevention with fluoride toothpaste in children: An update. European Archives of Paediatric Dentistry. 10 (3), pp.162-167

U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation. 2015. U.S. Public Health Service Recommendation for Fluoride Concentration in Drinking Water for the Prevention of Dental Caries. Public Health Reports, 130(4), pp.318–331.

Van Der Sluys Veer, J., Melman, A, Pot, B., Houwink, O. and Backer, D. 1975. Fluoridation of drinking water and bone mineral content, analyzed by monochromatic (125I) radiation absorptiometry. In: Calcium Metabolism, Bone and Metabolic Bone Disease, Springer-Verlag, Heidelberg, pp.138–142

Venkateswarlu, P. 1994. Determination of fluorine in biological materials: A review. Advances in Dental Research. 8(1), pp.80-86.

Walsh, T., Worthington, H., Glenny, A., Appelbe, P., Marinho, V. and Shi, X. 2010. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. Cochrane Database of Systematic Reviews 2010, Issue 1. Art. No. CD007868.
DOI: 10.1002/14651858.CD007868.pub2.

Watts, A. and Addy, M. 2001. Tooth discolouration and staining: a review of literature. British Dental Journal. 190(6), pp.209-316.

Whitford, G. 1987. Fluoride in dental products: safety considerations. Journal of Dental Research. 66(5), pp.1056-1060.

Whitford, G. 1992. Acute and chronic fluoride toxicity. Journal of Dental Research. 71(5); pp.1249-1254.

Whitford, G. 1994. Intake and metabolism of fluoride. Advanced Dental Research. 8(1), pp.5-1

World Health Organization. 2013. Oral Health Surveys: Basic Methods. 5th Edition. Geneva: WHO press.

Chapter 12 **List of Appendices**

Appendix A Literature review

A.1 Quality Assessment of Literature

A.2 Literature Review Summary Table

A.1 Quality Assessment of Literature

Table 11.10-1 Quality assessment of literature table.

No.	First Author (Year)	Inclusion criteria	Exclusion criteria	Randomisation	Examiner blinding	Sample size calculation	Key results
1	Bruun (1984)	No	No	No	No	No	**Effect of compound:** The fluoride concentration was initially highest for MFP for the first 10 minutes. The fluoride ion concentration was initially highest for NaF compound; however, this was not significant for samples collected after 3 minutes. **Effect of concentration:** Total Fluoride and fluoride ions in saliva were positively correlated to

No.	First Author (Year)	Inclusion criteria	Exclusion criteria	Randomisation	Examiner blinding	Sample size calculation	Key results
							fluoride concentrations in the intervention toothpastes.
2	Duckworth (1991)	No	No	No	No	No	**Oral clearance studies:** For all the three concentrations, the concentration of fluoride decreased in two distinct phases. The first phase being rapid and lasted for 40 – 80 minutes. During the second phase, the fluoride concentration continued to decrease slowly. The mean fluoride concentration during the second phase was significantly different between different toothpaste concentrations. **Equilibrium study:** Salivary fluoride concentration has

No.	First Author (Year)	Inclusion criteria	Exclusion criteria	Randomisation	Examiner blinding	Sample size calculation	Key results
							increased markedly during the study and returned back to baseline once the fluoridated toothpaste has stopped. Mean equilibrium salivary fluoride concentration tended to plateau at high fluoride concentration dose.
3	Attin (1996)	No	No	No	No	No	Fluoridated toothpastes results in significantly higher fluoride concentration than non-fluoridated toothpastes. Amine fluoride toothpaste had significantly higher fluoride concentration than sodium fluoride at 10 and 90 minutes. Rinsing with water after brushing reduces the amount of salivary fluoride

No.	First Author (Year)	Inclusion criteria	Exclusion criteria	Randomisation	Examiner blinding	Sample size calculation	Key results
							post-brushing.
4	Campus (2003)	No	No	Yes	Yes	No	Equilibrium studies: For all groups, the average concentration of fluoride was significantly higher than baseline, but no statistically significant difference was observed between intervention groups. Oral salivary clearance studies: fluoride concentrations were significantly different at each time interval, with AmF products having higher fluoride concentration post-brushing than after the use of NaMFP toothpaste.
5	Issa (2004)	No	No	Yes	Yes	No	The salivary fluoride levels for the high fluoride toothpaste concentrations

No.	First Author (Year)	Inclusion criteria	Exclusion criteria	Randomisation	Examiner blinding	Sample size calculation	Key results
							were higher than for the lower fluoride toothpaste concentrations. **For 1,000 ppmF toothpastes:** At 120 mintues interval; NaF resulted in higher salivary concentration than NaMFP with and without water rinsing. **For 1,400 and 1,450 ppmF toothpastes:** Fluoride levels of AmF were higher than NaF at all times with and without water rinsing. NaMFP displayed the lowest fluoride levels compared to NaF and AmF with and without water rinsing. **For 250 – 525 ppmF toothpastes:** At 1 minute, NaMFP had the showed

No.	First Author (Year)	Inclusion criteria	Exclusion criteria	Randomisation	Examiner blinding	Sample size calculation	Key results
							the highest fluoride concentration followed by NaF and AmF respectively, with water rinsing. All three formulations reached baseline levels at 120 minutes when followed by water rinsing. Salivary fluoride levels for NaF with no rinsing were the highest at all-time intervals. Salivary fluoride levels for NaMFP and AmF reached baseline after 2 hours.
6	Hirose (2015)	No	No	No	The study is described	No	Significant difference was found in fluoride concentration in saliva following brushing with NaF and

No.	First Author (Year)	Inclusion criteria	Exclusion criteria	Randomisation	Examiner blinding	Sample size calculation	Key results
					as single blinded, however, no mention on who was blinded.		NaMFP. Sodium fluoride retains significantly higher fluoride in saliva than sodium monofluorophosphate.
7	Nazzal (2016)	No	No	Yes	Yes	No	No significant difference was found between caries-prone and caries-free participants. No rinsing post-brushing resulted in significantly higher fluoride concentration than rinsing. Higher fluoride concentrations was associated with higher salivary fluoride concentrations.

No.	First Author (Year)	Inclusion criteria	Exclusion criteria	Randomisation	Examiner blinding	Sample size calculation	Key results
							Resulted retuned to baseline measurements at 90 minutes.

A.2 Literature Review Summary Table

Table 11.10-2 Literature review summary table

No.	First Author (Year)	Design	Aim	Intervention Test	Intervention Control	No. Of Subjects
1	Bunn (1984)	Crossover study	To study the effect of different F compounds and F concentrations in dentifrices on the availability of fluoride in whole saliva.	NaF toothpaste (1,500, 1,000, 500 ppm F) NaMFP toothpaste (1,500 and 1,000 ppm F)	-	9 dental students
2	Duckworth (1991)	Crossover study	Investigate the link between oral fluoride levels and applied fluoride dose from dentifrices.	NaMFP (1,000, 1,500 and 2,500 µg F/gram)	Non-fluoridated toothpaste for equilibrium study only	7 – 10 subjects
3	Attin (1996)	Crossover study	Comparison of salivary fluoride content after toothbrushing with sodium fluoride and amine fluoride	AmF toothpaste 0.125 % NaF toothpaste	0 ppmF fluoride toothpaste	24 dental students

No.	First Author (Year)	Design	Aim	Intervention Test	Intervention Control	No. Of Subjects
			toothpastes followed by two different mouthrinsing techniques.	0.125% Post-brushing rinse Vs. no post-brushing rinse		
4	Campus (2003)	Single-blinded randomised clinical trial	Evaluate the fluoride concentrations in saliva after use of different products containing different fluoride salts of different oral hygiene regimens in vivo.	Group A: NaMFP toothpaste 1,250 ppmF Group B: AmF toothpaste 1,250 ppmF Group C: AmF toothpaste 1,250 ppmF Group D: AmF toothpaste 1,250 ppmF + AmF	-	104 Medical students Median sample size in each group was 20 for equilibrium study. For salivary clearance study, median sample size in each group was 5.

No.	First Author (Year)	Design	Aim	Intervention Test	Intervention Control	No. Of Subjects
				mouthrinse 250 ppmF Group E: NaMFP toothpaste1,250 ppmF + NaMFP varnish 1,250 ppmF		
5	Issa (2004)	Crossover	To compare the oral fluoride retention in saliva in vivo: (1) different fluoride concentrations, formulations with and without water rinsing after brushing.	NaF toothpaste (500, 1000 and 1,450) ppmF NaMFP toothpaste (525, 1,000 and 1,450) ppmF AmF toothpaste (250, 1,400) ppmF	Fluoride-free toothpaste (0 ppmF)	10 healthy adult volunteers
6	Hirose (2015)	Single-blinded	To compare salivary fluoride concentrations following brushing	NaMFP (1,500 ppmF)	No control	8 healthy adults volunteers

No.	First Author (Year)	Design	Aim	Intervention Test	Intervention Control	No. Of Subjects
		Crossover	with sodium fluoride and sodium monofluorophosphate.	NaF (1,500 ppmF)		
7	Nazzal (2016)	Crossover Double-blinded Randomised	To compare salivary fluoride levels following brushing with different concentrations of amine fluoride toothpaste. To also compare rinsing results versus not rinsing post-brushing.	Amine fluoride (250, 500 and 1,250 ppmF) toothpastes. Post-brushing rinse versus no post-brushing rinse.	No control	32 children participants 17 caries-free 15 caries-prone

Appendix B : Ethical Approvals, Confirmations and Acknowledgments

B.1 University of Leeds (Sponsor) Approval

B.2 University of Leeds (Sponsor) Indemnity

B.3 Research Ethics Committee Approval Letter

B.4 Clinical Service Unit Approval

B.5 Research and Development Approval Letter

B.6 Substantial Amendment 1 Research Ethics Committee Approval Letter

B.7 Clinical Service Unit Substantial Amendment 1 Approval

B.8 Substantial Amendment 1 Research and Development Approval Letter

B.9 Declaration of the End of the Study Acknowledgment

B.1 University of Leeds (Sponsor) Approval

confirmation of sponsorship

Jean Uniacke

Mon 11/23/2015 11:20 AM

To:Marwah Albahrani <dnmalb@leeds.ac.uk>;

1 attachment (386 KB)

University of Leeds indemnity cert 2015 16 Liabilty Pl.pdf;

Dear Marwah,

We can now confirm University of Leeds sponsorship in principle for this study ' Salivary fluoride using different toothpaste formulations'. We will therefore proceed with electronic authorisation via IRAS. Please use the governance-ethics@leeds.ac.uk address for this. When you have booked into ethics and submitted your form via IRAS please send us a .pdf copy of the signed form populated with the NHS ethics reference number.
A copy of the University Indemnity certificate is attached to this email.

Please note; to simplify the process we recommend that you submit the REC and R&D forms for authorisation at the same time.

Role of the Research Sponsor under the Research Governance Framework for Health & Social Care (2005, 2nd Ed) and the Medicines for Human Use (Clinical Trials) Regulations 2004

I hereby confirm that the University of Leeds would be prepared to accept the role of research sponsor as currently defined in the *Research Governance Framework for Health & Social Care Version 2 (DoH 2005)* and the *Medicines for Human Use (Clinical Trials) Regulations 2004 (SI2004/1031),* in relation to the study:

Total salivary concentration of healthy adult subjects following tooth brushing with different formulations of fluoridated toothpaste with and without post-brushing water rinsing. A randomised controlled trial.

I have been informed that this study will be led by Mrs Marwah Albahrani a Professional doctorate of Paediatric Dentistry student at the University of Leeds under the supervision of Professor KJ Toumba and Professor Duggal of the University of Leeds.

For externally funded research projects, sponsorship is conditional upon an appropriate contract with the funding body being agreed and upon review and approval of the research by appropriate ethics, NHS and regulatory bodies.

To enable the sponsor to meet their responsibilities as listed in section 3.8 of the Research Governance Framework, Chief Investigators are required to adhere to their responsibilities as outlined in section 3.6 of the Framework www.dh.gov.uk/research. In line with this requirement Mrs Albahrani must ensure that all involved in the research project understand and discharge their responsibilities in accordance with the agreed protocol and any relevant management, ethical and regulatory approvals.

If you have any queries about sponsorship of this project then please address them to Mrs Clare Skinner, at governance-ethics@leeds.ac.uk or 0113 343 4897.

Yours,

Jean Uniacke

On behalf of Clare Skinner, Faculty Head of Research and Innovation Support.

NHS Research Ethics Administrator
Faculty of Medicine and Health
Room 10.110,Level 10
Worsley Building, Clarendon way
University of Leeds, LS2 9NL
Tel: 0113 3437587
j.m.uniacke@leeds.ac.uk

NB*: Please note I work from 9.30 to 2.00pm, Monday to Thursday.*

Appendix B.1 University of Leeds (Sponsor) Approval

B.2 University of Leeds (Sponsor) Indemnity

HENDERSON
INSURANCE BROKERS

Henderson Insurance Brokers Limited
Trueman House
Capitol Park
Leeds
LS27 0TS

Tel 0113 393 6300
Fax 0113 393 6363

www.hibl.co.uk

22 September 2015

To Whom it May Concern

Dear Sirs,

EVIDENCE OF INSURANCE – The University of Leeds &/or Subsidiary Companies

We are writing to confirm that we act as Insurance Brokers to the above client and that we have arranged liability insurance on their behalf as detailed below:

EMPLOYERS LIABILITY
Cover in respect of indemnity for claims made for death, injury or disease to any person arising out of and in the course of their employment.

INSURER	:	Zurich Municipal
POLICY NUMBER	:	NHE-03CA02-0015
PERIOD OF INSURANCE	:	29th September 2015 – 28th September 2016
LIMIT OF INDEMNITY	:	£40,000,000 each occurrence including costs and expenses

PUBLIC/PRODUCTS LIABILITY
Indemnity in respect of claims made for death, injury or disease to persons (other than employees) or loss or damage to third party property arising out of and in the course of the business.

INSURER	:	Zurich Municipal
POLICY NUMBER	:	NHE-03CA02-0015
PERIOD OF INSURANCE	:	29th September 2015 – 28th September 2016
LIMIT OF INDEMNITY	:	£40,000,000 each occurrence (and in the aggregate in respect of Products)

PROFESSIONAL INDEMNITY
Indemnity in respect of the Legal Liability to Third Parties for breach of professional duty due to negligent act, error or omission in connection with your business.

INSURER	:	Royal & Sun Alliance
POLICY NUMBER	:	RKK665002
PERIOD OF INSURANCE	:	29th September 2015 – 28th September 2016
LIMIT OF INDEMNITY	:	£10,000,000 each occurrence and in the aggregate

Subject to the policy terms, conditions, limitations, exclusions and cancellation provisions.

This document is issued as a matter of information only and confers no rights upon the document holder other than those provided by the policy. This document does not amend, extend or alter the coverage afforded by the policy or policies as described herein.

/Continued.....

HENDERSON
INSURANCE BROKERS

Notwithstanding any requirement, term or condition of any contract or other document with respect to which this document may be issued or pertain, the insurance afforded by the policy (policies) described herein is subject to all terms, conditions or exclusions of such policy (policies). Limits shown may have been reduced by paid claims.

If you should require any further information or the above please do not hesitate to contact us.

Yours faithfully,

David Galey
Broking Manager

Direct Dial: **0113 393 6825**
Email: david.galey@hibl.co.uk

B.3 Research Ethics Committee Approval Letter

NHS
Health Research Authority
Yorkshire & The Humber - Sheffield Research Ethics Committee
Jarrow Business Centre
Viking Business Park
Rolling Mill Road
Jarrow
Tyne and Wear
NE32 3DT

Telephone: 0191 4283564

12 February 2016

Mrs M Albahrani
Post-graduate student Paediatric dentistry
PhD Doctorate Student in Paediatric Dentistry
Leeds Dental Institute
Postgraduate room, Level 6
School of Dentistry, Worsley building
University of Leeds, Leeds, UK
LS2 9LU

Dear Mrs Albahrani

Study title:	**Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing.A randomised controlled Trial**
REC reference:	**16/YH/0015**
IRAS project ID:	**190951**

The Research Ethics Committee reviewed the above application at the meeting held on 01 February 2016. Thank you for attending with your Academic Supervisor to discuss the application.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Miss Kathryn Murray, nrescommittee.yorkandhumber-sheffield@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publicly accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non NHS sites

The Committee has not yet completed any site-specific assessment(s) (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Summary of discussion at the meeting

Social or scientific value; scientific design and conduct of the study

Members noted that this was a very thought out and presented project and commended the Student Investigator on the work which had been put into this application.

The Committee requested clarification around whether current guidance was for or against rinsing after brushing our teeth.

Professor Toumba confirmed that current guidance advised against rinsing after brushing.

The Committee further queried what the purpose if the trial was.

You advised that there were currently too many different varieties of toothpaste on the market and there needed to be research undertaken to prove which was the most effective one for use. You confirmed that the most effective was considered to be the toothpaste which kept the highest amount of fluoride in the mouth for the longest duration. She further advised that it was hoped that the trial would also provide evidence around the issue of whether to rinse or not, following brushing, or whether it could be a matter of preference when using the most effective toothpaste.

Professor Toumba further added that Sanis fluoride toothpaste, with very high fluoride levels, had just been relaunched on the UK market. He explained that this brand of toothpaste had previously been released in the 1960's; however, it was not compatible in its previous formulation and caused staining to the teeth.

The Committee queried that statistics which had been quoted for the project as it did not appear that there was sufficient numbers to generate statistically relevant findings.

You agreed and advised that this was something which had been picked up as part of the external review of the application, which you needed to address with the statistician supporting her on the project.

The Committee noted that the study was powered against a previous trial which involved a control arm and as such it was recommended that these numbers be removed and the proposed trial calculation be revisited. Clarification of the revised statistical elements was required along with any revised trial numbers. The Committee recommended that if the recalculated statistics showed that a change in study numbers was required, a substantial amendment would need to be submitted.

The Committee noted that the study aimed to find the toothpaste which left the highest concentration of fluoride in the mouth for the longest period of time; however, it was unclear whether the absorption rate of the fluoride was the more important measurement.

You explained that the purpose of the trial was to measure the concentration of the fluoride remaining in the mouth. Professor Toumba explained that it was not possible to measure how much fluoride was absorbed into the teeth and that as various toothpastes work in different ways, it was difficult to compare them in this fashion. You confirmed that that purpose of the project was to identify the best toothpaste from those being tested.

The Committee queried how this was determined.

Professor Toumba confirmed that this would be determined as the toothpaste which keeps the highest level of fluoride in the saliva for the longest duration post-brushing. He commented that the concentration of fluoride in the mouth and the active way it works was deemed to be the most effective protection.

The Committee queried whether it would be better to treat the drinking water with fluoride.

Professor Toumba confirmed that this would be a more efficient way of treating a large population but he explained that there were very few fluoridated cities in the country to make this workable.

The Committee queried whether the toothpastes to be trialled were available and recommended.

You advised that the toothpastes to be trialed were all Department of Health recommended, apart from one which was not currently available in the UK. Professor Toumba commented that this product was owned by a Swiss company which formed part of the Colgate group and it was expected that the product would be launched in the UK soon enough.

The Committee received the response and no further issues were raised.

Recruitment arrangements and access to health information, and fair participant selection

The Committee noted that participants would be paid £10 for their involvement in the study and Members queried who would provide the money when there was no funding for the project.

You confirmed that you would be paying from her PhD funds.

The REC queried how and by whom potential participants would be approached.

You explained that mailshot would be circulated attaching the information sheet to all students at the University of Leeds. You further explained that there would be posters displayed in waiting areas and leaflets available for collection by all visitors to the dental institute, so this could be NHS patients or those accompanying them to appointments.

The Committee queried why the participants needed to be fasted before attending for the trial.

You explained that it was known that some food and drinks contained levels of fluoride which had the potential to confound the trial results. In order to avoid this, you advised that participants would be requested to attend the trial visit fasted. You explained that it was agreed that two hours was sufficient time for any fluoride concentration from food or drinks to have passed.

The Committee queried how saliva flow was measured and if this exclusion criteria would make many individuals unsuitable for inclusion in the study.

You detailed that saliva flow was measured simply by asking the participant to drool in a beaker and timings them to see how long it takes to produce a certain amount of saliva. You confirmed that if a potential participant was unable to produce the required amount of saliva, they would be excluded but would still be reimbursed the £10.

The Committee received the response and no further issued were raised.

Suitability of the applicant and supporting staff

The Committee queried what relevance a trial in adult healthy volunteers had to a PhD in Paediatric Dentistry.

You confirmed that children were very difficult to work with, particularly in research, and you advised that you had approval that this study was relevant and would be considered as part of your PhD programme.

Appendix B.3 Research Ethics Committee Approval Letter

The response was received and no further issues were raised.

Other ethical issues were raised and resolved in preliminary discussion before your attendance at the meeting.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Research poster]	2	17 December 2015
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [University Indemnity certificate]		
Interview schedules or topic guides for participants [Medical questionnaire]	1	17 December 2015
Letter from sponsor [University Indemnity certificate]		
Letters of invitation to participant [Information sheet + invitation letter]	2	17 December 2015
Other [Co-supervisor CV]		
Other [Letter of appreciation]	1	17 December 2015
Other [Fluoride analysis participant slip]	1	17 December 2015
Other [Toothpastes labels]	1	17 December 2015
Other [thanks you card]	1	17 December 2015
Other [Research poster (email version)]	2	17 December 2015
Other [Reminder message]	1	17 December 2015
Other [Appreciation letter]	1	17 December 2015
Other [Appointment message]	1	17 December 2015
Participant consent form [Consent form]	2	17 December 2015
Participant information sheet (PIS) [Information sheet and invitation letter]	2	17 December 2015
REC Application Form [REC_Form_22122015]		22 December 2015
Research protocol or project proposal [Protocol]	1	17 December 2015
Summary CV for Chief Investigator (CI) [Cheaf investigator CV]	1	23 November 2015
Summary CV for student [Summary CV - Marwah]	1	23 November 2015
Summary CV for supervisor (student research)		30 November 2015

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

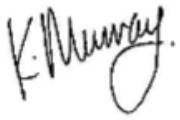
HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

16/YH/0015	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely



pp.
Professor Basil Sharrack
Chair

E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*

"After ethical review – guidance for researchers" [*SL-AR2 for other studies]*

Copy to: *Ms Ann Gowing, Leeds NHS R&D LTHT*

Appendix B.3 Research Ethics Committee Approval Letter

B.4 Clinical Service Unit Approval

RE: CSU approval -

Speirs Alastair (LEEDS TEACHING HOSPITALS NHS TRUST) <aspeirs@nhs.net>

Fri 1/8/2016 5:27 PM

To:Marwah Albahrani <dnmalb@leeds.ac.uk>;

Cc:Khan Mohammed (LEEDS TEACHING HOSPITALS NHS TRUST) <mohammed.khan38@nhs.net>; Vollans Deborah (LEEDS TEACHING HOSPITALS NHS TRUST) <deborah.vollans@nhs.net>; Sue Pavitt <S.Pavitt@leeds.ac.uk>;

Dear Marwah

I am happy to give Leeds Dental Institute CSU approval for your study, subject to ethics approval.
I would be grateful if you can send me confirmation once ethics approval has been granted and wish you well with the study.

Kind regards

Alastair Speirs
Clinical Director
Leeds Teaching Hospitals NHS Trust
0113 343 6186 | aspeirs@nhs.net

From: Marwah Albahrani [mailto:dnmalb@leeds.ac.uk]
Sent: 08 January 2016 12:17
To: Speirs Alastair (LEEDS TEACHING HOSPITALS NHS TRUST)
Cc: Khan Mohammed (LEEDS TEACHING HOSPITALS NHS TRUST)
Subject: CSU approval -

Dear Mr. Speirs,

I have been advised by the R&D department that I will require a CSU approval in order to grand an R&D approval for my research study which will be held at the Dental Translational and Clinical Reseach Unit (DentCRU) which is based at the Leeds Dental Institute (LDI).

Please find attached a copy of my Protocol and R&D form.

Please do not hesitate to contact me should you require further information or details regarding my study.

Many thanks

Kind regards,

Marwah Albahrani
Post-graduate student at the University of Leeds
Professional Doctorate Program
Contact No. 07597549869
Email: Dnmalb@leeds.ac.uk
Email: Marwahalbahrani@nhs.net

B.5 Research and Development Approval Letter

The Leeds Teaching Hospitals NHS
NHS Trust

Date: 15/02/2016

Our Ref: Mohammed Khan

Research & Innovation Department
34 Hyde Terrace
Leeds
LS2 9LN

Tel: 0113 392 0162
Email : leedsth-tr.lthtresearch@nhs.net

www.leedsth.nhs.uk/research

Marwah Albahrani
University of Leeds
Leeds Dental Institute
Postgraduate Room, Level 6
School of Dentistry, Worsley Building
Leeds
LS2 9LU

Dear Marwah Albahrani

Re: **NHS Permission at LTHT for: Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Tooth brushing with different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing. A randomised controlled Trial**
LTHT R&I Number: DT16/003
REC: 16/YH/0015

I confirm that *NHS Permission for research* has been granted for this project at The Leeds Teaching Hospitals NHS Trust (LTHT). NHS Permission is granted based on the information provided in the documents listed below. All amendments (including changes to the research team) must be submitted in accordance with guidance in IRAS. Any change to the status of the project must be notified to the R&I Department.

The study must be conducted in accordance with the *Research Governance Framework for Health and Social Care*, ICH GCP (if applicable), the terms of the Research Ethics Committee favourable opinion (if applicable) and NHS Trust policies and procedures (see http://www.leedsth.nhs.uk/research/) including the requirements for research governance and clinical trials performance management listed in appendix 1 and 2 . NHS permission may be withdrawn if the above criteria are not met including the requirements for clinical trials performance

The Leeds Teaching Hospitals NHS Trust participates in the NHS risk pooling scheme administered by the NHS Litigation Authority ""Clinical Negligence Scheme for NHS Trusts"" for: (i) medical professional and/or medical malpractice liability; and (ii) general liability. NHS Indemnity for negligent harm is extended to researchers with an employment contract (substantive or honorary) with the Trust. The Trust only accepts liability for research activity with NHS Permission

The Trust therefore accepts liability for the above research project and extends indemnity for negligent harm. Should there be any changes to the research team please ensure that you inform the R&I Department and that s/he obtains an appropriate contract, or letter of access, with the Trust if required.

Yours sincerely

Anne Gowing
Research Governance Manager

Approved documents
The documents reviewed and approved are listed as follows:-

Document	*Version*	*Date of document*
NHS R&D Form	5.2.0	18 December 2015
SSI Form	5.2.1	15 February 2016
CSU Approval		08 January 2016
REC Letter confirming favourable opinion		12 February 2016
Copies of advertisement materials for research participants [Research poster]	2.0	17 December 2015
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [University Indemnity certificate]		
Interview schedules or topic guides for participants [Medical questionnaire]	1.0	17 December 2015
Letter from sponsor [University Indemnity certificate]		
Letters of invitation to participant [Information sheet + invitation letter]	2.0	17 December 2015
Participant consent form [Consent form]	2.0	17 December 2015
Participant information sheet (PIS) [Information sheet and invitation letter]	2.0	17 December 2015
Research protocol or project proposal [Protocol]	1.0	17 December 2015

Appendix B.5 Research and Development Approval Letter

Conditions of NHS Permission for Research:

Appendix 1

Governance requirements:

Managerial approval within the Clinical Support Unit must be obtained before starting the study and healthcare staff should be suitably informed about the research their patients are taking part in and information specifically relevant to their care arising from the study should be communicated promptly.

Agreements must be in place with appropriate support departments.

Arrangements must be in place for the management of financial and other resources provided for the study, including intellectual property arising from the research.

All data and documentation associated with the study must be available for audit/monitoring by authorised Trust or external agencies.

All members of the research team, where applicable, have appropriate employment contracts or letter of agreement to carry out their work in the Trust.

Each member of the research team must be qualified by education, training and experience to discharge his/her role in the study. Students and new researchers must have adequate supervision, support and training.

The research must follow the protocol approved by the relevant research ethics committee. Any proposed amendments to or deviations from the protocol must be submitted for review (as necessary) by the Research Ethics Committee, the Research Sponsor, regulatory authority and any other appropriate body. Where the amendment has resource implications within the CSU, the Directorate research lead/clinical director and R&I should be notified.

Adverse Events in clinical trials of investigational medicinal products must be reported in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004.

Procedures should be in place to ensure collection of high quality, accurate data and the integrity and confidentiality of data during processing and storage in line with Trust Information Governance Policies and arrangements must be made for the appropriate archiving of data when the research has finished. Records must normally be kept for 15 years.

In compliance with the Health Research Authority (HRA) regulations, clinical trials (and other studies falling within the HRA definition) must be registered on a publically accessible database (such as https://clinicaltrials.gov/) prior to commencement. Studies sponsored by LTHT will be registered by the R&I Department.

Findings from the study should be exposed to critical review through accepted scientific and professional channels.

All members of the research team involved in seeking informed consent adheres to GCP standards. Investigators are directed to the R&I website for further information about training in consent for clinical trials.

Studies involving the use of human tissues must be performed in compliance with the code of practice of the Human Tissue Authority.

If you are not able to comply with these requirements, NHS permission to conduct the research in LTHT will be suspended.

Appendix B.5 Research and Development Approval Letter

Appendix 2

Commercially Sponsored and funded studies.

In line with Trust Standing Financial Instructions there must be a research agreement with the commercial funder signed by the R&I Department (on behalf of the Leeds Teaching Hospitals NHS Trust). Investigators do not have the authority to sign research agreements on behalf of the Trust.

NHS permission for this project to be carried out in the Trust is granted on the understanding that you:

Provide recruitment information when requested by R&I on the Clinical Trial Tracker (available on the CSU Research Hub)

Work with R&I to resolve blocks and delays on trials to ensure that LTHT meets the NIHR benchmarks.

NIHR Benchmarks for Performance in Initiating & Delivering Clinical Research
LTHT clinical trial performance is measured against 2 national benchmarks to improve the initiation and delivery of clinical trials approved by the Trust. NIHR funding to the Trust is conditional on meeting these benchmarks.

Initiation – it should take no more than 70 days from receipt of a valid research application (signed SSI form) by the R&I Department to the recruitment of (ie consenting) the 1st patient to the trial

Delivery – for all trials hosted by the Trust the agreed number of patients must be recruited within the agreed recruitment period

The Trust submits quarterly performance reports to the Department of Health setting out our performance.

For more information about the benchmarks and the work we are doing to support clinical trial management please see the R&I website.
http://www.leedsth.nhs.uk/research/

Appendix B.5 Research and Development Approval Letter

B.6 Substantial Amendment 1 Research Ethics Committee Approval Letter

NHS
Health Research Authority

Yorkshire & The Humber - Sheffield Research Ethics Committee
Jarrow Business Centre
Viking Business Park
Rolling Mill Road
Jarrow
Tyne and Wear
NE32 3DT

Tel: 0191 428 3561

16 March 2016

Ms Claire Skinner
University of Leeds
Faculty Research Ethics and Governance Administrator
Faculty Research Office, Room 10.110, Level 10
Worsley Building
Clarendon Way
Leeds
LS2 9NL

Dear Ms Skinner,

Study title:	**Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing. A randomised controlled Trial**
REC reference:	**16/YH/0015**
Amendment number:	**Substantial Amendment 1 - 25/2/16**
Amendment date:	**25 February 2016**
IRAS project ID:	**190951**

The above amendment was reviewed by the Sub-Committee in correspondence.

This amendment is to gain approval for the increase in sample size to at least 10 per group meaning that a total number of at least 120 participants will be recruited.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The Sub Committee did not raise any ethical issues.

Approved documents

The documents reviewed and approved at the meeting were:

Appendix B.6 Substantial Amendment 1 Research Ethics Committee Approval Letter

Document	*Version*	*Date*
Covering letter on headed paper	Email from Marwah Albahrani	25 February 2016
Notice of Substantial Amendment (non-CTIMP)	Substantial Amendment 1 - 25/2/16	25 February 2016
Other [PASS Results]	Version 2	24 February 2016
Research protocol or project proposal	Version 2	24 February 2016

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

16/YH/0015:	**Please quote this number on all correspondence**

Yours sincerely

pp

Professor Basil Sharrack
Chair

E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Ms Ann Gowing, Leeds NHS R&D LTHT*
Mrs M Albahrani, University of Leeds

A Research Ethics Committee established by the Health Research Authority

Appendix B.6 Substantial Amendment 1 Research Ethics Committee Approval Letter

Yorkshire & The Humber - Sheffield Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 10 March 2016 via correspondence.

Committee Members:

Name	*Profession*	*Present*	*Notes*
Professor Basil Sharrack	Consultant Neurologist	Yes	
Dr Steven Thomas	Consultant Vascular and Cardiac Radiologist	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Miss Kerry Dunbar	REC Assistant

B.7 Clinical Service Unit Substantial Amendment 1 Approval

Re: Substantial amendments approvals

Speirs Alastair (LEEDS TEACHING HOSPITALS NHS TRUST) <aspeirs@nhs.net>

Thu 3/17/2016 8:43 PM

To:Marwah Albahrani <dnmalb@leeds.ac.uk>;

Nothing attached to your message

On 17 Mar 2016, at 18:09, Marwah Albahrani <dnmalb@leeds.ac.uk> wrote:

Dear Dr. Speirs,

Please find attached copies of both RECs and R&Ds approval for the substantial amendments of my research study.

Regards,

Marwah Albahrai

Postgraduate in Paediatric dentistry

Leeds Dental School

RE: Ethical approvals substantial amendments

Speirs Alastair (LEEDS TEACHING HOSPITALS NHS TRUST) <aspeirs@nhs.net>

Fri 3/18/2016 7:18 PM

To:Marwah Albahrani <dnmalb@leeds.ac.uk>;

Many thanks Marwah and good luck with your study
Regards

Leeds Dental Institute

Alastair Speirs
Clinical Director
Leeds Teaching Hospitals NHS Trust
0113 343 6186 | aspeirs@nhs.net

From: Marwah Albahrani [mailto:dnmalb@leeds.ac.uk]
Sent: 18 March 2016 06:35
To: Speirs Alastair (LEEDS TEACHING HOSPITALS NHS TRUST)
Subject: Fw: Ethical approvals substantial amendments

Apologies for this.

Regards,

Marwah

Marwah

B.8 Substantial Amendment 1 Research and Development Approval Letter

The Leeds Teaching Hospitals NHS
NHS Trust

Date: 17/03/2016

Our Ref: Mobeen Fazal

Research & Innovation Department
34 Hyde Terrace
Leeds
LS2 9LN

Tel: 0113 392 0162
Email : leedsth-tr.lthtresearch@nhs.net

www.leedsth.nhs.uk/research

Marwah Albahrani
University of Leeds
Leeds Dental Institute
Postgraduate Room, Level 6
School of Dentistry, Worsley Building
Leeds
LS2 9LU

Dear Marwah Albahrani

Re: **LTHT R&I Number: DT16/003: Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing.A randomised controlled Trial**
REC: 16/YH/0015

Thank you for your letter email regarding an amendment **(Amendment date: 25 February 2016)** to the above research study.

The amendment may be implemented with immediate effect in the Leeds Teaching Hospitals NHS Trust under the existing NHS Permission. Please note that you may only implement the changes described in the amendment notice or letter

Continued NHS Permission for the project is subject to the following conditions:

Research Ethics Committee approval/regulatory approval for the amendment, if required, has been obtained
Any contractual arrangements relating to this change have been addressed
The Research Lead/Clinical Director for the Clinical Support Unit has approved any resource implications for the Directorate
Implications for support departments working on the project have been assessed and approved by the relevant support department.

If you have any queries about this acknowledgement please do not hesitate to contact the R&I Department on telephone (0113) 392 0162.

With kind regards

Yours sincerely

Richard Evans
R&I Manager

The documents reviewed and approved are listed as follows:-

Document	*Version*	*Date of document*
REC Letter Confirming Favourable Opinion for SA01	**Amendment date: 25 February 2016**	**16 March 2016**
Notice of Substantial Amendment (non-CTIMP)	Substantial Amendment 1 - 25/2/16	25 February 2016
PASS Results	Version 2	24 February 2016
Research protocol or project proposal	Version 2	24 February 2016

Appendix B.8 Substantial Amendment 1 Research and Development Approval Letter

B.9 Declaration of the End of the Study Acknowledgment

NHS
Health Research Authority

Yorkshire & The Humber - Sheffield Research Ethics Committee

Room 001
Jarrow Business Centre
Rolling Mill Road
Jarrow
Tyne & Wear
NE32 3DT

Tel: 0207 104 8282

16 June 2017

Mrs M Albahrani
Post-graduate student Paediatric dentistry
University of Leeds
Postgraduate room, Level 6
School of Dentistry, Worsley building
University of Leeds, Leeds, UK
LS2 9LU

Dear Mrs Albahrani

Study title:	**Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing.A randomised controlled Trial**
REC reference:	**16/YH/0015**
IRAS project ID:	**190951**

Thank you for sending the declaration of end of study form, notifying the Research Ethics Committee that the above study concluded on 31 May 2017. I will arrange for the Committee to be notified.

A summary of the final research report should be provided to the Committee within 12 months of the conclusion of the study. This should report on whether the study achieved its objectives, summarise the main findings, and confirm arrangements for publication or dissemination of the research including any feedback to participants.

In England, Wales and Northern Ireland, samples may be held after the declaration of the end of the trial, for analysis or verification of research data for up to one year. After this period legal authority to hold any human tissue under the ethical approval for this project will expire. To ensure that any continued storage is lawful, either the tissue must be held on premises with a storage licence from the Human Tissue Authority, or an application made for ethical approval of another project before the favourable ethical opinion of the existing project expires. Otherwise the tissue would need to be destroyed in accordance with the HTA Codes of Practice.

A Research Ethics Committee established by the Health Research Authority

Appendix B.9 Declaration of the End of the Study Acknowledgment

16/YH/0015:	Please quote this number on all correspondence

Yours sincerely

Kerry Dunbar
REC Assistant

Email: nrescommittee.yorkandhumber-sheffield@nhs.net

Copy to: *Mrs Clare Skinner, University of Leeds*
MS Ann Gowing, Leeds NHS R&D LTHT

A Research Ethics Committee established by the Health Research Authority

Appendix B.9 Declaration of the End of the Study Acknowledgment

Appendix C : Research Materials

C.1 Investigator Site File

C. 2 Blinding the Groups of the study - Instructions

C. 3 Blinding the Groups of the Study - Flow Chart

C.4 Random Assignment of Participants List

C.5 Advertisement Materials for Research Participants

C.6 Medical History Form

C.7 Appointment Message Example

C.8 Reminder Message Example

C.9 Participant Information Sheet

C.10 Participant Consent Form

C.11 Case Record Form

C.12 Appointment Checklist

C.13 Thank You Card

C.14 Cash Payment Receipt

C.15 Group Randomisation Code Breaker

C.16 Letter of appreciation

C.1 Investigator Site File

Table 11.10-3 Table showing contents of investigator site file.

SECTION	CONTENT/COMMENTS	DATE AND VERSION WERE APPLICABLE
Protocol / amendments	Current protocol	09 May 2016 Version 3
	Superseded protocols	24 Feb 2016 Version 2 17 Dec 2015 Version 1
Ethics approval documentation	REC application form (signed)	18 Dec 2015
	Ethics submission letter	30 Dec 2015
	Ethics approval letter	12 Feb 2016
	Ethics amendment notification letter	Version 2 03 March 2016 Version 3 09 May 2016
	Notification of amendments forms	Version 2 25 Feb 2016 Version 3 09 May 2016
	Ethics correspondence	Version 2 16 March 2016 Version 3 16 May 2016
R&D approval documentation	R&D application form (signed)	18 Dec 2015
	SSI application form (signed)	15 Feb 2016
	R&D submission letter	15 Feb 2016
	R&D approval letter	15 Feb 2016
	R&D substantial amendment notification letter	17 March 2016

SECTION	CONTENT/COMMENTS	DATE AND VERSION WERE APPLICABLE
	R&D correspondence	17 March 2016
CSU approval documentation	CSU approval	8 Jan 2016
	CSU substantial amendments approval	18 March 2016
Signed Delegation of Duties log		
Curriculum Vitae (signed and dated)	CVs for all research personnel listed in the Delegation of Duties log	
	GCP certificates for research personnel, where applicable	
	Informed consent certificates for research personnel, where applicable	
Patient Identification log	ID log contains list of all patients recruited onto the study	
Patient screening log	Contains list of all patients considered for the study	
Participant Data log	Participants data collection sheet	
Patient Information / Informed Consent form, Patient Invitation Letter and GP Letter	Current Patient Information / Informed Consent form	Version 2 17 Dec 2015
	Superseded Patient Information / Informed Consent form	Version 1 1 Oct 2015
	Current patient invitation letter	See PIS
	Recruitment advert / Recruitment documents Email and poster	Version 2 17 Dec 2015
	Completed Informed Consent Forms	
Sample CRF	Sample CRF	Version 1 12 May 2016
	Appointment Checklist	Version 1 09 September 2016

SECTION	CONTENT/COMMENTS	DATE AND VERSION WERE APPLICABLE
	Completed CRFs (If too bulky to put in file place file note in this section stating where it can be found)	
Data Summary	Salivary fluoride concentration / data	
Appointment card (for patient)	Appointment messages Reminder messages	Version 1 17 December 2015
Expenses documents	Expenses log	
	Sample expenses receipt form	
	Signed expenses receipt forms	
General Correspondence	File in chronological order all correspondence. File email communications. Include a separate section here for newsletters	
Meeting reports/minutes	Meeting log	
Notes of telephone calls	Document telephone call in relation to agreements or significant discussions regarding trial administration, trial conduct, adverse events or protocol violations	
Randomisation details	Instructions (if applicable)	
	Participant Randomisation Log	
	Toothpaste Randomisation Log	Kept in a sealed envelope
Instructions for handling trial medication and trial related materials	Shipping records/receipts	
	Product distribution Log (to patients)	
	Trial Related Materials	
Contracts	Proof of sponsorship	
	Indemnity	

SECTION	CONTENT/COMMENTS	DATE AND VERSION WERE APPLICABLE
	Financial disclosure letter (if applicable)	
Completed Data Queries		
Study Training Materials	Study specific SOPs	
	Proof of reading logs	
	Training log	
	Equipment manuals	
	Safety data sheets	
Miscellaneous	Risk assessment	
	Rinsing log	
	Research Study Timeline	
	Appointment diary	

C.2 Blinding the Groups of the study – Instructions

The Leeds Teaching Hospitals NHS

UNIVERSITY OF LEEDS

Dental Translational and Clinical Research Unit

A Double Blinded Randomised Controlled Trial

Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing

CENTRE: DenTCRU, Leeds Dental Institute **REC NUMBER:** *16/YH/0015*
CHIEF INVESTIGATOR: *Marwah Albahrani*

Double blinding the groups of the study

Formulation	Rinsing method	Group number
NaF	R	G
MFP	R	G
NaF + MFP	R	G
SnF + NaF	R	G
AmF	R	G
No fluoride	R	G
NaF	NR	G
MFP	NR	G
NaF + MFP	NR	G
SnF + NaF	NR	G
AmF	NR	G
No fluoride	NR	G

NaF Sodium fluoride **MFP** Sodium monofluorophosphate **Naf+MFP** sodium fluoride with sodium monofluorophsophate combined **SnF + NaF** stannous fluoride with sodium fluoride combined **AmF** Amine fluoride **R** Rinsing **NR** No rinsing

This will be done using the following website:

https://www.randomlists.com/team-generator

The name of the groups will be put in the items box as follows:

NaF – R

MFP – R

NaF + MFP – R

SnF + NaF – R

Amf – R

Version 1.0 01/12/16

The Leeds Teaching Hospitals NHS

UNIVERSITY OF LEEDS

Dental Translational and Clinical Research Unit

A Double Blinded Randomised Controlled Trial

Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing

CENTRE: DenTCRU, Leeds Dental Institute **REC NUMBER:** *16/YH/001*
CHIEF INVESTIGATOR: *Marwah Albahrani*

Double blinding the groups of the study

No fluoride – R

NaF – NR

MFP – NR

NaF + MFP – NR

SnF + NaF – NR

Amf – NR

No fluoride - NR

Quantity:

To be set as 12

Groups:

To be set as 12

Then click Refresh

This should randomly assign each toothpaste and rinsing method to a group (G1-G12).

Complete the table

Complete the group numbers above depending on the results of the random generator.

Version 1.0 01/12/16

Appendix C. 2 Blinding the Groups of the study - Instructions

C.3 Blinding the Groups of the Study - Flow Chart

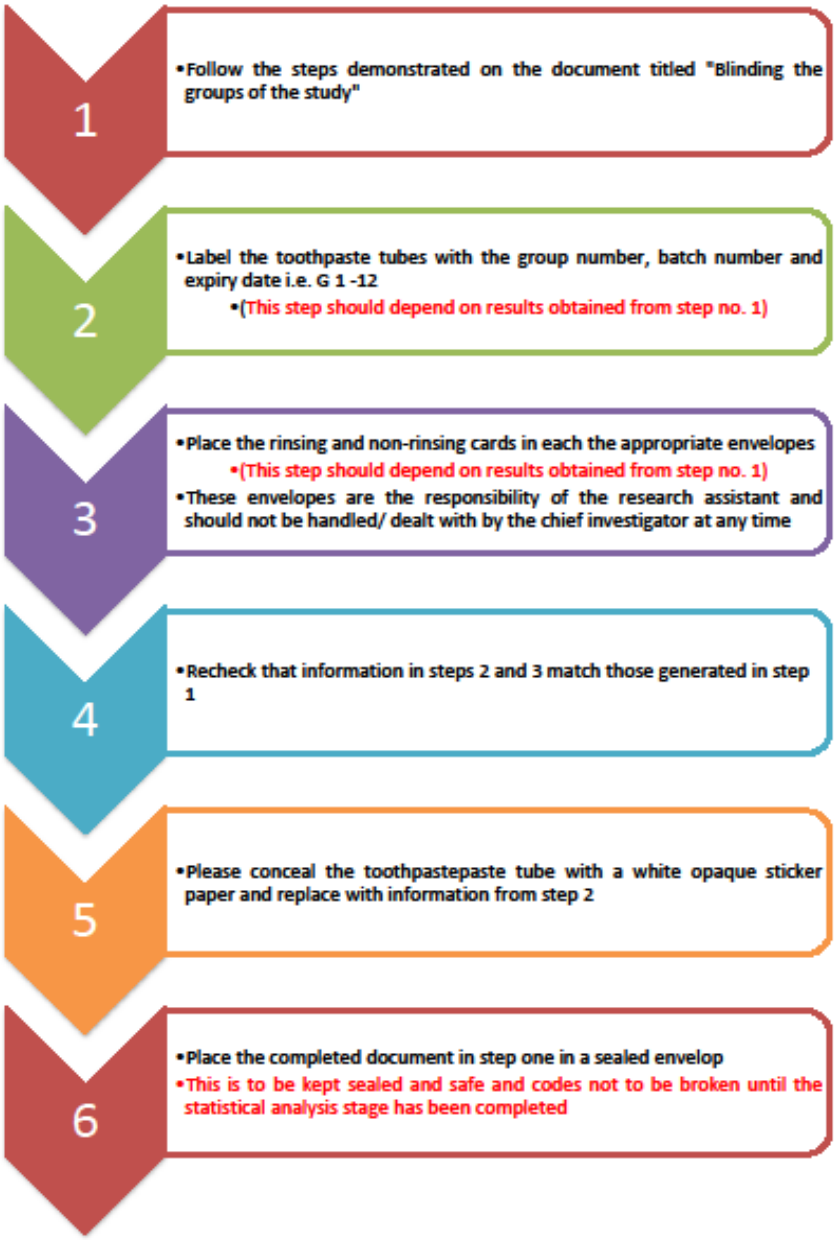
UNIVERSITY OF LEEDS

Dental Translational and Clinical Research Unit

A Double Blinded Randomised Controlled Trial

Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing

CENTRE: DenTCRU, Leeds Dental Institute **REC NUMBER:** *16/YH/0015* **CHIEF INVESTIGATOR:** *Marwah Albahrani*



Version 2.0 06/05/16

Appendix C. 3 Blinding the Groups of the Study - Flow Chart

C.4 Random Assignment of Participants List

The Leeds Teaching Hospitals NHS

UNIVERSITY OF LEEDS

Dental Translational and Clinical Research Unit

A Double Blinded Randomised Controlled Trial

Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing

CENTRE: DenTCRU, Leeds Dental Institute **REC NUMBER:** *16/YH/0015*
CHIEF INVESTIGATOR: *Marwah Albahrani*

Randomisation of all the 120 participant identification numbers (Participant ID) was performed using the following website:

https://www.randomlists.com/team-generator

The results of the randomisation were as follows:

Random Team Generator:

Group 1	*Group 2*	*Group 3*	*Group 4*
47.	48.	12.	73.
105.	23.	10.	22.
83.	100.	88.	114.
13.	54.	26.	9.
28.	21.	39.	91.
16.	68.	70.	31.
61.	51.	44.	79.
120.	107.	2.	19.
1.	41.	76.	52.
98.	56.	89.	3.

The Leeds Teaching Hospitals NHS

UNIVERSITY OF LEEDS

Dental Translational and Clinical Research Unit

A Double Blinded Randomised Controlled Trial

Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing

CENTRE: DenTCRU, Leeds Dental Institute **REC NUMBER:** *16/YH/0015*
CHIEF INVESTIGATOR: *Marwah Albahrani*

5. *Group 5*	6. *Group 6*	7. *Group 7*	8. *Group 8*
1. 37.	1. 118.	1. 99.	1. 81.
2. 63.	2. 112.	2. 5.	2. 113.
3. 64.	3. 106.	3. 62.	3. 94.
4. 34.	4. 110.	4. 7.	4. 38.
5. 6.	5. 115.	5. 96.	5. 65.
6. 103.	6. 57.	6. 95.	6. 116.
7. 67.	7. 53.	7. 117.	7. 4.
8. 80.	8. 111.	8. 87.	8. 50.
9. 29.	9. 84.	9. 42.	9. 78.
10. 86.	10. 108.	10. 55.	10. **74.**

The Leeds Teaching Hospitals NHS

UNIVERSITY OF LEEDS

Dental Translational and Clinical Research Unit

A Double Blinded Randomised Controlled Trial

Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing

CENTRE: DenTCRU, Leeds Dental Institute **REC NUMBER:** *16/YH/0015*
CHIEF INVESTIGATOR: *Marwah Albahrani*

9. *Group 9*	10. *Group 10*	11. *Group 11*	12. *Group 12*
1. 104.	1. 25.	1. 82.	1. 92.
2. 101.	2. 45.	2. 43.	2. 35.
3. 8.	3. 17.	3. 30.	3. 11.
4. 102.	4. 72.	4. 66.	4. 32.
5. 77.	5. 14.	5. 119.	5. 33.
6. 71.	6. 59.	6. 24.	6. 27.
7. 20.	7. 60.	7. 40.	7. 75.
8. 97.	8. 18.	8. 15.	8. 69.
9. 93.	9. 36.	9. 90.	9. 49.
10. 58.	10. 46.	10. 109.	10. 85.

C.5 Advertisement Materials for Research Participants

C.6 Medical History Form

Leeds Dental Institute
FACULTY OF MEDICINE AND HEALTH
UNIVERSITY OF LEEDS

Medical History Form V. 1 – 17/12/2015

Participant Reference No.	Yes	No
Are you currently taking any prescribed medications?	☐	☐
Are there any treatments or materials that you are allergic to or that make you unwell?	☐	☐
Do you have any problems with your heart?	☐	☐
Have you ever had a stroke or Transient Ischaemic Attack – TIA ('mini-stroke')?	☐	☐
Do you have any problems with your breathing?	☐	☐
Do you have Diabetes?	☐	☐
Do you have an underactive immune system?	☐	☐
Do you have epilepsy or suffer from any other seizures?	☐	☐
Do you have any other problems related to the nervous system?	☐	☐
Are you prone to fainting or panic attacks including hyperventilation?	☐	☐
Do you have any mental issues such as depression, anxiety, schizophrenia or dementia?	☐	☐
Do you have any problems with your kidneys?	☐	☐
Do you have any problems with your liver?	☐	☐
Could you have any infections that could be passed to others such as hepatitis, HIV or Tuberculosis?	☐	☐
Have you ever had cancer (including blood cancer – leukaemia)?	☐	☐
Have you ever had an operation?	☐	☐
Do you have a tendency to bleed after injury?	☐	☐

Is there anything else you think we should know about you that you feel might be important to help us manage you during an emergency?

ASA	1	2	3	4	5

History Completed by :	Date
History Checked on the day of experiment by:	Date

REC: 16/YH/0015

C.7 Appointment Message Example

Appointment Messages V.1-17/12/2025

Dear [Mr. / Mrs. / Ms] [Surname of participant], this is a confirmation of your appointment at [Time] on [Day], [Date] at the [Place]. If unable to attend please reply with the word CANCEL. [Sender of the text]. [Email]

Example Messages

Dear Mr. John Smith, this is a confirmation of your appointment at 07:45 on Thu, 16 of Jul at the DenTCRU, The University of Leeds School of Dentistry LS2 9LU. If unable to attend please reply with the word CANCEL. Regards Regards Marwah from the Leeds Dental Institute.dnmalb@leeds.ac.uk

C.8 Reminder Message Example

Reminder Messages V.1 – 17/12/2015

Don’t forget your appointment at (time) on (day) (date) at the (place). Please reply Y to confirm, N to cancel or STOP to quit. Please do NOT brush on the day of your appointment and do NOT eat or drink (no water as well) at least two hours prior to your appointment. Regards Marwah from the Leeds Dental Institute.dnmalb@leeds.ac.uk

Example Messages

Don’t forget your appointment at 07:45 on Thursday 16 of July at DenTCRU, The University of Leeds School of Dentistry LS2 9LU. Please reply Y to confirm, N to cancel or STOP to quit. . Please do NOT brush on the day of your appointment and do NOT eat or drink (no water as well) at least two hours prior to your appointment. Regards Marwah from the Leeds Dental Institute. dnmalb@leeds.ac.uk

C.9 Participant Information Sheet

REC: 16/YH/0015

School of Dentistry

FACULTY OF MEDICINE & HEALTH

UNIVERSITY OF LEEDS

PARTICIPANT INFORMATION SHEET V.2 – 17/12/2015

You are invited to take part in this research project. Please read the following information sheet. If you have any questions or queries, please do not hesitate to contact the researcher. Contact details are provided at the end of this information sheet.

Title of Research:

Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpaste With and Without Post-brushing water rinsing.

What is the purpose of the research?

This research is being conducted as part of a degree dissertation project for a postgraduate student in the University of Leeds (Professional Doctorate in Paediatric Dentistry).

Why are we conducting this research?

The regular use of fluoridate toothpastes has led to marked reduction in dental decay incidence in the UK during the past 3 – 4 decades. Data from 2008 – 2012 shows there was a reduction in the number of 5 year-old-children with dental decay in the UK. Nearly, a third of 5 year-old-children in the UK had dental decay in 2012. On average, these children had more than 3 teeth that were decayed, missing or filled (at age five, children normally have 20 baby teeth)[i]. We aim to reduce those figures even further by providing oral hygiene advice that is based on actual research. With your help we can achieve that.

What will I be asked in the research?

You will only need to attend one visit, which will last for 2 hours. You will be asked to brush with one of six toothpastes, with or without water rinsing after brushing. The type of toothpaste and rinsing method will be determined randomly by online randomisation software. Six saliva samples will be collected before brushing and at different intervals up to 90 minutes. This will help us determine how long can fluoride last in the mouth after brushing with different toothpastes. Your saliva samples will then be destroyed according to the Human Tissue Authority's Code of Practice.

How will you use my personal details?

When you sign your consent sheet, you will need to provide us with your name, contact number and mailing address. If decided to take part, we only need to contact you to tell you about when and where your appointment will be. A day before you appointment, you will receive a reminder text message. We will not share your information with third parties and will not contact you for anything else. After we have analysed our data, we will write to you informing you about our results. Your details will remain confidential until they are destroyed according to the University of Leeds policy.

What shall I do on the day of the research?

We would kindly ask you:

1. Not to brush on morning of the experiment.
2. Not to eat or drink for at least 2 hours before your appointment.

REC: 16/YH/0015

3. Not to eat or drink during the experiment.

Will my information be kept confidential?

All your information will be kept highly secure and confidential and you will not be identified in any reports or publications. When the dissertation is submitted, all the information will be destroyed according to the University of Leeds Policy.

Is this research funded?

This research project is not funded. To cover out your pocket expenses, including travel and food, each volunteer will receive 10 pounds at the end of the experiment.

Do I have to take part?

It is up to you to decide. We will describe the study and go through the information sheet, which we will give to you. We will then ask you to sign a consent form to show you agreed to take part. You are free to withdraw at any time, without giving a reason. Your withdrawal will not affect the standard of care you receive.

Please do not hesitate to contact us if you have any questions or queries:

Mrs Marwah Albahrani
Post-graduate student
Paediatric department
University of Leeds
LS2 9LU

Tel: 07984420031
Email: dnmalb@Leeds.ac.uk

Professor K. J. Toumba
Honorary professor in Paediatric dentistry
Paediatric department
University of Leeds
LS2 9LU

Tel: 0113 343 6141
Email: k.j.toumba@Leeds.ac.uk

* Public Health of England (2012) National Dental Epidemiology Programme for England: Oral health survey for five-year-old children. A report on the prevalence and severity of dental decay. Available at: [http://www.nwph.net/dentalhealth/Oral%20Health%205yr%20old%20children%202012%20final%20repor%20gateway%20approved.pdf].

C.10 Participant Consent Form

REC: 16/YH/0015

School of Dentistry

FACULTY OF MEDICINE & HEALTH

UNIVERSITY OF LEEDS

PARTICIPANT CONSENT FORM V. 2 – 17/12/2015

This research is conducted by Mrs M. Albahrani, **under the supervision of** Professor K. J. Toumba.

Title of Research
Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing.

This consent form establishes that you have read and understood what taking part in this study will involve. After reading the enclosed "Participant Information Sheet V.2" then initial all boxes that apply.

I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.	☐
I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason. If decided to withdraw, this should not carry any prejudice to my current and / or future dental treatment at Leeds Dental Institute.	☐
I understand that you will use your reasonable endeavours to preserve my anonymity and any personal information collected is confidential.	☐
I understand that it is very unlikely that I will be harmed as a result of taking part in this study.	☐
I understand that the study will include brushing with one of six toothpastes and I might be asked to either rinse or not with water after brushing.	☐
I understand that my saliva will be collected at six different times during the study. After the analysis, I understand that my saliva will be disposed safely and confidentially according to the Human Tissue Authority's Code of Practice.	☐
I understand that in case of data publication of this research, my personal details will still remain anonymous.	☐
I consent (agree) to take part in this study.	☐
I agree to the research team having the following details for the purpose contacting me directly to arrange a research appointment, send me a reminder appointment, send me a letter of appreciation and contacting me regarding the results of this study.	☐

Volunteer's Name: ..... Signature: .....

Contact number: ..... Date: .....

Volunteer's Mailing Address: .....

.....

Name of person taking consent: .....Signature: .....

C.11 Case Record Form

School of Dentistry

FACULTY OF MEDICINE & HEALTH

UNIVERSITY OF LEEDS

Case Record Form (CRF)

Participant Initials	__ __ __
Participant DOB	_ _ / _ _ / _ _ _ _
Participant Screening Number	**S** __ __ __
Participant ID Number	__ __ __
Randomisation Group Number	__ __ __

A Double Blinded Randomised Controlled Trial

Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing

Chief Investigator: Mrs Marwah Albahrani

Principle Supervisor: Professor K. J. Toumba

Co-supervisors: Dr. M. Malinowski, Professor M. Duggal

Version 1.0 : 27 May 2016

REC NUMBER: 16/YH/0015

Participant Initials	___
Participant DOB	__/__/____
Participant Screening Number	S ___

Screening Visit

Date of Visit __/__/____

Participant's information sheet (PIS) Version __ Dated __/__/____	Date participant given copy of PIS: __/__/____ PIS should have been provided / read by participant at least 48 hours before informed consent can be obtained.

***Note: If PIS has not been given / read by participant at least 48 hours prior to screening visit, then informed consent cannot be obtained and the participant should be discontinued from the study as "deviation from protocol" or "other" on the study conclusion page.**

Pre-appointment instructions Checklist

Has the participant followed the instructions by NOT brushing their teeth the morning of the study date?	**Yes**	**No**
Has the participant appropriately fasted; did not eat or drink in the past 2 hours? Time of last food / drink __:__ (hh:mm)	**Yes**	**No**

***Note: If any of the above questions are answered "No", the participant should be discontinued from the study as "screening failure" on the study conclusion page.**

Medical History Form Completed and Signed ☐ Yes ☐ No

Participant's Consent Sheet (PCS) Version __ Dated __/__/____	Date of informed consent: __/__/____ Time of Informed consent __:__ (hh:mm)

Chief Investigator's Signature ..... **Date** __/__/____

Participant Initials	**_ _ _**
Participant DOB	**_ _ / _ _ / _ _ _ _**
Participant Screening Number	**S _ _ _**

Screening Visit

Date of Visit _ _ / _ _ / _ _ _ _

Dental Examination (Salivary Flow Rate)

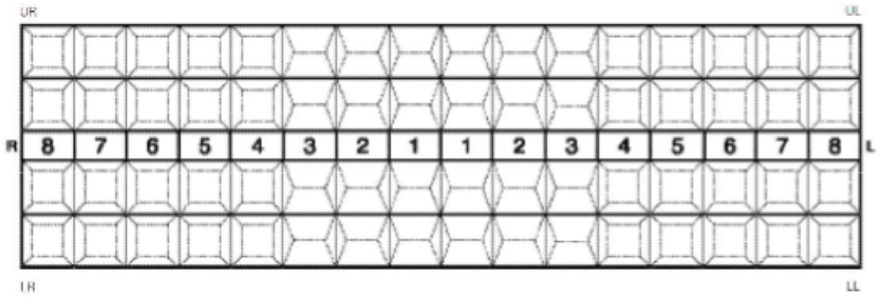
Unstimulated salivary flow rate _ . _ _ ml / 2 min

(Must be ≥ 0.1 ml / min) _ . _ _ ml / 1 min

***Note: If the salivary flow rate is < 0.1 ml/min, the participant should be discontinued from the study as "screening failure" on the study conclusion page.**

Time Saliva Sample disposed _ _ : _ _ (hh:mm)

Dental Examination (Teeth Chart)



DMFS	**DS**	**MS**	**FS**
DMFT	**DT**	**MT**	**FT**

Chief Investigator's Signature ..... **Date** _ _ / _ _ / _ _ _ _

Participant Initials	___
Participant DOB	__/__/____
Participant Screening Number	S ___

Inclusion Criteria Checklist

Is the participant 18 years or over?	**Yes**	**No**
Is participant's salivary flow rate ≥ 0.1 ml / min?	**Yes**	**No**
Is participant's ASA grade I or II?	**Yes**	**No**

***Note: If any of the above questions are answered "No", the participant should be discontinued from the study as "screening failure" on the study conclusion page.**

Exclusion Criteria Checklist

Is the participant edentulous?	**Yes**	**No**
Is the participant's ASA grade of III or higher?	**Yes**	**No**
Is the participant allergic to any of the materials used in the study	**Yes**	**No**
Is the participants wearing an orthodontic device / brace?	**Yes**	**No**
Is the participants salivary flow rate of < 0.1 ml / minute?	**Yes**	**No**
Is the participants incapable of fasting for 4 hours?	**Yes**	**No**
Does the participant want to have control over which group to be in?	**Yes**	**No**
Is the participant incapable of retaining the toothpaste without rinsing (ie. gagging due to toothpaste taste)?	**Yes**	**No**

***Note: If any of the above questions are answered "Yes", the participant should be discontinued from the study as "screening failure" on the study conclusion page.**

Chief Investigator's Signature ..... **Date** __/__/____

Participant Initials	___
Participant DOB	__/__/____
Participant Screening Number	S___

Fitness and Eligibility to Participate in the Research Study

In the investigator's opinion, on the basis of the Pre-appointment instructions checklist and inclusion and exclusion criteria checklists, is the participant eligible to participate / take part in the study?

☐ **Yes** ☐ **No**

Chief Investigator's Signature ..... **Date** __/__/____

Participant Initials	___
Participant DOB	__/__/____
Participant Screening Number	S ___

Fluoride Analysis Study

Date of Visit __/__/____

Fluoride Analysis Table

Participant Inclusion Number ___

Group Randomisation Number ___

Tooth brush weight (grams)	Dispensed toothpaste weight (grams) ≈ 1.0 g	Toothbrush and toothpaste weight (grams)

Participant brushed for 2 full minutes **Yes** **No**

Brushing time (hh:mm) __:__

Participant followed rinsing / non-rinsing procedures appropriately according to the randomisation group number (please refer to the rinsing log for more details). **Yes** **No**

***Note: participant rinsing, should rinse with 10 ml of distilled water for 5 seconds.**

Sex	F	M	**Age**		**Caries** +	-
Saliva Sample Interval	Pre-brushing	1 mins post-brushing	15 mins post-brushing	30 mins Post-brushing	60 mins Post-brushing	90 mins post-brushing
Time	__:__	__:__	__:__	__:__	__:__	__:__
Fluoride Concentration						
Disposal Time of Sample	__:__	__:__	__:__	__:__	__:__	__:__

Chief Investigator's Signature ..... **Date** __/__/____

Participant Initials	___
Participant DOB	__/__/____
Participant Screening Number	S___

Study Conclusion

Has the participant completed the entire study? ☐ **Yes** ☐ **No**

If yes, date completed __/__/____

If no, please complete the following (please tick as appropriate):

☐ Screen failure

☐ Adverse event failure

Protocol deviation (please specify details) ☐

Withdrawal of participant (please specify details) ☐

Other (please specify details) ☐

Chief Investigator's Signature ..... **Date** __/__/____

Participant Initials	___
Participant DOB	__/__/____
Participant Screening Number	S ___

Chief Investigator Signature

I confirm that I have reviewed all the data collected in this Case Report Form (CRF) and take responsibility that the information is accurate and complete.

Chief Investigator's Name .....

Chief Investigator's Signature .....

Date __/__/____

C.12 Appointment Checklist

The Leeds Teaching Hospitals NHS

UNIVERSITY OF LEEDS

Dental Translational and Clinical Research Unit

A Double Blinded Randomised Controlled Trial

Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing

CENTRE: DenTCRU, Leeds Dental Institute **REC NUMBER:** *16/YH/0015*

CHIEF INVESTIGATOR: *Marwah Albahrani*

Participant Initials	_ _ _
Participant DOB	_ _ / _ _ / _ _ _ _
Participant Screening Number	**S** _ _ _

Appointment checklist

The following forms have been completed and signed:

Case record form (CRF)	[ ]
Medical history form (MH)	[ ]
Participant's consent sheet (PCS)	[ ]

The participant has been given:

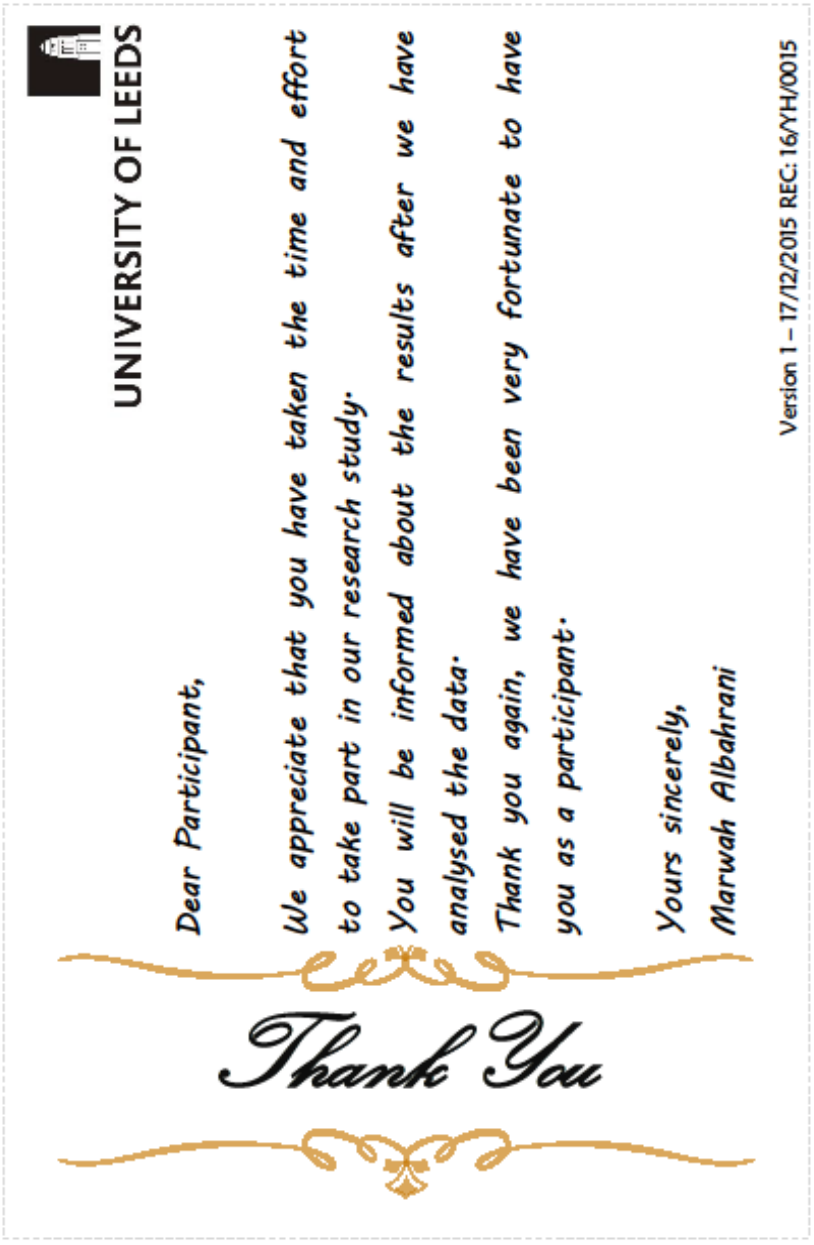
Copy of participant's consent	[ ]
Cash payment of 10.00 GBP	[ ]
Copy of cash payment reciept	[ ]
Thank you card	[ ]

Chief Investigator's Signature ..... **Date** _ _ / _ _ / _ _ _ _

Version 1.0 09/09/16

C.13 Thank You Card

Cut along dotted line and fold at center (US Size card 5. 5 x 4.25 inches)



C.14 Cash Payment Receipt

The Leeds Teaching Hospitals NHS

UNIVERSITY OF LEEDS

Dental Translational and Clinical Research Unit

A Double Blinded Randomised Controlled Trial
Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing water rinsing

CASH PAYMENT RECEIPT

PARTICIPANT NAME: _____

PARTICIPANT SCREENING NUMBER: S ___

DATE (dd/mm/yyyy): __/__/____

RECEIVED THE SUM OF: £

COMMENTS (E.g. visit name/number): _____

_____

_____

Participant has been informed that they will need to declare the payment to HMRC via self-assessment

PARTICIPANT SIGNATURE: _____

CLINICAL RESEARCH NURSE SIGNATURE: _____

Version1.0 23.04.16

C.15 Group Randomisation Code Breaker

RandomLists

Custom List

Random Team Generator:

Group 1

1. No fluoride - NR

Group 2

1. Amf – R

Group 3

1. NaF + MFP – R

Group 4

1. Amf – NR

Group 5

1. MFP – R

Group 6

1. MFP – NR

Group 7

1. NaF + MFP – NR

Group 8

1. NaF – R

Group 9

1. NaF – NR

Group 10

1. SnF + NaF – NR

Group 11

1. SnF + NaF – R

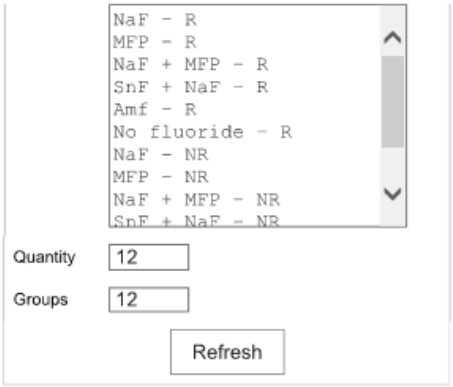
Group 12

1. No fluoride – R

[Updated at: September 13, 2016 5:36:51 AM]

Options:

Items



How to create randomized groups

Enter each item on a new line, choose the amount of groups unders settings, and click the button to generate your randomized list. Don't like the first team? Just click again until you do.

Random pairing generator

Need your list separated into pairs? Easy. Just choose "2" as the amount of items per group.

Fairly pick teams without bias. No need to draw names out of a hat. No need to do a grade school style draft or put hours of thought into the most balanced teams. The most fair dividing method possible is random.

Mix up your to-do list by generating random groups out of them. For example, enter all your housecleaning activities and split them into seven groups, one for each day or one for each person.

Just want a single group? Use the list randomizer.

Similar to this:

- Custom List

Looking for something else?
Create a random list, generate a random team, browse the site, search or browse randomly!

Policies | Suggestions | Contact

C.16 Letter of appreciation

School of Dentistry

FACULTY OF MEDICINE & HEALTH

UNIVERSITY OF LEEDS

Letter of Appreciation V.1-17/12/2015

[Day/Month/Year]

Name of Participants
Address Line 1
Address Line 2
Post Code

Mrs. Marwah Albahrani
Paedaitric Department
University of Leeds
LS2 9LU
Tel: 07984420031
Email: dnmalb@leeds.ac.uk

Dear Mr. / Mrs. / Ms. [Last Name of Participant],

As you may recall, you previously volunteered to be in our study

"Total Salivary Fluoride Concentration of Healthy Adult Subjects Following Toothbrushing with Different Formulations of Fluoridated Toothpastes With and Without Post-brushing Water Rinsing"

We appreciate that you have taken the time and effort to take part in our research study. Without your participation, this research would have not been possible.

Taking part in such research studies is one of the most important ways that we can improve our dental services and care. The results of this study will be published in the medical literature.

I have enclosed a summary of our results and conclusions from this study.

Thank you again, we have been very fortunate to have you as a participant.

Yours sincerely,

Marwah Albahrani

Appendix D : Statistical analysis

D.1 Descriptive statistics of salivary fluoride concentration for individual groups.

D.2 P-values tables comparing between non-rinsing groups.

D.3 P-values tables comparing between rinsing groups.

D.1 Descriptive statistics of salivary fluoride concentration for individual groups.

Table 11.10-4 Descriptive statistics (Mean and standard deviation (SD)) of the salivary fluoride concentrations.

Group Number.	Baseline time interval Mean ± SD	1 minutes time interval Mean ± SD	15 minutes time interval Mean ± SD	30 minutes time interval Mean ± SD	60 minutes time interval Mean ± SD	90 minutes time interval Mean ± SD
G01	0.106 ± 0.154	0.032 ± 0.193	0.050 ± 0.071	0.0407 ± 0.053	0.039 ± 0.060	0.041 ± 0.056
G02	0.059 ± 0.058	16.865 ± 9.286	1.650 ± 1.169	0.561 ± 0.414	0.312 ± 0.295	0.174 ± 0.160
G03	0.078 ± 0.080	12.285 ± 6.486	1.356 ± 0.849	0.443 ± 0.459	0.186 ± 0.259	0.100 ± 0.120
G04	0.173 ± 0.205	33.760 ± 17.507	2.784 ± 2.214	1.216 ± 1.044	0.500 ± 0.365	0.324 ±0.221
G05	0.046 ± 0.043	8.976 ± 4.519	0.867 ± 0.578	0.260 ± 0.137	0.107 ± 0.058	0.058 ± 0.029
G06	0.172 ± 0.143	12.775 ± 4.871	1.905 ± 1.281	0.537 ± 0.371	0.180 ± 0.101	0.113 ± 0.062
G07	0.046 ± 0.023	18.118 ± 10.066	1.512 ± 1.452	0.369 ± 0.292	0.149 ± 0.104	0.105 ± 0.086
G08	0.063 ± 0.043	15.104 ± 9.497	1.701 ± 0.856	0.452 ± 0.210	0.213 ± 0.104	0.138 ± 0.096
G09	0.048 ± 0.025	35.500 ± 18.351	3.322 ± 2.504	0.787 ± 0.523	0.299 ± 0.229	0.158 ± 0.095
G10	0.153 ± 0.117	21.919 ± 11.677	1.054 ± 0.673	0.272 ± 0.154	0.116 ± 0.058	0.071 ± 0.034
G11	0.087 ± 0.068	17.710 ± 9.433	2.245 ± 1.800	0.506 ± 0.342	0.175 ± 0.121	0.078 ± 0.042
G12	0.129 ± 0.120	0.037 ± 0.038	0.039 ± 0.049	0.023 ± 0.024	0.031 ± 0.048	0.020 ± 0.030

Table 11.10-5 Descriptive statistics (Upper and lower bonds 95 % confidence interval) of the salivary fluoride concentrations.

	95 % confidence interval											
	Baseline interval		1 min. interval		15 min. interval		30 minutes interval		60 min. interval		90 min. interval	
	LCI	UCI	LCI	UCI	LCI	UCI	LCI	UCI	LCI	UCI	LCI	UCI
G01	0.040	0.172	-6.320	6.385	-0.794	0.895	-0.226	0.308	-0.075	0.154	-0.024	0.105
G02	-0.007	0.125	10.512	23.218	0.805	2.494	0.294	0.828	0.197	0.426	0.110	0.238
G03	0.011	0.144	5.932	18.637	0.511	2.200	0.176	0.710	0.072	0.300	0.035	0.163
G04	0.107	0.239	27.407	40.113	1.939	3.628	0.949	1.483	0.386	0.615	0.260	0.389
G05	-0.020	0.112	2.623	15.329	0.022	1.711	-0.007	0.527	-0.008	0.221	-0.006	0.122
G06	0.106	0.238	6.422	19.128	1.061	2.749	0.270	0.804	0.066	0.294	0.050	0.178
G07	-0.020	0.113	11.765	24.471	0.667	2.356	0.102	0.637	0.035	0.263	0.041	0.169
G08	-0.003	0.129	8.751	21.457	0.857	2.546	0.185	0.719	0.099	0.328	0.074	0.203
G9	-0.019	0.114	29.147	41.853	2.477	4.166	0.520	1.055	0.185	0.414	0.094	0.222
G10	0.087	0.219	15.566	28.272	0.210	1.898	0.005	0.539	0.002	0.231	0.006	0.135
G11	0.021	0.153	11.357	24.063	1.401	3.090	0.249	0.783	0.061	0.290	0.014	0.143
G12	0.063	0.195	-6.316	6.390	-0.806	0.883	-0.244	0.290	-0.084	0.145	-0.045	0.084
	LCI	Lower bond confidence interval					UCI	Upper bond confidence interval				

D.2 P-values tables comparing between non-rinsing groups.

Key:

Highlighted cell	Denotes significant p-values ≤ 0.005

Table 11.10-6 P-values of non-rinsing groups at 1 minutes time interval.

	G01	G04	G06	G07	G09
G04	0.000				
G06	0.204	0.004			
G07	0.021	0.065	0.925		
G09	0.000	1.000	0.002	0.029	
G10	0.003	0.275	0.560	0.982	0.150

Table 11.10-7 P-values of non-rinsing groups at 15 minutes time interval.

	G01	G04	G06	G07	G09
G04	0.004				
G06	0.117	0.822			
G07	0.334	0.489	0.994		
G09	0.000	0.974	0.368	0.134	
G10	0.726	0.169	0.840	0.987	0.029

Table 11.10-8 P-values of non-rinsing groups at 30 minutes time interval.

	G01	G04	G06	G07	G09
G04	0.000				
G06	0.282	0.053			
G07	0.716	0.007	0.978		
G09	0.025	0.445	0.887	0.473	
G10	0.917	0.002	0.861	0.998	0.244

Table 11.10-9 P-values of non-rinsing groups at 60 minutes time interval.

	G01	G04	G06	G07	G09
G04	0.000				
G06	0.560	0.005			
G07	0.784	0.002	0.999		
G09	0.036	0.182	0.719	0.488	
G10	0.942	0.000	0.974	0.999	0.270

Table 11.10-10 P-values of non-rinsing groups at 90 minutes time interval.

	G01	G04	G06	G07	G09
G04	0.000				
G06	0.678	0.001			
G07	0.783	0.001	1.000		
G09	0.185	0.017	0.947	0.892	
G10	0.990	0.000	0.952	0.982	0.498

D.3 P-values tables comparing between rinsing groups.

Key:

Highlighted cell	Denotes significant p-values ≤ 0.005

Table 11.10-11 P-values of rinsing groups at 1 minute time interval.

	G02	G03	G05	G08	G11
G03	0.735				
G05	0.179	0.916			
G08	0.995	0.956	0.441		
G11	1.000	0.576	0.105	0.968	
G12	0.000	0.006	0.091	0.000	0.000

Table 11.10-12 P-values of rinsing groups at 15 minutes time interval.

	G02	G03	G05	G08	G11
G03	0.988				
G05	0.541	0.895			
G08	1.000	0.975	0.470		
G11	0.789	0.398	0.046	0.845	
G12	0.012	0.064	0.479	0.009	0.000

Table 11.10-13 P-values of rinsing groups at 30 minutes time interval.

	G02	G03	G05	G08	G11
G03	0.955				
G05	0.259	0.766			
G08	0.967	1.000	0.730		
G11	0.999	0.995	0.436	0.997	
G12	0.003	0.039	0.522	0.033	0.009

Table 11.10-14 P-values of rinsing groups at 60 minutes time interval.

	G02	G03	G05	G08	G11
G03	0.602				
G05	0.112	0.912			
G08	0.811	0.999	0.750		
G11	0.515	1.000	0.951	0.996	
G12	0.009	0.367	0.926	0.200	0.447

Table 11.10-15 P-values of rinsing groups at 90 minutes time interval.

	G02	G03	G05	G08	G11
G03	0.489				
G05	0.081	0.923			
G08	0.958	0.935	0.405		
G11	0.224	0.996	0.996	0.710	
G12	0.007	0.420	0.942	0.069	0.729