THE IMPACT OF THE DOWNWARD ADJUSTMENT OF FLUORIDE CONCENTRATION IN THE MALAYSIAN PUBLIC WATER SUPPLY ON DENTAL FLUOROSIS AND CARIES

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Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

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SUMMARY

In Malaysia, the public water supply has been artificially fluoridated since 1972 at an optimum level of 0.7 ppm fluoride as a public health measure to control dental caries. However, concerns arose that a fluoride concentration of 0.7 ppm was too high given increasing exposure to other sources of fluoride. That prompted a downward adjustment of the fluoride concentration from 0.7 to 0.5 ppm in 2005. In addition to Malaysia, there has recently been a movement towards the downward adjustment of fluoride concentration in the water in the United States, Hong Kong, Singapore and Ireland. However, little is known about the impact of such adjustments on oral health.

This thesis aimed to evaluate the outcome of the downward adjustment of fluoride concentration in the Malaysian public water supply from 0.7 to 0.5 ppm in relation to dental fluorosis and dental caries.

Two projects were conducted. The first project comprised a systematic review to critically appraise the literature on stopping the addition of fluoride or reducing the level of fluoride in public water supply on dental caries and fluorosis. This review highlighted the gaps in knowledge and several methodological issues such as lack of examiner blinding and control of confounders.

The second project was a cross sectional survey involving life-long residents aged 9 and 12 year-olds in fluoridated and non-fluoridated areas in Malaysia (*n*=1155). In the fluoridated area, children aged 12 years and 9 years were exposed to 0.7 and 0.5 ppmF respectively at the times when maxillary central incisors enamel developed. Fluoride exposures were assessed by questionnaire. Standardized photographs of maxillary central incisors were blind scored for fluorosis using Dean’s Index. Caries prevalence was examined using ICDAS-II criteria. The key findings indicated that the change in fluoride level from 0.7 to 0.5 ppm has reduced fluorosis and maintains caries preventive effect. The change in fluoridation concentration has also had a significant impact on caries prevalence at different thresholds of severity.

The findings support the policy initiative of a lower fluoride concentration in the Malaysian public water supply. It also highlights the need for modification of oral health advice with regards to fluoride exposure in maximising caries prevention while minimising fluorosis.

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List of abbreviations

BLC	Prof Barbara L. Chadwick *(supervisor)*
CAST	Caries Assessment Spectrum and Treatment
CFI	Community Fluorosis Index
CS	Cross sectional survey
DDE	Developmental defect of enamel
DFF	Dr Damian F. Farnell *(statistician)*
DI	Dean's Index
DMFS/dmfs	Decayed, missing, filling surfaces
DMFT/dmft	Decayed, missing, filling teeth
F	Fluoridated
FDI	World Dental Federation
ICDAS	International Caries Detection Assessment System
IGC	Prof Ivor G. Chestnutt *(supervisor)*
MYR	Malaysian Ringgit
NAMN	Nor Azlida Mohd Nor *(candidate)*
n.d	No date
NF	Non-fluoridated
NHNES	National Health and Nutrition Examination Survey
NSFEO	National Survey of Fluoride Enamel Opacities
NIDR	National Institute of Dental Research
NOHSS	National Oral Health Survey of School Children
ppm	Parts per million
ppmF	Parts per million fluoride
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PUFA	Pulp-Ulcer-Fistula-Abscess Index
Ref	Reference group
SD	Standard Deviation
SDS	School dental service
SPSS	Statistical Package for the Social Sciences

STPM	*'Sijil Tinggi Pelajaran Malaysia'*, Malaysian education qualification which is equivalent to Pre-University certificate
TF	Thylstrup-Fejerskov Index
TSIF	Tooth surface index of fluorosis
UK	United Kingdom
USA	United States of America
USD	United States Dollars
WHO	World Health Organization

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1 Introduction and literature review

1.1 Introduction to the thesis

This thesis consists of two main projects. The first project is a systematic review looking at the impact of stopping and reducing fluoride level in the water supply on caries and fluorosis. This is reported in Chapter 2. The second project is the main study, which evaluated the effect of a downward adjustment of fluoride level from 0.7 ppm to 0.5 ppm in the Malaysian water supply on caries and fluorosis. This comprised the main body of the thesis.

The thesis consists of seven chapters and is structured as follows:

Chapter 1 is an introduction to the thesis and a literature review that focuses on key evidence with regards to fluoride and its relation to dental caries and fluorosis. Chapter 2 describes the systematic review of the literature on the impact of stopping or reducing fluoride level in the water on dental caries and fluorosis. A discussion related to the key findings of the systematic review is also included. Chapter 3 presents the rationale for the main study followed by the research questions and aims and objectives. Chapter 4 describes the material and methods employed to address the study objectives. Chapter 5 reports the results of the main study. Chapter 6 presents a general discussion that draws together overall findings from the main study, how it is linked to the findings from the systematic review and the implications to the PhD project as a whole. Chapter 7 concludes the overall projects and provides the implications on practice and direction for future research.

1.2 Literature review

The literature review in this chapter is divided into four main sections. The first section gives an overview of fluoride in our environment and how it is related to oral health. The following sections review the key literature on dental fluorosis and dental caries. The last section presents an overview of fluoride exposure in Malaysia.

1.2.1 Fluoride and oral health

1.2.1.1 Availability, absorption, excretion and metabolism of fluoride

Fluoride is the ionic form of fluorine, a member of the halogen group. Fluorine is the most reactive and the most electronegative of the elements in the periodic table. However, fluorine is not found as its element form, it is found as the fluoride ion in soils, rocks and water in different concentrations (Smith and Ekstrand, 1988, Whitford, 1999, Dhar and Bhatnagar, 2009, Buzalaf and Whitford, 2011).

Following ingestion, fluoride is absorbed systemically from the gastrointestinal tract. Fluoride is taken-up in higher proportions from liquids than solids, approximately 85-97% from water and 80-90% from food (Trautner and Einwag, 1989, Whitford, 1999, Buzalaf and Whitford, 2011). The amount of fluoride absorbed is influenced by the concentration of cations such as calcium, magnesium and aluminium. High levels of cations bind with the fluoride ion and form insoluble substances which are less likely to be absorbed (Whitford, 1996). The rate of the absorption is also inversely related to the acidity of the gastric contents. The higher the acidity of the gastric content, the faster the fluoride absorption from the stomach (Messer and Ophaug, 1993). Of the fluoride that remains in the body, approximately 99% is deposited in bones, enamel and dentine.

Enamel fluoride concentrations are usually lower than dentine and bone. Enamel fluoride mainly reflects the levels of fluoride exposure during the tooth formation stage, whereas dentine and bone fluoride levels are generally the result of the dynamic metabolism of fluoride (Buzalaf and Whitford, 2011).

Fluoride is eliminated from the body through urine, faeces and sweat. The main fluoride excretion route is exclusively through urine, with about half of the absorbed fluoride being excreted within 24 hours. Approximately 10-25% of the total daily fluoride intake is not absorbed systemically and is consequently excreted through faeces (Ekstrand et al., 1994, Whitford, 1996, Maguire and Zohoori, 2013). Sweat is considered a minor route of fluoride excretion under most environmental conditions approximately 1-3:µmol/L (Smith and Ekstrand, 1996, Whitford, 1996, Buzalaf and Whitford, 2011).

1.2.1.2 Sources of fluoride exposure

Fluoride is found naturally in soil, rocks and plants and to a certain extent fluoride is present in water and food (Smith and Ekstrand, 1988, McGrady et al., 2010). Therefore everyone has some potential for fluoride ingestion on a daily basis. Fluoride may be ingested from different sources such as drinking water, salt, milk, food and beverages as well as from dental products such as toothpastes and mouth rinses (Dhar and Bhatnagar, 2009, Buzalaf and Levy 2011). The total intake of fluoride is a risk factor for fluorosis development. The details of fluorosis risk factors are described in Section 1.2.2.3.

1.2.1.3 Discovery of fluoride in caries prevention

The discovery of fluoride in caries prevention is attributed to the investigations carried out in the United States during the early decades of the 20th century (McKay, 1928, Dean, 1938, Dean, 1942). These studies were originally concerned with identifying the cause of the endemic condition of 'mottled enamel' or 'fluorosis' among children in areas where the water supply contained relatively high concentrations of natural fluoride. Data showed that children with 'mottled enamel' generally had a lower caries experience than those in areas without fluoride. These discoveries lead to the widespread introduction of artificial fluoridation of water supply in the United States and other countries worldwide.

Later research moved towards alternative methods of fluoride delivery such as fluoridated toothpaste (Fanning et al., 1968, Hollender and Koch, 1969, Marthaler, 1974). The use of fluoride toothpaste now constitutes the most common method of fluoride delivery and has been reported as the main reason for the reduction of caries rate in many industrialised countries since the 1970s (Bratthall et al., 1996).

1.2.1.4 Mechanism of fluoride in caries prevention

The anti-caries effect of fluoride has been well established. While in the mid-twentieth century the systemic effect of fluoride (i.e. incorporation into enamel during tooth formation) was thought to be crucial, it is now accepted that the primary effect of fluoride in caries prevention is post-eruptive (Burt, 2004). This includes fluoride delivered systemically (i.e. water fluoridation, fluoride supplements) or topically (i.e. fluoridated toothpaste, fluoride varnish/gel). In order to interfere in the dynamics of dental caries formation, fluoride must be constantly present in the oral environment at low concentrations. In the presence of fluoride, it has three principal topical mechanisms of action in caries prevention. These are inhibition of demineralisation, enhanced remineralisation, and inhibition of growth of plaque bacteria (Featherstone, 2000, Featherstone, 2004a).

In order to understand the mechanism of action, it is important to know the normal composition of tooth structure. This is because the structural dissimilarities between enamel and dentine have an effect on caries and fluoride activity within these tissues. Details of normal tooth composition are described below followed by the description of how fluoride plays a role in caries prevention. The aetiology of dental caries is described in depth in Section 1.2.3.1.

Normal composition of tooth structure

Teeth are formed from the calcium phosphate mineral hydroxyapatite. The solubility of hydroxyapatite depends on the pH level and ionic-levels of the hydroxyapatite components (calcium & phosphate) of the surrounding environment (Ten Cate and Featherstone, 1991, Ten Cate, 2013). Under normal oral physiological conditions, saliva and dental biofilms have a neutral pH 7.

Dental hard tissue consists of enamel and dentine, both of which have different compositions and structures. Enamel is the most highly mineralised tissue and is mainly comprised of hydroxyapatite crystallites (85% by volume), which are organized in long and thin apatite crystals. The space between the structure of enamel prims and hydroxyapatite crystals is filled with water (12% by volume) and organic material (3% by volume) (Ten Cate and Featherstone, 1991, He and Swain, 2008, Buzalaf and Levy, 2011). Enamel is the hardest tissue in the human body and it has a glossy surface and varies in colour from light yellow to greyish white (Chun et al., 2014).

Dentine is a mineralised, elastic, yellow-white, avascular tissue enclosing the central pulp chamber. Dentine consists of apatite crystals (47% by volume), organic components (33% organic components) and water (20% by volume) (Marshall et al., 1997). Dentine has less mineral (hydroxyapatite) than enamel and the crystallites have much smaller dimension than those in enamel. Although dentine is harder than bone, it is softer than enamel, so dentine is more prone to caries attack than enamel. The characteristic feature of dentine is its permeation by closely packed tubules traversing its entire thickness and containing odontoblasts cells. Odontoblasts located near the pulp chamber can be simulated to repair dentine when under caries attack (Nanci, 2007).

Fluoride inhibits demineralization

Dental caries is simply described as "demineralization, or loss of mineral from the tooth" (Featherstone, 2004a, Featherstone, 2004b). Acid produced by the bacteria when they ferment dietary carbohydrate dissolve the acid soluble dental mineral and produce soluble calcium and phosphate. These minerals then diffuse out from the tooth and lead to cavitation if the process is not stopped or reversed. When fluoride is present in an acidic solution surrounding enamel crystals, it is readily incorporated on to the surface of carbonated apatite and inhibits mineral loss (Ten Cate and Featherstone, 1991, Featherstone, 2000).

Fluoride enhances remineralisation

Following demineralisation, the natural repair process for carious lesions may take place (Zero, 1999, Featherstone, 2004b). This is known as remineralisation and is the process of placing back the lost mineral. Saliva is supersaturated with calcium and phosphate ions that can stimulate the mineral to re-enter the tooth structure. The partially demineralized surface of the enamel acts as a nucleus for new crystal growth. In the presence of fluoride during the remineralisation, it adsorbs to the crystal surface and attracts calcium and phosphate ions to form a new mineral (fluorapatite). The newly formed mineral has a stronger resistance to dissolution by acid than the original carbonated apatite (Featherstone, 2000). In addition the buffering capacity of saliva neutralised bacteria derived acids and favours the remineralisation process.

Fluoride inhibits bacteria growths

Fluoride ions act on the physiology of oral bacteria through several complex mechanisms. In its ionic form fluoride is not able to cross the cell wall and membrane. However in the form of hydrogen fluoride, it can penetrate the cariogenic bacteria cell membrane. When the pH in the plaque drops as the bacteria produce acids, the fluoride present in the plaque fluid combines with hydrogen ions to form hydrogen fluoride. It then rapidly diffuses into the cariogenic bacterial cells. Inside the cell, the hydrogen fluoride dissociates, acidifying the cell and releasing fluoride ions that inhibit bacterial enzyme activity (Featherstone, 2000). Although the anti-glycolotic effects of fluoride on oral bacteria metabolism are frequently cited, the degree to which this accounts for the caries protective effect of fluoride compared with the mineral effects discussed above are debatable (Ten Cate, 1999, Lussi et al., 2012).

1.2.1.5 The effect of fluoride on fluorosis development

Balancing the benefits and risks of fluoride is crucial because excessive exposure to fluoride during a critical period of tooth development is often associated with fluorosis. This section describes how fluoride acts on enamel and the way it induces dental fluorosis. The next Section (1.2.1.6) describes the critical period of development when teeth are most at risk of fluorosis.

Fluoride ingested during tooth development can result in changes to dental enamel form and structure due to alteration of the composition of the enamel matrix resulting in altered apatite crystal growth (Bronckers et al., 2009, Den Besten and Li, 2011). Several mechanisms have been suggested to explain how dental fluorosis occurs. These include the systemic effect of fluoride on calcium homeostasis, altered protein secretion, impaired matrix biosynthesis, direct effects on extracellular proteins and proteinases and specific effects on cell function and metabolism (LeGeros and Tung, 1983, Browne et al., 2005). The effect of fluoride on cell function is the mechanism that is most widely accepted and discussed in the literature. Fluoride is believed to have direct effects through interactions with the developing ameloblasts or interactions with the extracellular matrix (Den Besten, 1999, Bronckers et al., 2009).

Fluorosis occurs when fluoride interacts with mineralizing tissues, causing alterations in the mineralization process. The earliest sign is an increase in tissue hypomineralization (porosity) along the striae of Retzius (Fejerskov et al., 1994). This would appear as diffuse lines of opacity following the perikymata on the enamel surface. Severity increases with increased exposure to fluoride during enamel development. The surface and, in particular, the subsurface enamel becomes increasingly hypomineralized and increasingly porous. This subsurface porosity is most likely caused by a delay in the hydrolysis and removal of enamel proteins, particularly amelogenins during the enamel maturation stage (Den Besten, 1999). The diffuse lines of opacity appear widened and begin to merge to produce diffuse patches on the enamel. These patches appear as confluent chalky white areas of opacity and extend toward the dentine-enamel junction as severity increases. In the mild form, it mostly affects the coronal region at the outer half of the enamel and in the most severe form it may affect the entire enamel. The development and severity of the fluorosis depends on fluoride dose, timing and duration (Den Besten, 1999).

1.2.1.6 Timing of fluoride intake in relation to development of fluorosis in maxillary central incisors

Knowledge of the risk periods associated with the development of fluorosis is important not only for the understanding of the processes involved, but also to assist in minimising the risk of fluorosis when prescribing fluoride for caries prevention.

In order to understand when developing teeth are most at risk of fluorosis, it is crucial to know when calcification and eruption of primary and permanent teeth occurs (Table 1.1 and Table 1.2).

Calcification of permanent incisors begins at 3-4 months and is completed at 4-5 years. Completion of the crowns of primary molars overlaps with commencement of calcification of permanent incisors at around four months of age (Berkowitz et al., 1992). Enamel is no longer susceptible to fluorosis once its pre-eruptive maturation is complete (Institute of Medicine, 1997).

Table 1.1 Chronology of permanent teeth calcification and eruption

	Central incisors		Lateral incisors		Canines		First molars	
	U	L	U	L	U	L	U	L
Calcification commences	3-4m	3-4m	10-12m	3-4m	4-5m	4-5m	Birth	Birth
Completion of crown	4-5y	4-5y	4-5y	4-5y	6-7y	6-7y	2.5-3y	2.5-3y
Appearance in mouth	7-8y	6-7y	8-9y	7-8y	11-12y	9-10y	6-7y	6-7y

U, upper jaw: L, lower jaw; m, months; y, years. Adapted from Berkowitz et al. (1992).

Table 1.2 Chronology of primary teeth calcification and eruption

	Incisors	Canines	First molars	Second molars
Calcification commences	3rd-5th month IU	5th month IU	5th month IU	6th-7th month IU
Completion of crown	Age 4-5 months	Age 9 months	Age 6 months	Age 10-12 months
Appearance in mouth	Age 6-8 months	Age 16-20 months	Age 12-16 months	Age 21-30 months

IU, In utero. Adapted from Berkowitz et al. (1992).

Which period in tooth development might be most susceptible to the adverse effects of exposure to elevated levels of fluoride is a matter of debate in the literature. In the late 1980s Evans and Stamm (1991a) examined a series of epidemiologic ‘windows’ or time frames of differing lengths to determine the critical period during which developing maxillary central incisors are most prone to fluoride challenge. These authors found that “the most susceptible period was associated with a critical 4-month period commencing at 22 months after birth”. The authors also concluded that “fluoride exposure during the months prior to this period carry less risk than continued exposure for up to 36 months beyond this critical time”. However, these findings were only relevant to the risk period for the maxillary incisors and should not be used to infer the risk of fluorosis in relation to the whole dentition.

Recently, Buzalaf and Levy (2011) conducted a review of studies that examined the window of a maximum susceptibility to the development of dental fluorosis in the permanent maxillary central incisors (Table 1.3). The studies were divided into two categories: studies involving subjects exposed to fluoride starting at different ages during tooth development (Holm and Andersson, 1982, Osuji et al., 1988, Lalumandier and Rozier, 1995, Ismail and Messer, 1996, Wang et al., 1997, Bardsen and Bjorvatn, 1998, Hong et al., 2006a, Hong et al., 2006b) and subjects exposed to fluoride from birth and then experiencing a sudden reduction in daily fluoride intake at different ages during the amelogenesis period (Ishii and Suckling, 1986, Evans and Darvell, 1995, Bardsen, 1999, Burt et al., 2000, 2003). The majority of the studies agreed that the risk period for fluorosis for central incisors is the first two years of life. More recent studies reported the risk is up to the first three years of life. There is also evidence to suggest a gender difference between risk period of developing fluorosis from 15 to 24 months in males and 21 to 30 months in females (Evans and Stamm, 1991a, Evans and Darvell, 1995). However there is lack of evidence in terms of possible fluorosis development for the whole permanent dentition. It has been reported that the age during which children are considered to be susceptible to the development of fluorosis in the whole dentition (excluding the third molars) is from birth to 8 years of life (Hong et al., 2006a, Hong et al., 2006b).

Table 1.3 Window of a maximum susceptibility to the development of dental fluorosis in the permanent maxillary central incisors

Study type	Sample size	Window of maximum susceptibility	Fluoride source	References
1	86	6-23 months	toothpaste, supplements	Holm and Andersson, 1982
2	16	35-42 months	water	Ishii and Suckling, 1986
1	139	First 2 years	toothpaste	Osuji et al., 1988
2	1, 062	22-26 months	water	Evans and Stamm, 1991a
2	1085	15-24 months (males) 21-30 months (females)	water, toothpaste, supplements	Evans and Darvell, 1995
1	113	First 2 years	toothpaste	Lalumandier and Rozier, 1995
1	48	First year	water	Ismail and Messer, 1996
1	383	0-20 months	toothpaste, supplements	Wang et al., 1997
1	66	First 2 years	water, toothpaste, supplements	Bardsen and Bjorvatn, 1998
1 and 2[a]	n.a	First 2 years (but duration of exposure more important)	variable	Bardsen, 1999
2	1896	First 3 years	water	Burt et al., 2000, 2003
1[b]	579	First 2 years	total intake	Hong et al., 2006a
1[b]	628	First 3 years	total intake	Hong et al., 2006b

Study type 1=Individuals introduced to fluoride at different ages.
Study type 2= Populations exposed from birth then experienced an abrupt reduction in intake.
[a]Meta-analysis.
[b]Longitudinal design.
Table adapted from Buzalaf and Levy (2011).

1.2.1.7 Summary

It is established that a low level of fluoride in the oral environment helps to interfere in the dynamics of dental caries formation. However, excessive exposure of fluoride during tooth development increases the risk of developing fluorosis. The risk periods of fluorosis for central incisors is the first two to three years of life. Evidence for the risk periods of fluorosis for the whole dentition is scarce and available evidence considered the risk from birth to eight years of life. The key literature on the risk factors associated with fluorosis development is considered in the following section.

1.2.2 Dental Fluorosis

The earlier Section (1.2.1.5) has described how fluorosis develops. This section now reviews in depth the definition and presentation of fluorosis, risk factors associated with the development of fluorosis, methods of measurement and trends in fluorosis prevalence.

1.2.2.1 Definition

In 1934, Dean originally defined dental fluorosis as “hypomineralization of tooth enamel or dentine by prolonged ingestion of excessive amounts of fluoride during tooth development” (Dean, 1934). Other fluorosis definitions proposed by several authors in the 1980s (Møller, 1982, Murray, 1986, Fejerskov et al., 1988) reported consensus agreement that fluorosis is a developmental condition caused by excessive concentration of fluoride disrupting normal amelogenesis.

1.2.2.2 Clinical and histological presentation of dental fluorosis

A brief explanation of the histological presentation has been described earlier when explaining fluorosis development (Section 1.2.1.5). The spectrum of clinical and histological presentations for fluorosis is broad. Clinically, in its mild form, fluorosed enamel manifests as white striations or has a white parchment-like appearance. At this stage, the tooth functions normally. In more severe cases, fluorosed enamel appears pitted and discoloured and the tooth is prone to wear and fracture (Fejerskov et al., 1990, Mascarenhas, 2000, Browne et al., 2005, Buzalaf and Levy, 2011,). Dental fluorosis may also present as a diffuse opacity. This typically is symmetrically distributed about the mid-line, a feature which can be used to differentiate diffuse opacities attributable to fluorosis from diffuse opacities arising from other causes. (Ellwood et al., 1994, Buzalaf and Levy, 2011).

Histologically, fluorosed enamel is characterised by hypomineralisation and subsurface porosity (Fejerskov et al. 1977). In the mild form, the structural arrangement of the crystals in the outer layer of enamel is normal, but is more porous or in other words, the inter-crystalline space is larger than normal. In more severe forms, the hypomineralised lesion is located deeper to a well mineralised surface zone which is very fragile and susceptible to mechanical stress that leads to breakdown of the enamel surface (Baelum et al., 1986, Fejerskov et al., 1990).

1.2.2.3 Risk factors for dental fluorosis

It is established that fluorosis results from excessive exposure to fluoride during enamel development (Hong et al. 2006b, Bronckers et al. 2009, Buzalaf and Levy 2011). To a certain extent, any source of systematic fluoride ingested during this stage may pose a level of risk for the development of fluorosis. To date, systematic reviews have identified four major risk factors for fluorosis: fluoridated water (McDonagh et al., 2000, Australian National Health and Medical Research Council, 2007, Iheozor-Ejiofor et al., 2015), fluoride supplements (Ismail and Bandekar, 1999), fluoride toothpaste (Wong et al., 2010) and infant formulae (Hujoel et al., 2009). Some other sources such as food and beverages may also be important contributors to total daily fluoride intake (Bronckers et al., 2009). However, this section focuses on discussing the key evidence for the major risk factors.

Water fluoridation

The most widely recognised systematic review of water fluoridation was published in 2000 which is also known as York Review (McDonagh et al., 2000). Other published reviews on water fluoridation such as an Australian review (Australian National Health and Medical Research Council, 2007) and a Cochrane review (Iheozor-Ejiofor et al., 2015) aimed to update this review and adopted different methods in certain areas. All of these reviews have acknowledged that the benefits of fluoridation in terms of dental caries prevention and fluorosis is the only entity that has been identified as a side effect of fluoridation, when fluoride is present at the level for the prevention of dental caries (0.5-1.0 ppmF).

In the early 1940s, Dean and co-workers (Dean, 1942) reported a higher prevalence of dental fluorosis (10%) in children resident in areas where the level of fluoride naturally present in the water was 1.0 ppm compared to children in areas with a negligible level of fluoride in the water, where 1% of children were affected by fluorosis. Most of the cases were diagnosed with mild or very mild fluorosis. This degree of prevalence was recorded when fluoridated drinking water was the sole significant source of fluoride intake. During the latter half of the 20th century, studies reported that although the prevalence of fluorosis remains higher among populations in fluoridated areas, the risk of developing fluorosis in non-fluoridated areas had increased. This phenomenon can be explained by multiple exposures to different sources of fluoride such as fluoride toothpaste as well as foods and beverages processed using fluoridated water and transported to non-fluoridated areas.

The York review included 88 studies on the association of water fluoridation and dental fluorosis (McDonagh et al., 2000). Data reported that at a fluoride level of 1 ppm, the prevalence of fluorosis of aesthetic concern was 12.5% (95% CI 7.0% to 21.5%). This percentage increases to 48% (95% CI 40% to 57%) when considering fluorosis at any level. The Australian review identified 10 additional studies and reported a pooled relative risk of 2.54 (95% CI 1.52-3.56) of developing any fluorosis and 4.01 (95% CI 3.15-5.10) of developing aesthetic fluorosis at water fluoride levels between 0.8-1.2 ppm (Australian National Health and Medical Research Council, 2007). Although there was a four-fold risk of developing fluorosis of aesthetic concern with optimal versus suboptimal water fluoridation, the absolute increase in prevalence was very small, approximately 4-5%. In a more recent review by Cochrane, authors analysed different number of studies based on the fluorosis definition used. Authors reported the percentage of participants with fluorosis of aesthetic concern was 12% (95% CI 8% to 17%; 40 studies) at a fluoride level of 0.7 ppm (Iheozor-Ejiofor et al., 2015). This percentage increased to 40% (95% CI 35% to 44%; 90 studies) when considering fluorosis of any level. All three reviews used the same definition of any fluorosis and aesthetic fluorosis. Any fluorosis was defined as Developmental Defect of Enamel (DDE), Tooth Surface

Index of Fluorosis (TSIF), Thylstrup and Fejerskov (TF) Index score greater than zero or Dean’s classification of ’questionable’ or higher. Aesthetic fluorosis was defined as TSIF ≥2 or TF ≥3 or Dean’s mild or higher.

In terms of fluorosis studies included in the above-mentioned reviews, many authors have raised concerns about the quality of the original studies. Many studies were not blinded in terms of fluoride exposure status of the studied population, the prevalence was overestimated by different indices used and confounding factors were not controlled during analysis (McDonagh et al., 2000, Iheozor-Ejiofor et al., 2015).

Toothpaste

There are mixed findings regarding fluoride toothpaste as a risk factor for fluorosis. In particular in countries that have combinations of fluoride modalities in place to prevent dental caries. Several studies have reported a significant association between the use of fluoride toothpaste in the first two years of life and fluorosis (Mascarenhas and Burt, 1998, Pendrys, 2000, Pereira et al., 2000, Maupome et al., 2003). Studies reported that early use of toothpaste (Maupome et al., 2003, Pereira et al., 2000), higher brushing frequency (more than once per day) (Pendrys et al., 1994), a larger quantity of toothpaste (3/4 of brush head) (Evans, 1991), swallowing toothpaste in infancy (Riordan, 1993a) and higher fluoride toothpaste concentration have all been reported as risk factors for fluorosis. Two randomised control trials (Holt et al., 1994, Tavener et al., 2006) found toothpaste with a higher fluoride concentration was significantly associated with increased fluorosis prevalence. With regards to fluoride toothpaste concentration, a study conducted on Australian children living in fluoridated areas (Do and Spencer, 2007) found a significant decline in fluorosis prevalence after introduction of low concentration

(400-550ppmF) fluoridated toothpaste. However, a Cochrane review could not confirm an association between the use of fluoride toothpaste and the prevalence of fluorosis (Wong et al., 2011). The authors concluded that the benefit of fluoride toothpaste was only significant in caries prevention for concentrations of 1000ppm and above and there was only weak evidence that early use of fluoride toothpaste for children under 12 months was associated with an increased risk of fluorosis. The authors emphasized that the decision of what fluoride levels to use for children under six years should be balanced between the risk of developing caries and mild fluorosis (Wong et al., 2011).

Fluoride supplements

Fluoride supplements have been used to prevent dental caries in areas where fluoride levels in the water supply were deficient. They are available as tablets or drops, intended to be swallowed, as tablets for chewing or lozenges intended to be sucked or dissolve slowly in the mouth. The availability of the supplements varies by country either upon prescription, over-the counter sales or through public health programmes.

In terms of dosage, several guidelines have been published in relation to prescription of fluoride supplements (Banting, 1999, Ismail and Hasson, 2008, Buzalaf and Levy, 2011). The recommendation is for daily use based on the child’s age and fluoride level in the drinking water. However, it has been shown that frequently the guidelines were not followed or were used inappropriately (Banting, 1999, Sohn et al., 2007). Recommendations on the use of fluoride supplements vary across the world depend on the need of the specific population. For example, in the US the current recommendations are 0.25mg fluoride/day from age 6 months to 3 years for children living in areas containing less than 0.3ppm fluoride in drinking water. In contrast, Canada and

Switzerland do not recommend fluoride supplements under 3 years of age. Fluoride supplement use has been linked with low compliance, particularly in those from low socio-economic backgrounds and thus at greatest risk of dental caries. Fluoride supplementation using tablets and drops are increasingly regarded as a poor public health measure (Ismail and Bandekar, 1999, Tubert-Jeannin et al., 2011). As a result fluoride supplements as a means of caries prevention on a population basis have been re-appraised in several countries (Oganessian et al., 2007, Rozier et al., 2010).

In the 1990s there were several reviews published by Riordan (Riordan, 1993b, 1996, 1999), Ismail (Ismail 1994, Ismail and Bandekar 1999) and Burt (Burt, 1999) to answer questions regarding the efficacy of fluoride supplements in caries prevention. Those reviews were updated by Ismail and Hasson in 2008 and the Cochrane collaboration in 2011 (Tubert-Jeannin et al., 2011). Ismail and Hasson (2008) included more study designs, which lead to a total of 85 articles included in their review. Out of 85 articles, 20 were clinical trials, 9 were cohort studies, 22 were cross-sectional studies and 8 retrospective studies were included. In contrast, the Cochrane review had more stringent inclusion criteria and only included randomised controlled trials with a minimum 2 years of follow-up. As a result, just 11 studies of randomized or quasi-randomized trials were included in that review (Tubert-Jeannin et al., 2011). There is a consistent agreement reported by all reviews that fluoride supplements help in reducing caries in permanent teeth, however the effect of the fluoride supplements on primary teeth was unclear.

In terms of risk of fluoride supplements and fluorosis mixed findings were reported by Ismail and co-workers and the Cochrane review. Results from the meta-analysis in the 1999 review reported odds ratios of the association between any use of fluoride supplement and fluorosis of 2.3 (95% CI: 1.5-3.4) and 6.6 (95% CI: 2.9-15.2) in the cross-sectional/case control and follow-up studies, respectively (Ismail and Bandekar, 1999). No meta-analysis was reported in the follow-up review in 2008. The 2008 review included five additional studies. These reported that the use of fluoride supplements increased the risk of mild to moderate fluorosis. However, these results should be treated with caution because the majority of studies were assessed as of low study quality and high risk of bias. For example low compliance of fluoride tablets among study participants, lack of standardisation of method of fluoride tablet delivery (supervision vs non-supervision), high rate of subjects' withdrawal and lack of examiner blinding (Banting, 1999, Ismail and Bandekar, 1999).

The later review by the Cochrane group (Tubert-Jeannin et al., 2011) reported that there was insufficient information to determine the risk of fluoride supplements and fluorosis or other adverse effects. Only one trial was available for analysis (Driscoll et al., 1974). Data from this study reported that a slight increase in fluorosis prevalence in the fluoride interventions group (20% in the group with one acidulated phosphate fluoride (APF) tablet per day and 22% in the group with two APF tablets per day) in comparison to the placebo control group (15%).

Infant formula

Infant formula is a major source of nutrition during infancy. It can be divided into three categories; milk-based products, soy-based products and ready-to-feed formulas. Powdered and liquid concentrate formula require reconstitution with drinking water, whereas ready-to-feed formulas do not need to be reconstituted.

Historically, infant formulas contained high concentrations of fluoride. Prior to the 1970s in the United States a high level of fluoride in infant formula was associated with high fluorosis prevalence in fluoridated and non-fluoridated areas (Silva and Reynolds, 1996, Mascarenhas, 2000). Studies of risk factors for fluorosis among children who were born before 1979 reported that children in fluoridated areas were at a seven times higher risk than those in non-fluoridated areas (Pendrys and Katz, 1989, Pendrys et al., 1994). The reported risk prompted a call for manufacturers to reduce and control the concentration of fluoride in their products in several countries such as in the US since 1979 (Singer and Ophaug, 1979, Dabeka et al., 1982) and Australia and New Zealand since 1992-1993 (Do et al., 2012). After reduction of the fluoride level in infant formula, fluorosis was often associated with sources of water used to reconstitute infant formula. However, a recent study in Australia reported different findings where infant formula was associated with a high prevalence of fluorosis in non-fluoridated areas but not in fluoridated areas. The association persisted after controlling for other fluoride sources (Do et al., 2012). The authors suggested that the unexpected results could be due to exposure to other sources of fluoride such as food and beverages. Also there is a possibility of formula powdered might have still contained a considerable level of fluoride (Do et al., 2012).

Evidence from a systematic review of infant formula and fluorosis summarised that there was a weak evidence to support fluoride in infant formula causing fluorosis (Hujoel et al., 2009). However, infant formula intake may be associated with some detectable level of fluorosis depending on the level of fluoride of the water used to reconstitute them (OR 1.8, 95% CI 1.4-2.3). Seventeen studies reported in the review reported odds ratio (OR). A meta-regression analysis indicated that the ORs associating infant formula with enamel fluorosis increased by 5% for each 0.1ppm increase in the reported levels of fluoride in the water supply (OR 1.05, 95% CI 1.02-1.09). In terms of the quality of the studies included in the review, less information was available about the extent of exposure to infant formula, the type of infant formula consumed, the fluoride concentration of the formula and the level of fluoride in the water with which the infant formula was reconstituted. The authors were unable to determine whether the increased risk was caused by fluoride intake from infant formula, fluoridated drinking water or other sources of fluoride such as toothpastes or fluoride supplements (Hujoel et al., 2009). Further research was recommended to address this issue.

Variation between countries in relation to fluoride level in both drinking water and the infant formula itself makes advice regarding use of infant formula more complex. In Canada, where the fluoride level in the public water supply ranges from 0.5 to 0.8 ppm, the Canadian Dental Association (Canadian Dental Association, 2007) has made no recommendation regarding infant formula preparations and fluorosis. In the United States, where water fluoride levels typically range from 0.7 to 1.2 ppm, the guideline by the American Dental Association (Berg et al., 2011) suggested that those who are concerned about their children’s exposure to fluoride should use ready-to-feed formula or should reconstitute the formula with water that has no or low levels of fluoride.

1.2.2.4 Measuring Fluorosis

Clinical measurement

Several epidemiological indices have been developed and used to describe the clinical appearance of dental fluorosis. Many researchers have extensively discussed and criticised each index. Two distinct groups of indices have been proposed for measuring fluorosis. These can be divided into specific fluorosis indices and descriptive indices:

i) Specific fluorosis indices: These indices specifically measure fluoride induced enamel changes, and report the extent and severity of the fluoride induced changes in dental enamel. Examples are: Dean’s Index (Dean, 1942), Thylstrup and Fejerskov (TF) Index (Thylstrup and Fejerskov, 1978) and TSIF Tooth Surface Index of Fluorosis (Horowitz et al., 1984).

ii) Descriptive indices: The indices include all types of enamel defects and are not specific to dental fluorosis. The indices record enamel defects based on descriptive criteria without assumptions about the aetiology of the defects. Examples are: Developmental Defects of Enamel (DDE) Index (FDI, 1992) and classifications described by Al-Alousi et al (Al-Alousi et al., 1975).

All the measurements and indices described above have been developed from relatively different rationales. No one Index has emerged as the agreed standard criteria to measure fluorosis, and the most suitable index of recording fluorosis to a degree depends upon the objective of the study.

This section highlights the most commonly used indices when reporting fluorosis in the literature. Dean’s Index was commonly reported in older studies and national surveys. Many European studies favoured the use of the TF Index. The DDE Index is also popular in several national surveys, where the main aim is to measure enamel defects and diffuse opacities commonly considered as ‘fluorosis’. Variation in the indices used in fluorosis measurement makes comparison difficult across studies. Different studies used different cut off points of fluorosis definition for the individual index. The most common categorisation used in the literature is ‘any fluorosis’ and ‘aesthetic fluorosis’. Any fluorosis defined by DDE, TSIF, TF score greater than zero or Dean’s classification of ‘questionable’ or greater. Aesthetic fluorosis defined by as TSIF ≥2 or TF ≥3 or Dean’s mild or higher.(McDonagh et al., 2000, Iheozor-Ejiofor et al., 2015). However some researchers argued that the cut-off point used to define any fluorosis and aesthetic is arbitrary. Population perceptions on aesthetic impact of fluorosis may be the key indicator to define level of ‘aesthetic fluorosis’ which again varies across studies (Chankanka et al., 2010).

A description of the most commonly used Indices together with their advantages and disadvantages is presented in Table 1.4 .

Table 1.4 Summary of the advantages and disadvantages of fluorosis indices

Index/ Reference	Description	Advantages	Disadvantages
Dean's Index (Dean et al., 1942)	The initial Dean's classification in 1934 (Dean, 1934) had 7 classifications (0=normal, 0.5=questionable, 1=very mild, 2=mild, 3=moderate, 4=moderately severe, 5=severe). This original classification was modified in 1942 into 6 categories (0=normal, 1=questionable, 2=very mild, 3=mild, 4=moderate, 5=severe). The 'moderately severe' category in the original scale was removed and combined with the 'severe' category (Dean, 1942). Dean's Index results in a single score for an individual. If fluorosis is present, the individual will be scored based upon the two most affected teeth. If the two teeth were not equally affected, the less affected tooth is scored.	Simple to use; accepted at global level; long track record of use supported by literature; allows historical comparison with old studies; recognized by World Health Organization for use in oral health surveys basic method; teeth are examined wet -more relevance to concerns in a public health context.	Only measure the two most severely affected teeth, does not allow measurement of fluorosis on different tooth surfaces; no information about location of affected teeth; the diagnostic category for 'questionable' in the classification is unclear and lacks precision; the index lacks sufficient precision to distinguish different degrees of fluorosis; teeth are examined wet-may overlook minor opacities (Horowitz, 1986, Clarkson, 1989, Rozier, 1994).
Community Fluorosis Index (CFI), (Dean, 1946)	Dean also developed the CFI which aims to compare the average severity of fluorosis between different groups. The CFI is calculated for a geographic location based on the mean of all scores for individuals examined. The CFI can be obtained from statistical weight (ranging from 0 to 4) to each category within the classification. This index awards weights to the different scores in Dean's Index. Normal is awarded 0, 0.5 to questionable and 1,2,3,4 to very mild, mild, moderate and severe respectively. CFI scores below 0.4 is not considered as public health significance. Scores that ranged between 0.4 and 0.6 were borderline significance and CFI scores above 0.6 were of increasing public health significance.	CFI calculates average severity of fluorosis between different groups and the range of value will determine public health effect of fluorosis.	The statistical basis for using the arithmetic mean to calculate the CFI is questionable on the grounds that the classification is based on an ordinal and not an interval scale; the weights assigned to each category are arbitrary (Horowitz, 1986, Clarkson, 1989, Rozier, 1994).

Table 1.4 (continued)

Index/ Reference	Description	Advantages	Disadvantages
Thylstrup-Fejerskov (TF) Index, (Thylstrup and Fejerskov, 1978).	The TF index was developed in order to refine, modify and extend the use of Dean's index. The aim of the index is to record histological changes that occur in dental fluorosis based on an ordinal scale. Enamel changes observed on single tooth surfaces are divided into 10 categories which range from 0 (normal) to 9 (severe condition). Unlike Dean's Index, TF scores are applied to the buccal, lingual and occlusal surfaces. In order to improve sensitivity at low diagnostic thresholds, teeth are examined after cleaning and drying to emphasize the appearance of fluorotic change.	Record histological changes that occur in dental fluorosis based on an ordinal scale which allow sufficient precision to distinguish different degrees of fluorosis; TF index has been validated clinically and histologically; TF index is as commonly used as Dean's Index and is particularly favoured in European studies; teeth are examined dry - improved diagnostic sensitivity.	Difficult to standardize tooth dryness; the effect of drying may reveal a short period of changes which have less aesthetic or public health importance; the criteria for score 1 and 2 describe only very minor changes (Clarkson, 1989).
Developmental Defect of Enamel (DDE) Index, (FDI, 1992).	The DDE Index was developed by a Working Group of the Federation Dentaire Internationale (FDI) to provide an internationally acceptable classification system for developmental enamel defects. The original index was criticised as time-consuming and complicated to use and analyse. To overcome the weaknesses of the index, it was subsequently modified and presented in three types of defects: demarcated, diffuse and hypoplastic. Many studies are in agreement that the diffuse opacities category probably contain most of the fluoride-related opacities and usually considered a close approximation of fluorosis (Ellwood et al., 1994, Mohamed et al., 2010, Wong et al., 2014).	Detailed measurement that includes a broad range of defects with information on the distribution and location; teeth are examined wet -more relevance to concerns in a public health context (FDI, 1992, Ellwood et al., 1994).	Teeth are examined wet-may overlook minor opacities; time-consuming to conduct due to large volume of information collected.
Tooth Surface Index of Fluorosis (TSIF), (Horowitz et al., 1984).	Researchers at the National Institute for Dental Research in the United States developed the TSIF index, in an attempt to improve on Dean's Index. The TSIF primarily aims to measure the public health effect or aesthetic concern of fluorosis in a population. The TSIF requires that the teeth are examined wet and a score is given to all surfaces (labial, lingual and occlusal surfaces).	A score is given to all surfaces instead of individual teeth (labial and lingual surfaces and occlusal surface of posterior teeth); the index improves diagnostic sensitivity for fluorosis in	Scoring all surfaces may increase surface-to-surface variation between examiners; scoring lingual and hard to see surfaces may reduce examiner consistency; possibility of

Table 1.4 (continued)

Index/ Reference	Description	Advantages	Disadvantages
TSIF (continued)	In the TSIF, the 'questionable' category of Dean's index was removed and the remaining four categories of the index expanded to seven categories. Categories 1-3 (parchment white fluorosis) are differentiated by the surface area of the enamel involved. Categories (4-7) represent different degrees of staining and quantitative loss of enamel.	severe categories; the index permits a distinction between discrete pitting and more advanced confluent pitting and staining alone and staining with pitting; the index is useful especially in populations where severe fluorosis is prevalent; teeth are examined wet - more relevance to concerns in a public health context.	losing data on occlusal surfaces because of restorations; teeth are examined wet-may overlook minor opacities (Rozier, 1994)
Fluorosis Risk Index (FRI), (Pendrys, 1990).	The index was developed to determine the association between age-specific exposure to fluoride sources and risk of developing fluorosis The scoring system for this index is based on different enamel surface zones which were divided into four zones: occlusal/ incisal edge, incisal one third, middle one third and cervical one third. The index then divides the enamel surfaces into two groups based on their time of formation and mineralisation: classification I zones are 10 surface zones that begin formation during the first year of life; classification II zones are 48 zones that begin formation during the third year through to the sixth year of life. The unassigned enamel surface zones are categorized as questionable (54 surface zones). In total, approximately 112 zones are scored using this index. Each zone is scored as either negative for fluorosis (score 0), questionable (score 1), positive for mild to moderate fluorosis (score 2), or positive for severe fluorosis (score 3). The rationale for this classification was that different fluoride exposures may have different effects on fluorosis experience on surface zones that are mineralised at different times during an individual's life.	The scoring system of different zones of a tooth surface; allows identification of risk factors of fluorosis; useful for analytical epidemiology studies because it allows identification of age-specific exposure to fluoride sources and development of enamel fluorosis (Rozier, 1994).	The index is complex for its biological perspective and application; suitable to estimate the relative risk of fluorosis rather than fluorosis prevalence; the many surface zones to be scored may lead to the possibility of misclassification and increase surface variation both within and between examiners; teeth need to be examined either 'dry' or 'wet' is not clearly stated (Rozier, 1994).

Photographic assessment of dental fluorosis

Clinical measurement has several limitations such as lack of standardised examination method, examiner bias and the different indices used make comparison between studies difficult. A way of overcoming these shortcomings is development of a standardized photographic method for capturing a permanent record of the condition of the enamel.

There are however, advantages and disadvantages of photographic assessment in comparison with the clinical measurement of dental fluorosis. The major benefits of photographs are that they capture a permanent record and allow blind scoring between different examiners. In addition, the same method can be used by different investigators in multi-site epidemiology studies and allow repeated objective assessments of the photographs (Ellwood et al., 1994, Cochran et al., 2004a, Soto-Rojas et al., 2008).

The disadvantages of using photographs are firstly variation in photographic technique between different studies such as variation in equipment, lens, lighting system and quality of image produced (Cochran et al., 2004a). Secondly, difficulties in capturing teeth images due to lack of accessibility especially for posterior teeth mean that photographs have only been used to record the subject’s anterior teeth, this could result in under reporting of the prevalence of dental fluorosis. On the other hand, the greater detail provided by photographs may well result in over reporting prevalence (Soto-Rojas et al., 2008)

Imaging techniques in assessing fluorosis can be divided into conventional and digital photography. In the early introduction of imaging techniques for fluorosis assessment, conventional photography was often used (Nunn et al., 1993, Ellwood et al., 1994, Sabieha and Rock, 1998). Although photographic methods have evolved from conventional transparencies using film to digital images, some researchers still prefer to use conventional photography to enable data comparison with different studies that used the same standardized methods (Cochran et al., 2004a, Cochran et al., 2004b, Wong et al., 2014).

In recent years, several researchers have reported fluorosis assessment using digital photography (Tavener et al., 2007, Martins et al., 2009, Cruz-Orcutt et al., 2012, Golkari et al., 2011). The main benefits of using digital photography are that it allows the examiner to evaluate the quality of the image captured during the clinical examination immediately post exposure. It can therefore be repeated if the quality of the image is not acceptable. In addition, digital photography also allows the examiner to zoom and adjust to capture the best image instead of using a fixed barrel lens (Golkari et al., 2011). It is suggested that digital photography can more easily accommodate patient confidentiality and can be stored in digital systems. Images are, produced instantaneously and do not require developing of negatives and printing.

1.2.2.5 Trends in the prevalence of dental fluorosis

Global trends in the prevalence of dental fluorosis

Several indices have been used to measure fluorosis prevalence and may not be directly comparable. Comparison between studies was made with this limitation in mind. To aid in consistency in data reporting, fluorosis prevalence described in this section is defined by, Deans≥2 (very mild or greater), TF≥1, TSIF≥1 and diffuse opacities from DDE Index unless stated otherwise. A summary of fluorosis prevalence among children in selected countries is presented in Table 1.5.

The United States (US) was the first country to introduce community water fluoridation programmes to prevent dental caries. It has been established that the prevalence of fluorosis increases with increasing levels of water fluoride. However there is a trend of increase in fluorosis in the US over the previous 30 years not only in fluoridated areas but also in non-fluoridated areas (Beltrán-aguilar et al., 2002). Data from the US national survey using Dean's Index reported that fluorosis increased among children from 22.6% in the 1986-1987 survey to 40.6% in the surveys conducted between 1999-2004 surveys. Similar trends have been reported in Ireland. The percentage of 8 and 15 year-old children having fluorosis (very mild or higher) was 1% in fluoridated areas in 1984, but this increased to 11.8% and 18% respectively in 2002. The same trend has observed in the non-fluoridated areas whereby none of the children had experience of fluorosis in 1984 but the prevalence has increased to 3.3% for the 8 year-olds and 6.5% for the 15 year-olds in 2002 (Whelton et al., 2004a, Whelton and O'Mullane, 2012).

Unlike in the USA and Ireland, an opposite trend has been observed in the UK. There is a trend of decreasing prevalence of diffuse enamel opacities among 12 year-olds in the UK from 2003 to 2013. These data were based on the Children's Dental Health surveys that were conducted using the DDE index. In terms of individual country, a higher prevalence of diffuse enamel opacities was observed in England (2003: 18%, 2013: 16%) followed by Northern Ireland (2003: 11%, 2013: 8%) and Wales (2003: 9%, 2013: 5%) (Pitts et al., 2015). An opposite trend observed in the UK may be due to only 10% of the population receiving a fluoridated water supply and the main fluoride delivery is through fluoridated toothpaste. Another reason could be the different index used to measure fluorosis. Another study in the UK used TF index to measure fluorosis and blinded photographic scoring. Results from this study reported a higher fluorosis prevalence in fluoridated Newcastle upon Tyne (55%) and non-fluoridated Manchester (27%) than the UK national prevalence of diffuse opacities (McGrady et al., 2012a).

In Australia, data in 2003/2004 reported the prevalence of fluorosis using blinded photographic scoring as 26.9%. Further analysis across different birth cohorts indicate a marked decline in the prevalence of fluorosis (TF≥ 2) among children born after 1993 (8.3%) in comparison to children born before that (17.9%) (Do and Spencer, 2007). The decline was reported to be mainly linked with the reduction in the concentration of fluoride in the children toothpaste (400-550 ppm) introduced in 1993 and a combined effect of fluoride level in the water at 0.6 to 1.1 ppm.

Neighbouring South-East Asia countries like Singapore and Thailand also reported a high fluorosis prevalence. For example in Singapore, Lo and Bagramian (1996) reported 82.6% fluorosis prevalence at 0.7 ppm optimal fluoridated among 9-16 year-old children. However in Thailand where some areas have high natural fluoride in the water (0.35-2.22 ppm), data indicate a high fluorosis prevalence among the studied population (70.9%) (McGrady et al., 2012b).

Table 1.5 International prevalence of fluorosis in children from selected countries

Countries (Area)	Year of survey	Age	Fluorosis prevalence^{+}	Mean F level*	Index	Reference
USA						(Beltrán-Aguilar et al., 2010)
National survey (NIDR)	1986/87	12-15	22.6	0.7-1.2	Dean's	
National survey (NHNES)	1999-2004	12-15	40.6	0.7-1.2	Dean's	
Ireland						
National survey	1984	8	F:1.0 NF: 0	0.8-1	Dean's	(Whelton et al., 2004a, Whelton and O'Mullane, 2012)
		15	F:1.3 NF: 0			
National survey	2002	8	F: 11.8 NF:3.3	0.8-1	Dean's	
		15	F:18.0 NF: 6.5			
UK						(Pitts et al., 2015)
England	2003	12	18	10% of population in England have water fluoridation	DDE	
Northern Ireland			11			
Wales			9			
England	2013	12	16		DDE	
Northern Ireland			8			
Wales			5			
UK						(McGrady et al., 2012a)
Newcastle upon Tyne	2008/09	11-13	55	1	TF	
Manchester			27			
South Australia	2002/03	8-13	26.9	1	TF	(Do and Spencer, 2007)
Singapore	1986	9,12,16	82.6	0.7	Dean's	(Lo and Bagramian, 1996)
Thailand (Chiang Mai)	Not stated	8-13	Overall: 70.9 F(>0.9):85.1 F (<0.9):60.0	0.35-2.23 (naturally fluoridated)	TF	(McGrady et al., 2012b)

$^{+}$Fluorosis prevalence defined by, Deans≥2, TF≥1, DDE: Diffuse opacities

*Mean fluoride (F) level in the water in fluoridated area

NIDR: National Institute of Dental Research, National Survey of Oral Health in U.S. School Children, 1986–1987.

NHNES: National Health and Nutrition Examination Survey 1999-2004.

Fluorosis trends in Malaysia

A number of different studies have reported on the degree of fluorosis and enamel defects in Malaysia. These findings are tabulated in Table 1.6 and Table 1.7. The prevalence of fluorosis reported in Malaysia ranges from 20.3% to 67.7%, while the presence of diffuse opacities were reported as ranging from 42.2% to 88.6% in fluoridated areas. Most of the fluorosis studies were conducted in 1990s and in the early millennium. In the 1991, the prevalence of fluorosis was reported as only 32.8% (Esa and Razak, 2001). However data from the National Survey of Enamel Opacities reported an increased prevalence of fluorosis is in particularly in those living in fluoridated communities (62.3%) (Oral Health Division Ministry of Health Malaysia, 2011). The national findings were consistent with a later study in fluoridated Selangor by Tan et al., (2005). In contrast, in the same year, another local study conducted in fluoridated Negeri Sembilan reported slightly lower prevalence of fluorosis (27.8%) than the national prevalence (Mohd et al., 2008). However this study had a low sample size and only involved children in three schools in one district (Kuala Pilah). The most recent study in 2003/2004 reported fluorosis prevalence in sub-optimally fluoridated (≤0.4 ppm) areas using TSIF index (Shaharuddin et al. 2010). The overall prevalence in the selected three cities was 31.6%. Although the sample size is very small to infer to the state population, this finding is rather unexpected for a sub-optimal fluoridated area.

Table 1.6 Studies on prevalence of fluorosis in Malaysia

Areas	Age (years)	Sample size	Fluorosis Prevalence* (%)	Index	Fluoride level (ppm)	Year of survey	Authors/year
Selangor	12-13	1519	32.8	Dean's	0.7	1991	(Esa and Razak, 2001)
National survey				Dean's			
F areas (overall)	16-17	2153	62.3		0.7	1999	(Oral Health Division Ministry of Health Malaysia, 2001)
NF areas (overall)	16	756	3.0		-		
Selangor	10-11	1343	58.7	Dean's		2003	(Tan et al., 2005)
N.Sembilan	16-17	431	27.8		0.7	2003	(Mohd et al., 2008)
Sub-optimal F areas (overall)	12-13	147	31.6	TSIF	0.29±0.18	2003/2004	(Shaharuddin et al., 2010)
Kota Kinabalu, Sabah	12-13	79	20.3		0.08 ±0.06	2003/2004	
Pasir Mas, Kelantan	12-13	85	27.1		0.44±0.12	2003/2004	
Kuala Terengganu	12-13	83	47.0		0.34-±0.13	2003/2004	

F, Fluoridated; NF, Non-fluoridated

*Fluorosis prevalence defined by (Dean's score: very mild or greater, TSIF: score one or higher)

Table 1.7 Studies on the prevalence of enamel defects in Malaysia using the modified Developmental Defects of Enamel (DDE) Index

Area	Age (years)	Sample size	Enamel defects (%)		% Diffuse Opacities (DO)		% Bilateral DO		Year of survey	Authors/year
			Mouth	Tooth	Mouth	Tooth	Mouth	Tooth		
Johor (overall)	11-12	2388	83.1	29.9	72.1	26.2	-	-	n/a	(Dental Division Johor Malaysia, 1986)
Fluoridated	11-12		88.4	38.4	81.2	34.9	-	-		
Non-fluoridated	11-12		73.8	15.1	56.0	11.1	-	-		
Petaling Jaya	11-12	1024	72.5	40.0	67.1	-	-	-	n/a	(Razak and Nik,1986)
Johor	adult	203	75.6	13.1	42.2	6.2	18.0	4.3	n/a	(Majid et al.,1995)
Penang	12-15	229	76.4	19.1	60.2	16.3	41.5	9.0	n/a	(Majid et al., 1996)
Penang	16	1024	67.1	64.5	88.6	-	-	-	1996	(Sujak et al., 2004)
Malaysia	16	4085	56.0	21.8	53.5	20.1	41.0	13.1		(Oral Health Division Ministry of Health Malaysia,1998)
Fluoridated	16	2195	69.6	30.7	67.4		54.2	19.3		
Non-fluoridated	16	1639	38.6	9.7	35.8		23.4	4.7		
Kuala Lumpur	11-12	957	90.7	45.1	88.6	-	77.0	-	1997	(Yusoff et al., 2008)
Selangor	10-11	1343	-	-	-	-	58.7	30.1	2003	(Tan et al., 2005)

Adapted from (Tan et al., 2005)
n/a= information not available

1.2.2.6 Summary

There are established risk factors associated with fluorosis such as water fluoridation, fluoridated toothpaste, fluoride tablets and infant formula. However there is some disagreement between systematic review findings due to different inclusion and exclusion criteria used in each review. Usually a Cochrane review tends to have a more stringent inclusion criteria and eventually lead to insufficient information to synthesise. Several indices have been developed to measure fluorosis. There is no consensus about the best Index to measure fluorosis but rather depends on individual study objectives. Within the most commonly used indices in the literature, there is some agreement on fluorosis case definition to enable comparisons across studies.

1.2.3 Dental Caries

A fluoride based preventive strategy aims for caries prevention. Therefore it is important to understand the aetiology of the disease and how dental caries is measured and reported. This section will start by discussing the aetiology of dental caries, followed by a description of caries measurement and a report on caries trends internationally and in Malaysia.

1.2.3.1 Aetiology of dental caries

The term ‘caries’ can be used to refer to both the caries process and caries lesion (Kidd and Fejerskov, 2004). Dental caries is a complex interaction of dental biofilms and dietary sugar with tooth structure (Fejerskov, 1997, Ten Cate, 2013). Following eruption into the oral cavity, the enamel surface will be covered by numerous microbial deposits and undergoing modification by contact with the oral environment. The bacteria produce acids and matrix biofilms from sugar metabolism. Organic acids formed in the dental biofilms (plaque) will reduce the pH level and penetrate into the enamel and lead to selective dissolutions inside the tooth. This process is known as demineralisation or ‘caries attack’. Cumulative demineralization processes with prolonged acid challenges will gradually dissolve and weaken the tooth structure and become a cavity. The initial stage of demineralization can be reversed with natural repair mechanism known as remineralisation (Section 1.2.1.4).

After an acid challenge, saliva buffers the acid produced by bacteria. During the remineralisation process, saliva neutralises the pH level by the deposition of calcium and phosphate ions (Manji et al., 1991, Kidd and Fejerskov, 2004, Ten Cate, 2013). The acid production in the biofilm can be reduced by several local factors in the environment such as salivary flow rate and the concentration of fluoride ions in the oral fluid. Therefore the caries process has been conceptualised as a “delicate balance…...determined by the relative weight of the sums of pathological factors [acid-producing bacteria, fermentable carbohydrates] and protective factors [saliva, calcium, phosphate and fluoride]” (Featherstone, 1999).

1.2.3.2 Caries measurement

Various carious assessment systems have been developed since the late 19th century. This section focuses on the most common caries measurement indices used in epidemiology surveys, namely the DMF Index and ICDAS system. A description of other caries classification systems such as Caries Assessment Spectrum and Treatment (CAST) (Frencken et al., 2011), Significant Caries Index (Bratthall, 2000) and Pulp-Ulcer-Fistula-Abscess (PUFA) index (Monse et al., 2010) is beyond the scope of this thesis.

DMF Index

The most commonly used caries index is the DMF (Decay, Missing, Filling) developed by Klein et al., (1938) and subsequently endorsed by the World Health Organization (1997). The index is used separately for the deciduous and the permanent dentition. Upper-case letters (DMF) are used for permanent dentition and lower-case letters (dmf) for the primary dentition. In terms of index variation, the tooth surfaces (DMFS or dmfs index) are used as assessment unit as opposed to the tooth. Data in epidemiology surveys using this index can be used to report the prevalence and severity of the disease at population level. The prevalence is usually measured by the proportion of the children in the population who have at least one decayed, missing or filled tooth (% DMFT>0). The severity of the disease is usually measured by the average number of decayed, missing or filled teeth (mean DMFT) per child. The advantages of the DMF index are reported to be a system that is easy to use, valid and reliable, allows comparison of caries prevalence in various populations and it recognized by the majority of countries for national oral health survey purposes. However one of the major disadvantages of DMF index is that it only records cavitated or restored lesions and does not record non-cavitated lesions (i.e. caries in its early stages, when still confined to dental enamel or non-cavitated dentine caries). In addition, some researchers have noted limitations with the index, including the assumption that filled and missing teeth are assumed to have been carious, and the equal weighting assigned to decayed, filled and missing teeth (Broadbent and Thomson, 2005).

ICDAS

In the year 2001, a new caries assessment system was developed and the ICDAS-International Caries Detection Assessment System (Pitts, 2004, Ismail et al., 2007). This system was developed to facilitate caries epidemiology, research and appropriate clinical management (Pitts, 2004). Unlike DMF index, the ICDAS system records stages of caries lesion development which include cavitated and non-cavitated lesions and active or inactive lesions. The initial development of ICDAS-I included detection of coronal caries and lesion activity without root caries assessment. In 2009, the ICDAS coordination committee expanded the discussion and came up with ICDAS-II. The index has described caries assessment for coronal and root surface and caries assessment associated with restorations and sealants. The code for coronal caries range from 0 to 6, indicating severity of the lesion (Appendix 1). Subsequently ICDAS-II became a two-digit scoring method, where the first digit records restorations/sealants as denoted by a specific code, followed by the appropriate caries code. The strengths of ICDAS-II are it includes stages of carious lesion progression in the enamel, the caries assessment can be carried our through visual/tactile sensation and it has found to be valid and reliable. Inclusion of stages of enamel carious lesion is important particularly to manage the lesion progression using caries preventive agents and to assess lesion progression. The limitations of ICDAS include excessive amount of information collected may result to difficulty in reporting data in meaningful way, and overestimation of seriousness of dental experience (Frencken et al., 2011). Some investigators claim it is not practical to dry surfaces to assess for early enamel caries (code 1) particularly during epidemiology fieldwork (Frencken et al., 2011, Fisher et al., 2012). However, in terms of drying the surface, the index has allowed some epidemiology modification, which still enable assessment of enamel caries without drying. In terms of analysis, full ICDAS detection codes can be collapsed to make them equivalent to the traditional DMFT index to enable comparison across studies (Appendix 2).

1.2.3.3 Caries trends in children

International trends in the prevalence of dental caries

Globally, the prevalence of dental caries has declined since the 1970s. The largest decline has been seen in industrialised countries. In terms of differences in trends between the two dentitions, the caries reduction in permanent teeth was greater than that in the primary teeth. However the decline seemed to have reached a plateau in both dentitions. For example in the UK, in 5 year-old children caries prevalence in the primary dentition reduced significantly from 72% (1973) to 50% (1983) and to 45% (1993), but showed less improvement in the following decade from 43% (2003) to 31% (2013). Similar patterns were observed in the permanent dentition among the 12 and 15 year-old children where the rate of caries reduction continued to slow in the last decade compared to the preceding twenty years (Pitts et al., 2015). However the decline in improvement has changed to an increase in caries trend in the US (Dye et al., 2007), Norway (Haugejorden and Birkeland, 2006) and Australia (Mejia and Ha, 2011). For instance, the United Sates National Health and Examination Survey (NHANES) data from 1988-1994 and 1999-2004 indicated that prevalence of caries in primary teeth among children aged 2-5 years increased from 24% to 28% (Dye et al., 2007). Data reported from Norway for the period 1985-2004 showed a 15-year trend of caries reduction in permanent teeth of 12 year-olds. However starting in 2000, an increase of 3.3% per year was reported (Haugejorden and Birkeland, 2006). Table 1.8 presents trends of caries prevalence among children in selected countries.

Table 1.8 International prevalence of caries in children from selected countries

Countries	Year of survey	Age	Index	Caries prevalence %	Mean caries experience	Reference
USA	1988-1994	2-5 Primary	dmft	24	1.01	(Dye et al., 2007)
	1984-2004			28	1.17	
Australia	1977	6 Primary	dmft	-	3.13	(Australian Institute of Health and Welfare et al., 2016)
	1987			-	1.91	
	1997			-	1.50	
	2007			-	1.95	
	1977	12 Permanent	DMFT	-	4.79	
	1987			-	1.75	
	1997			-	0.86	
	2007			-	0.95	
UK$^+$	1973	5 Primary	dmft	72	-	(Pitts et al., 2015)
	1983			50	-	
	1993			45	-	
	2003			43	-	
	2013		ICDAS	31		
England, Wales, Northern Ireland$^+$	2003	12 Permanent	DMFT	43	1.0	
	2013		ICDAS	34	0.8	
New Zealand	2004	5 Primary	dmft	48.9	2.18	(Schluter and Lee, 2016)
	2009			44.9	2.01	
	2013			43.3	1.93	
	2004	8 Permanent	DMFT	55	1.60	
	2009			48.4	1.39	
	2013			46.1	1.15	
Norway	1997	5 Primary	d3mft	30	1.1	(Haugejorden and Birkeland, 2005)
	2001			40	1.6	
	2003			36	1.4	
	1985	12 Permanent	D3MFT	81	3.4	(Haugejorden and Birkeland, 2006)
	2000			52.2	1.5	
	2004			59.8	1.7	
China	1983	12 Permanent	DMFT	38.2	0.8	(Wang et al., 2002)
	1995/95			45.8	1.0	

$^+$Trends comparison was made based on obvious decay experience

Caries trends in Malaysia

Similar trends in caries experience have been reported in Malaysia. According to the National Oral Health Survey for Schoolchildren (NOHSS), the dental caries prevalence for the 12-year-olds declined from 60.9% in 1997 to 41.5% in 2007 (Oral Health Division Ministry of Health Malaysia, 2010). Data from the School Dental Services reported caries continued to decrease in 2013 (36.8%), however in a much slower rate Table 1.9. (Oral Health Division Ministry of Health Malaysia, 2014). In the primary dentition, a consistent pattern of caries decline was observed from 1995 (87.1%) to 2013 (65.8%) (Oral Health Division Ministry of Health Malaysia, 2009). However, the rate of reduction was lower than the permanent dentition (Table 1.10).

Although all states in Malaysia show a reduction in caries experience, there is a wide variation in terms of caries prevalence and severity across states in Malaysia. The highest caries prevalence states were among less affluent states with a negligible concentration of fluoride in the public water supply namely the states of Kelantan and Sabah. The more affluent states with established fluoridation programmes such as Kuala Lumpur, Johor and Selangor have a lower caries prevalence than national average.

Table 1.9 Caries prevalence among 12 year-old children in Malaysia by state

		Percentage caries prevalence %		
	State	NOHSS 1997	NOHSS 2007	SDS 2013
West Malaysia	Johor	51.0	25.7	25.8
(Peninsular)	Perak	72.4	40.2	29.5
	Kedah	62.8	38.0	28.5
	N.Sembilan	52.9	32.8	23.9
	Pahang	69.4	43.5	40.7
	Perlis	65.2	42.2	29.0
	Melaka	49.5	32.4	36.0
	Terengganu	73.8	49.5	57.1
	Kuala Lumpur	39.8	27.1	18.1
	Selangor	44.0	30.2	21.2
	Penang	52.9	38.5	30.0
	Kelantan	67.7	62.7	65.4
East Malaysia	Sarawak	72.6	47.1	50.9
(Borneo)	Sabah	80.5	73.3	66.8
	MALAYSIA	**60.9**	**41.5**	**36.8**
	(Mean DMFT)	**(1.9)**	**(1.12)**	**(0.91)**

NOHSS: National Oral Health Survey of School Children 2007 (Oral Health Division Ministry of Health Malaysia, 2010)
SDS: National data from the School Dental Service (Oral Health Division Ministry of Health Malaysia, 2014)

Table 1.10 Dental caries status of 6 year-old preschool children in Malaysia

	Peninsular Malaysia^ 70/71	Peninsular Malaysia^ 1988	Malaysia NOHSS 97	Malaysia NOHSS 2007	Malaysia SDS 2013
Caries prevalence	95.7	89.3	80.9	74.5	65.8
Mean dmft	6.3	6.2	4.1	3.9	n/a

NOHSS: National Oral Health Survey of School Children (Oral Health Division Ministry of Health Malaysia, 2010).
SDS: National data from the School Dental Service (Oral Health Division Ministry of Health Malaysia, 2014).
^National data for Peninsular Malaysia (excluding the East Malaysia, Sabah and Sarawak states in Borneo).

1.2.3.4 Summary

A dramatic improvement of dental caries has been reported since the mid-twentieth century. However recent data often suggest either slowing down in the rate of improvement or indeed an increase again. The ICDAS index is a new validated index that enable detection of enamel and dentine caries which would contribute to the improvement of caries recoding and reporting.

1.2.4 Overview of fluoride exposure and caries prevention approach in Malaysia

This Section considers the approach to preventing dental caries in Malaysia, with a particular emphasis on the role of fluoride. Specifically the role played by water fluoridation is discussed as is methods to monitor quality standards and additional sources of fluoride.

1.2.4.1 Exposure to fluoride from water

Malaysia implemented a water fluoridation programme in 1972 with an optimum fluoride level of 0.7 ppm. The fluoride level in the water has subsequently been reduced to 0.5 ppm in 2005 (Oral Health Division Ministry of Health Malaysia, 2006). The reasons for this reduction were an increase in the prevalence of fluorosis and concerns over the contribution of water fluoridation to total fluoride exposure. Concerns had also been raised about the higher water intake among the population in a tropical country like Malaysia with average temperature of 27 to 30 degree Celsius (Malaysian Metrological Department, 2017). Evidence on the relationship between climatic conditions and fluorosis levels in the water was first established by Galagan et al. (1957). These authors found that variation between fluorosis levels could be attributed to different volumes of water consumed by people living in different temperatures (Galagan et al., 1957). Therefore climate factors also play a role when considering the recommendation of the optimal fluoride concentration in the drinking water.

1.2.4.2 Exposure to fluoride from other sources

Since the 1980s fluoridated toothpaste use has become widespread in Malaysia. Similar to many other countries, the standard fluoride toothpaste concentration is 1000-1500 ppm for adults and 500 ppm for children. The previous standard recommendations for children with regards to amount of toothpaste under six years of age was to use a small (smear to pea) size and under three years a smear of toothpaste (Oral Health Division Ministry of Heath Malaysia, 2003a). However this guideline was revised in 2007 (Oral Health Division Ministry of Health Malaysia, 2007, Malaysian Dental Council, 2009). The modification to the guideline includes; children under two years of age should have their teeth brushed without fluoridated toothpaste; a smear size of toothpaste for children aged two to four years; and pea size of toothpaste is for children aged four years and above. Professionally applied fluoride varnish/gel is recommended for children who are at high risk in developing caries. Some local initiatives took place to improve the oral health status in non-fluoridated areas. For example a school-based fluoride mouth rinsing programme in the Sarawak area (Chen et al., 2010). Another preventive strategy adopted in Malaysia is a school-based fissure sealant programme for seven year-old school children (Oral Health Division Ministry of Health Malaysia, 2003b). This programme was established in 1999 and is still carried out in both fluoridated and non-fluoridated areas as part of the school dental service.

1.2.4.3 Monitoring of fluoride levels in public water supply

There are three major agencies involved in monitoring fluoride levels in the Malaysian public water supply. These agencies are the Oral Health Division, Public Health Department and water treatment plant management.

At the national level, Oral Health Division, the Ministry of Health is responsible for monitoring the fluoridation programme by setting a standard in the National Indicator Approach to ensure safety and effectiveness of water fluoridation programme (Oral Health Division Ministry of Health Malaysia, 2006). The standard level for fluoride in drinking water is incorporated as a policy into the National Guidelines for Drinking Water Quality document. At the state level, the District Dental Officer is responsible for monitoring fluoride levels both at the water treatment plant sampling point and reticulation points in the district. Fluoride level is measured using test equipment such as Colorimeters/ ionic colorimeter.

The Public Health Department, Ministry of Health is authorised to monitor quality of drinking water. Every three months, water samples are collected from sampling points by relevant Health Inspectors and tested for fluoride levels by the Chemistry Department. Any violation on standards should be rectified in time to ensure safety and effectiveness of the programme. Periodic reports of fluoride levels are disseminated to relevant departments such as Engineering Division, Ministry of Health, the State Health Department and the District Health Officer.

The management of the water treatment plants in public and private sector is responsible for complying with the standard and ensuring that fluoride levels are maintained at recommended level at all time.

1.2.4.4 Summary

Malaysia has a strong public health policy in fluoride based caries prevention. In 2005 there was a change in the public health policy with regards to concentration of fluoride in the water from 0.7 ppm to 0.5 ppm. This policy change aims to achieve benefit of fluoride in caries prevention and minimise the risk of fluorosis. The next chapter systematically reviews the existing literature on the impact of reducing or stopping water fluoridation on dental caries and fluorosis.

2 The impact of stopping the addition or reducing the level of fluoride in public water supply: a systematic review

This chapter presents a systematic review that examines the impact of stopping or reducing the addition of fluoride to public water supply on dental caries and fluorosis. Standard of reporting in this review is based on the PRISMA guidelines for systematic reviews that evaluate health care interventions (Liberati et al., 2009).

2.1 Introduction

Systematic reviews have acknowledged the benefits of water fluoridation as a whole population approach to caries prevention (McDonagh et al., 2000, Australian National Health and Medical Research Council, 2007, Iheozor-Ejiofor et al., 2015). According to the British Fluoridation Society, water fluoridation is currently practiced in 25 countries worldwide (The Birtish Fluoridation Society, 2012). Although water fluoridation has proved a successful approach in caries prevention, over time a number of countries have reviewed their fluoridation policy in light of alternative means of fluoride delivery. There are a number of countries where fluoridation was used either for a short time on an experimental basis or having been used for a longer period was stopped. Countries and areas which have been fluoridated in the past but have removed fluoride from the water include Scotland (Wigtownshire) (Attwood and Blinkhorn, 1989), Wales (Anglesey) (Thomas et al., 1995), Finland (Kuopio) (Seppa et al., 1998), Cuba (La, Salud) (Kunzel and Fischer, 2000), Japan (Okinawa) (Kobayashi et al., 1992), China (Gongzhou) (Wei and Wei, 2002), South Korea (Cheongju) (Cho et al., 2014) and Canada (Calgary) (McLaren et al., 2016). Reasons for cessation are discussed further in Section 2.6.4.

In a number of countries, rather than cease fluoridation completely, the level of fluoride added to the water has been adjusted downwards. For example the US Public Health Services recommended lowering fluoride levels in public water supply from the previously agreed range of 0.7 to 1.2 ppm (parts per million) fluoride (F) to a level of 0.7 ppm (Federal Panel on Community Water Fluoridation, 2015). In Europe, Ireland has lowered the fluoride concentration in the water from 1.0 ppm to a new range 0.6 - 0.8 ppm, with a target concentration of 0.7 ppm in 2007 (Parnell et al., 2009, Whelton and O'Mullane, 2012). In Asia, authorities in Hong Kong have reduced the fluoride concentration in their public water supply twice, from 1ppm to 0.7 ppm in 1978 and then a further reduction to 0.5 ppm in 1988 (Wong et al., 2014). In Southeast Asia, Singapore has taken similar action by reducing the concentration of fluoride in drinking water twice from 0.7 to 0.6 ppm in 1992 and further to 0.5 ppm in 2008 (Petersen et al., 2012). Of particular relevance to the work reported later in this thesis, in 2005 the Malaysian Ministry of Health reduced the fluoride level in the public water supply from 0.7 ppm to a target concentration of 0.5 ppm (Oral Health Division Ministry of Health Malaysia, 2006). Reasons for lowering the 'optimum' fluoride level relate mainly to rising concern over an increased prevalence of dental fluorosis. The relationship between water intake and local climatic conditions and the contribution of fluoride in drinking water to total fluoride exposure have also impacted on decisions by authorities on optimal fluoride levels in the water supply. However the impact of reducing the optimum fluoride concentration in the water supply has been questioned (Spencer and Do, 2016), as discussed further in Section 2.7.2.

Existing systematic reviews of water fluoridation have evaluated the effectiveness of water fluoridation in terms of caries prevention (McDonagh et al., 2000, Australian National Health and Medical Research Council, 2007, Iheozor-Ejiofor et al., 2015).

Previous reviews have also examined the effect of the total cessation of water fluoridation, but have not been comprehensive in their inclusion of cessation studies. For example, the York review considered eight cessation studies, which included studies with negative and positive control groups (McDonagh et al., 2000). However, the recently published Cochrane review had more stringent inclusion criteria and included only a single study with a positive control (Iheozor-Ejiofor et al., 2015). The York review suggested that the prevalence of dental caries increased following the withdrawal of water fluoridation. The Cochrane review concluded that 'there is insufficient information to determine the effect of stopping community water fluoridation on caries levels'. A very recent systematic review by McLaren and Singhal (2016) included fifteen articles on the impact of fluoridation cessation on dental caries. These authors emphasised the methodological limitations of assessing fluoridation cessation and highlighted the value of including studies with a historical control. In addition to the effects of total removal of fluoride from the public supply, McLaren and Singhal discussed the decision-making circumstances that have surrounded cessation, but were unable to establish any studies reporting on this topic. Additionally they did not examine the impact of cessation on fluorosis.

Research on the effects of lowering the optimum fluoride level in the water is less common than studies that have examined total cessation. However this is important because as stated above, reduction rather than total cessation appears to be occurring more frequently in recent years. To date there have been no systematic reviews looking at the impact of fluoride reduction as opposed to total cessation. The work reported in this Chapter aims to systematically review the impact of stopping or reducing the fluoride level in the water on dental caries and fluorosis.

In this review the terms cessation and reduction are used. Cessation refers to stopping the addition of fluoride to the public water supply. Reduction implies a downward change in the concentration at which the water is fluoridated.

2.2 Aims

To systematically review the impact of stopping or reducing the fluoride level in the water on dental caries and fluorosis.

2.3 Review Questions

This review sought to answer the following questions:

1. What are the effects of cessation of water fluoridation on the prevalence of dental caries?
2. What are the effects of cessation of water fluoridation on the prevalence of dental fluorosis?
3. What are the effects of the reduction of fluoride level in the water on the prevalence of dental caries?
4. What are the effects of the reduction of fluoride level in the water on the prevalence of dental fluorosis?

2.4 Materials and Methods

2.4.1 Water fluoridation cessation and dental caries and fluorosis

2.4.1.1 Type of studies

When reviewing the impact of stopping water fluoridation, the following types of study were included in the review.

- Studies with a historical comparison, populations receiving fluoridated water then subsequently having fluoride discontinued from the water (pre and post study with no control group)
- Studies comparing at least two populations with one previously fluoridated, the other with non-fluoridated water (negative control); and
- Studies comparing at least two populations with groups from fluoridated areas at baseline, with one group subsequently having fluoride removed from the water and the control group remained fluoridated (positive control).

2.4.2 Type of interventions

The review looked at both the permanent or temporary cessation of fluoride in the water supply in at least one of the study areas. The intervention had to be in place at least for 12 months to allow a meaningful effect of the intervention on caries or fluorosis. Areas with a natural fluoride level of less than 0.3ppm were regarded as “non-fluoridated”. Exposure to other sources of fluoride (e.g. fluoridated toothpaste) were not considered as these were assumed to be similar across fluoridated and non-fluoridated communities. If no specific information was available for other sources of fluoride, any studies conducted after 1975 in industrialised countries were assumed to have been conducted in the presence of fluoridated toothpaste use in the communities involved.

2.4.2.1 Type of participants

There were no age limits or other demographic restrictions applied to the populations included in the review.

2.4.3 Reduction of fluoride in the water on dental caries and dental fluorosis

The review of studies examining a reduction in the concentration of fluoride in the water supply (rather than cessation) was conducted in a similar fashion to that for cessation studies (Section 2.4.1.1). The only difference was related to the type of study included for fluorosis outcome. Taking consideration of the contrasting aetiology between fluorosis and caries, studies that used birth cohort analysis was deemed valuable to be included in this review. Any studies that compared fluorosis prevalence across multiple age groups that correlated with the change in fluoride level during enamel development were included. The requirement of ‘two point in time’ was extracted based on change in exposure to fluoride level during tooth development that occurred at different age. When reviewing fluorosis, this additional type of study was included in addition to the studies described earlier.

2.4.3.1 Outcome measures

The primary outcomes were changes in caries prevalence and the presence of dental fluorosis. The measures deemed suitable for inclusion in the systematic review are described below:

Dental caries

Measures of dental caries were as follows:

- A change in the number of decayed, missing and filled deciduous and permanent surfaces and teeth (dmfs/DMFS and dmft/DMFT)
- The percentage of caries free children

Dental fluorosis

Dental fluorosis was measured as the percentage of children affected by fluorosis using the following indices:

- Dean's Fluorosis Index
- Tooth Surface Index of Fluorosis (TSIF)
- Thylstrup and Fejerskov (TF) Index
- Developmental Defects of Enamel (DDE)

For measurement of fluorosis, the percentage prevalence was based on the index used in the individual studies. Subjects were defined as having fluorosis with a DDE, TSIF, TF score greater that zero or Dean's classification of 'questionable' or greater as described in the York Review (McDonagh et al., 2000).

2.4.3.2 Other effects of fluoridation

For the context of this review, only dental fluorosis was recorded. Any other adverse effects (e.g. skeletal fluorosis, hip fractures, cancer, congenital malformations, mortality) are outside the scope of this review and are not reported.

2.5 Search Methods for Identification of Studies

2.5.1 Electronic searches

For this review, detailed search strategies were developed combining controlled vocabulary and free text terms for each database searched. In collaboration with a professional dental subject librarian, the search covered research published from their starting date to 11th February 2016. The detail of each search strategy and the keywords used are shown in Appendix 3 to Appendix 6. All publications were searched with no language restrictions on the language of publication. The searched databases were as follows:

- EMBASE via OVID (1947 to 11th February 2016; Appendix 3)
- MEDLINE via OVID (1947 to 11th February 2016; Appendix 4)
- The Cochrane Central Register of Controlled Trials (start date to 11th February 2016;Appendix 5)
- The Web of Science (1990 to 11th February 2016; Appendix 6)

2.5.1.1 Additional search strategies

The reference list of the eligible papers was also hand searched. Attempts were made to contact authors for unpublished papers if necessary (Appendix 7). During the conduct of the review and beyond the formal end date for the database search a new paper relevant to this work was published. This and two subsequently identified papers, identified outside the formal search strategy were identified and are also included in the review.

2.5.2 Data collection and analysis

2.5.2.1 Selection of studies

The author of this thesis (NAMN) screened the titles and abstracts based on all references identified by the electronic searches. Initial exclusions made based on titles and abstracts were agreed with IGC. For studies appearing to meet the inclusion criteria or for which there were insufficient data in the title and abstract to make a clear decision, full text articles were obtained. Two reviewers assessed all full text articles independently and disagreement was resolved by discussion. The excluded studies and reasons for their exclusion were recorded as described in Section 2.6.3.

2.5.2.2 Data extraction and management

Three reviewers (NAMN, IGC, BLC) were involved in the data extraction exercise. Reviewers were paired for designated articles using data extraction forms (Appendix 8). Any disagreements were resolved by discussion. The data extraction forms were piloted on three papers and necessary amendments were made before their use to screen all papers.

For each study, the following data were recorded (Table 2.1)

Table 2.1 Key information extracted from each study

- Year of publication, country of origin and source of study funding.
- Details of the participants including demographic characteristics (socioeconomic status, ethnicity), age, deciduous or permanent dentition and inclusion and exclusion criteria.
- Details of type of intervention, comparator and co-interventions.
- Details of outcomes reported
- Details of the confounding factors considered (potential confounders of relevance to this review include sugar consumption/dietary habits, SES, ethnicity and the use of other fluoride sources).
- Details on comparability of groups with regard to confounding factors.
- Details on methods used to control for confounding.
- Details relating to both adjusted and unadjusted effect estimates.
- Reasons for cessation or reduction of fluoride level in the water.

2.5.2.3 Assessment of study validity

Each study included in this review was assessed using a validity assessment checklist (a validity score and level of evidence) that used in the York Review (NHS Centre for Reviews and Dissemination, 1996, McDonagh et al., 2000)

Each study was assigned a level of evidence using definitions as described in Table 2.2 and a validity score based on the checklist (Appendix 9). The maximum score was 8 for all study designs.

Table 2.2 Level of evidence score, adapted from the York Review (McDonagh et al., 2000)

Level A Highest quality of evidence, minimal risk of bias	• Prospective studies that started within one year of discontinuation of water fluoridation and have a follow up of at least two years for positive effects and at least five years for negative effects. • Studies either randomised or addressing at least three possible confounding factors and adjusting for these in the analysis where appropriate. • Studies where the fluoridation status of participants is unknown to those assessing outcomes.
Level B Evidence of moderate quality, moderate risk of bias	• Studies that started within three years of discontinuation of water fluoridation, with a prospective follow up for outcomes. • Studies that measured and adjusted for less than three but at least one confounding factor. • Studies in which fluoridation status of participants was known to those assessing primary outcomes, but other provisions were made to prevent measurement bias.
Level C Lowest quality of evidence, high risk of bias	• Studies of other designs (e.g. cross sectional), prospective or retrospective, using concurrent or historical controls, that meet other inclusion criteria. • Studies that failed to adjust for confounding factors. • Studies that did not prevent measurement bias.

2.5.3 Data synthesis and outcome measurement

2.5.3.1 Caries

An excel spreadsheet was created to record data (mean, S.D/S.E, percentage prevalence, sample size) for all study types (study with no control, study with negative control, study with positive control). The following caries indices were included in the synthesis: DMFT/DMFS in the permanent dentition, dmft/dmfs in the primary dentition, percentage of children who were caries free in the permanent dentition and the percentage of children who were caries free in the primary dentition.

Studies with no control group

For the caries outcome, in studies with no control group, the comparison of interest was the difference between post-intervention and pre-intervention score in the mean caries prevalence (post-pre). A positive difference shows that caries increased after intervention (cessation or reduction). A negative difference shows that caries decreased after intervention. However, the interpretation of negative or positive difference of participants is reversed when percentage caries free is the outcome measure. This is because while an increase in dmf/DMF is undesirable the percentage caries free increasing is desirable.

Studies with a control group

For those studies with a control group, only cessation studies were available for analysis. The measure of effect was the mean caries change found between (intervention-control) at baseline, and between (intervention-control) at follow up. A larger mean difference in dmf/DMF (at baseline) indicates a beneficial effect of water fluoridation (positive difference). A smaller mean difference indicates a non-beneficial effect of water fluoridation (negative difference). This also holds true when percentage caries free is the outcome measure.

Of the studies included in the review, some were not usable because either the mean, or the standard deviation or the number of participants was missing. The data were compiled and presented in a descriptive table based on the available information indicating the general effect of stopping or reducing fluoride concentration in the water. Quantitative analysis using meta-analysis focused on studies with a control group. To be eligible for inclusion in the meta-analysis, studies required non-missing information and a minimum of two studies using the same outcome measure. Details of how the meta-analysis was conducted are discussed in the following section.

Assessment of heterogeneity

The I^2 score and chi-square analyses were used to test for differences between studies (heterogeneity). This test was use to assess whether the observed variability in study results (measure of effect) is greater than that expected to occur by chance. If the test of heterogeneity was not significant (I^2: towards 0%, chi-square: $p \geq 0.05$), fixed-effect models were used. Whereas, if the test of heterogeneity was significant (I^2: towards 100%, chi-square: $p<0.05$), the random-effect models were used. The analyses were carried out using STATA Version 13.

Ideally meta-regression will be used to investigate and explain sources of heterogeneity, however due to lack of data this analysis is not able to perform. The same principle applied for caries and fluorosis outcomes.

A meta-analysis for caries outcome

In this review, only cessation studies with a negative control group had a sufficient data to permit the conduct of a meta-analysis for caries outcome. The comparison of interest was the difference in mean caries change found between (intervention-control) at baseline, and between (intervention-control) at follow-up. This is an analysis of the difference of differences of means, which is slightly different to the usual approach that forms a simple difference of means between two groups (e.g., control and intervention) using meta-analysis. Thus, appropriate estimates of the standard deviation and sample size are therefore necessary with respect to these differences in each arm (i.e., control and intervention) for meta-analysis. Here the pooled estimate of the standard deviation based on the pre- and post-standard deviations in each arm (control and intervention) are used, and the harmonic means of the sample sizes in each arm are found. The formula used to calculate the difference of the difference was as follows: $[(\text{PostCessation}_I - \text{PostCessation}_C) - (\text{PreCessation}_I - \text{PreCessation}_C)]$. Data were analysed using (STATA Version13) software to produce a pooled estimate effect.

Results are presented as Forest plots, which show both the results of each individual study and the pooled results of meta-analysis. The pooled results are identified by the diamonds within the Forest plot, where the middle of the diamond gives the pooled point-value estimate for the Standardised Mean Difference (SMD) and its edges give the associated 95% confidence interval (CI).

2.5.3.2 Fluorosis

The percentage prevalence of fluorosis, measured using the indices described in Section 2.4.3.1 was used to determine the impact of fluoride level change. The percentage change in fluorosis prevalence was calculated as the difference between post-intervention and pre-intervention (post-pre). A positive difference showed that the fluorosis prevalence increased after the intervention (cessation or reduction). A negative difference shows that the fluorosis prevalence decreased after the intervention (cessation or reduction).

A meta-analysis for fluorosis outcome

All of the studies included in the meta-analysis for fluorosis outcome were from studies without a control group (pre and post studies). The meta-analysis for fluorosis was divided into three parts. The first analysis combined individual studies on the effect of reducing fluoride level and fluorosis. The second analysis combined individual studies on the effect of stopping fluoridation and fluorosis. A third analysis combined studies from both interventions (stopping or reducing) fluoride level for pooled estimates of effect across time points.

For the prevalence of fluorosis, outcomes are binary and results are given only for pre to post-fluoride cessation/reduction. A simple approach is to use meta-analysis based on odds ratios, which utilises the number of cases of fluorosis pre and post-fluoride cessation/reduction and overall sample sizes in order to form a pooled estimate effect. Data were analysed using STATA Version 13 software. Results are presented as Forest plots, which show both the results of each individual study and the pooled results of meta-analysis. The pooled results are identified by the diamonds within the Forest plot, where the middle of the diamond gives the pooled point-value estimate for the odds ratio and its edges give the associated 95% confidence interval (CI).

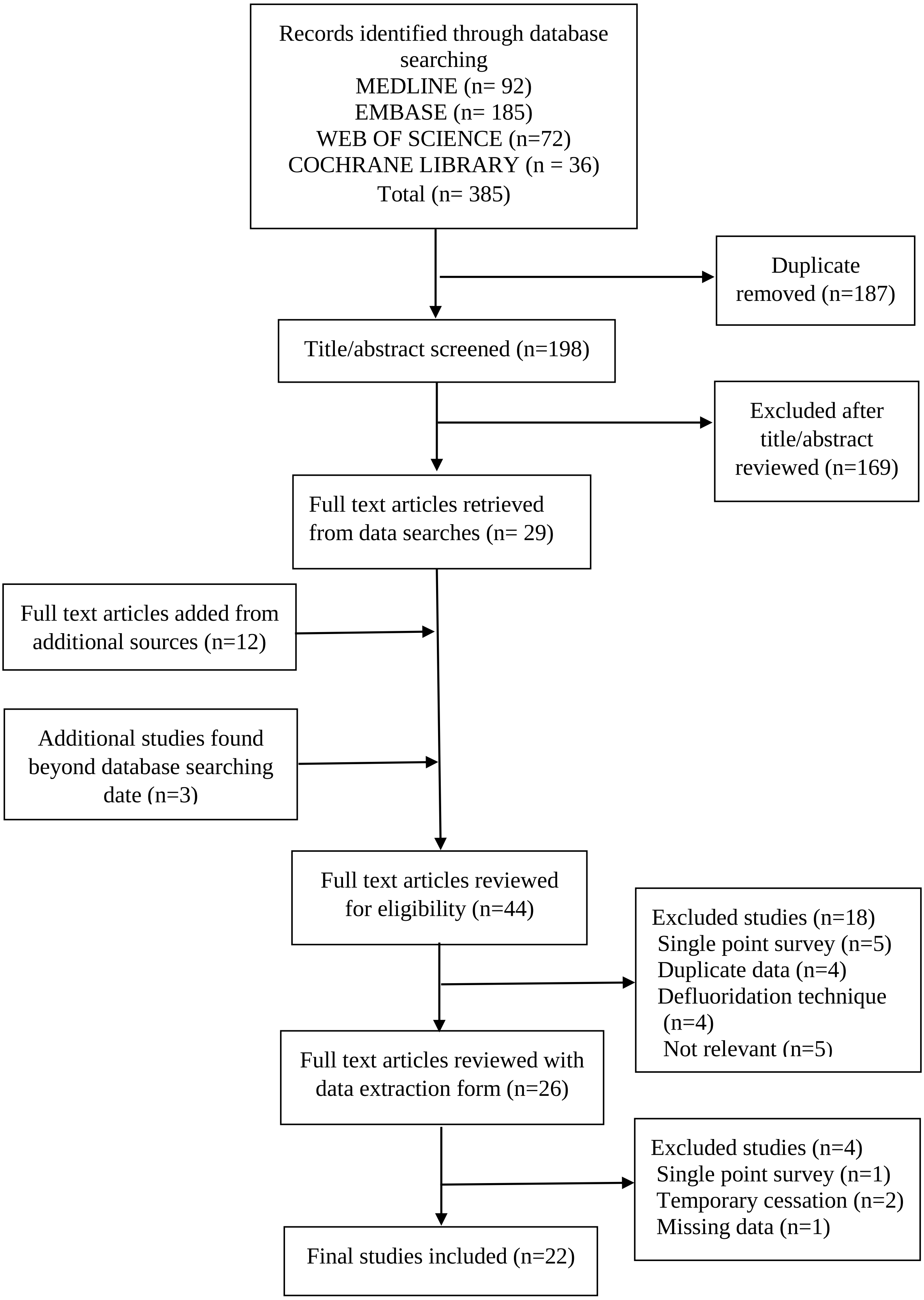
The meta-analysis used for fluorosis outcome differed from that used in caries studies because of the different study design. The analysis used for fluorosis compared pre and post intervention, rather than comparing the differences found within (intervention-control) at baseline and follow up survey.

2.6 Results

2.6.1 Results of the search

A total of 385 studies were identified in the database searches as shown in the PRISMA diagram (Liberati et al., 2009) Figure 2.1. Titles and abstracts were screened and 187 records were excluded as duplicates. A further 169 were deemed irrelevant and excluded. That left 29 articles for full text review and this was added to by 15 additional articles identified from other sources. In total, 44 full text articles were screened for eligibility. Eighteen records were excluded at this stage, leaving 26 papers for data extraction. In the course of data extraction it became apparent that four of these studies were unsuitable. Reasons of exclusion are described in section 2.6.3. A total of 22 publications were included in the review.

Figure 2.1. PRISMA Flow Diagram



2.6.2 Included studies

A total of sixteen publications on fluoride cessation and six publications on fluoride reduction were included in this review. No studies were reported as evidence level A (high quality, bias unlikely). The majority of the studies were cross-sectional in nature. Study designs were divided into three categories; studies with a positive control group, studies with a negative control group and studies without a concurrent comparison group (i.e. studies that relied on a historical control). Summaries of individual study designs with full details on findings are presented in Appendix 10, characteristics of studies (Appendix 11) and validity scores are presented in Appendix 12.

Year of publication year ranged from 1962 to 2016. The included studies were divided into stopping or reducing fluoride level. The study types are explained in Table 2.3 together with the number for each outcome measure (caries and fluorosis). The details of the included studies are described in the following sections.

Table 2.3 Number and type of studies categorised by change of fluoride level in the water and the main outcome measure

Change of fluoride level	Study type	Definition of study type	Number of studies for each outcome measure	
			Caries	Fluorosis
Cessation	No Control	Study that has pre and post-cessation data in one or more populations.	7[ϕ]	2[ϕ]
	Negative control	Study that used a non-fluoridated area as a control. The intervention group was exposed to artificial fluoridation at baseline and subsequently fluoride was removed from the water.	6	0
	Positive control	Study when the intervention group was exposed to artificial fluoridation at baseline and subsequently fluoride was removed from the water at follow-up, while the control group remained artificially fluoridated at both time points.	2	0
Reduction	No control	Study that has pre and post-fluoride reduction in one or more populations.	1	5

[ϕ] One publication reported both outcomes (caries and fluorosis) thus the above Table presents 23 publications.

2.6.2.1 Included studies for caries outcome measure

Sixteen publications met the inclusion criteria for the caries outcome. Fifteen of these publications assessed the effect of stopping fluoridation (Jordan, 1962, DHSS, 1969, Lemke et al., 1970, Stephen et al., 1987, Attwood and Blinkhorn, 1989, Kalsbeek et al., 1993, Kunzel and Fischer, 1997, Seppa et al., 1998, Kunzel and Fischer, 2000, Kunzel et al., 2000, Seppa et al., 2000a, Seppa et al., 2000b, Maupome et al., 2001a, Wei and Wei, 2002, McLaren et al., 2016) and only one study focused on the effect of reducing fluoride level in the water on caries (Kunzel, 1980).

Four publications were funded by research grants from research organisations, health authorities and government organisations (Seppa et al., 1998, Kunzel and Fischer, 2000, Maupome et al., 2001a, McLaren et al., 2016) while the other publications did not state their funding sources.

Cessation study (caries outcome)

Of the fifteen publications on caries outcome, eight publications had a control group (two publications with positive control, six publications with negative control) and the remaining seven publications were without a control group.

The occurrence of water fluoridation cessation varied by geographic location (USA, Germany, Scotland, Netherlands, Finland, China and Canada). Four publications were scored as evidence level B (moderate quality) (Kalsbeek et al., 1993, Maupome et al., 2001a, Seppä et al., 2000a, McLaren et al., 2016) and the remaining eleven publications were scored as evidence level C (lowest quality) (Jordan, 1962, DHSS, 1969, Lemke et al., 1970, Stephen et al., 1987, Attwood and Blinkhorn, 1989, Kunzel and Fischer, 1997, Seppa et al., 1998, Seppä et al., 2000b, Kunzel and Fischer, 2000, Kunzel et al., 2000, Wei and Wei, 2002).

Reduction study (caries outcome)

Only one publication conducted in Germany, looked at effect of lowering fluoride level on dental caries prevalence (Kunzel, 1980). This study had no comparison group and was rated as evidence level C.

2.6.2.2 Included studies for fluorosis outcome measure

Seven publications met the inclusion criteria for the fluorosis outcome (Horowitz and Heifetz, 1972, Horowitz et al., 1972, Evans, 1989, Evans and Stamm, 1991b, Wei and Wei, 2002, Clark et al., 2006, Wong et al., 2014). Five publications looked at the effect of reducing fluoride level in the water and two publications assessed the effect of stopping fluoridation on fluorosis.

Two studies were funded by research grants from research organisations, health authorities and government organisations (Clark et al., 2006, Wong et al., 2014), while the other studies did not state their funding sources.

Cessation studies (fluorosis outcome)

Two studies assessed the effect of stopping fluoridation on fluorosis. These were conducted in Gongzhou, China (Wei and Wei, 2002) and British Columbia, Canada (Clark et al., 2006). The Chinese study reported fluorosis prevalence using Dean’s Index and the Canadian study used the TF Index. The Chinese study was scored as evidence level C and the Canadian study was scored as evidence level B.

Reduction studies (fluorosis outcome)

Out of five studies that assessed the effect of reducing fluoride level in the water, three were conducted in Hong Kong and two in USA. Two Hong Kong studies (Evans, 1989, Evans and Stamm, 1991b) were linked publications conducted in multiple districts in Hong Kong, which compared fluorosis prevalence across multiple age groups that were exposed to different fluoride levels and change of fluoride level occurred during enamel development. The remaining publications were cross sectional studies without a control group.

Four publications reported fluorosis prevalence using Dean’s Index and the other used the DDE Index. Only one study was evidence level B and the remaining four publications were evidence level C.

2.6.3 Excluded studies

Of 44 studies that were assessed for eligibility, 18 studies were excluded as irrelevant (Horowitz et al., 1964, Walvekar and Qureshi, 1982, Attwood and Blinkhorn, 1988, King et al., 1986, King and Wei, 1986, King, 1989, Seaman et al., 1989, Kobayashi et al., 1992, Treasure and Dever, 1992, Treasure and Dever, 1994, Liang, 1998, Angelillo et al., 1999, Wu et al., 2000, Maupome et al., 2001b, Seppa et al., 2002, Wong et al., 2006, Mohapatra et al., 2009, Cho et al., 2014). The reasons for exclusion were as follows (Table 2.4).

Table 2.4 Excluded studies and reasons for exclusion following the first stage assessment of study eligibility

References	Reasons for exclusion
(Kobayashi et al., 1992, Treasure and Dever, 1992, Treasure and Dever, 1994, Seppa et al., 2002, Cho et al., 2014).	Studies that used a single post-intervention cross-sectional design.
(Horowitz et al., 1964, Attwood and Blinkhorn, 1988, Maupome et al., 2001b, Wong et al., 2006).	Series of publications that refer to the same data already included in this review.
(Walvekar and Qureshi, 1982, Liang, 1998, Wu et al., 2000, Mohapatra et al., 2009).	Studies that focus on defluoridation technology.
(King et al., 1986, King and Wei, 1986, King, 1989, Seaman et al., 1989, Angelillo et al., 1999).	Studies that were not relevant to stopping or reducing fluoride level in the water supply.

In the process of data extraction, a further four studies were excluded. Reasons for exclusion were as follows (Table 2.5).

Table 2.5 Excluded studies and reasons for exclusion following data extraction stage

References	Reasons for exclusion
(Burt et al., 2000, Burt et al., 2003)	Temporary cessation of fluoridation for less than 12 months. Further the data were presented in a manner which made data extraction impossible.
(Buzalaf et al., 2004)	A single post-intervention cross-sectional design. The date when the study was conducted was not stated. Duration of post-cessation exposure was not clear.
(Thomas et al., 1995)	A study with no concurrent follow-up data in the comparison group.

2.6.4 Date and reasons for change in fluoride level

The year of fluoridation cessation ranged from 1956 to 2011. The year of reducing fluoride level ranged from 1970 to 1988. All publications focused on children, aged between 5 years to 15 years.

Several studies reported reasons for water fluoridation cessation and the reasons varied across studies. These included: technical issues (Thomas et al., 1995), significant political/economic event (Kunzel and Fischer, 1997, Kunzel et al., 2000), lack of clarity about pertinent laws (Attwood and Blinkhorn, 1989), observed increases in dental fluorosis (Wei and Wei, 2002), public vote in favour of cessation (Maupome et al., 2001a) and opposition or anti-fluoridation movements (Seppa et al., 1998).

Reasons for reduction of fluoride level in the water were related to an increase prevalence of fluorosis, relationship between water intake and climate condition (Evans, 1989, Wong et al., 2014) and technical issues (Kunzel, 1980).

2.6.5 Results synthesis

The included studies varied by study design, outcome measure, duration of intervention (stopping or reducing fluoride level) and differences in fluoride concentration being compared.

For caries outcome, there were more studies published on stopping water fluoridation than those reporting a reduction. For the fluorosis outcome measure, there were more studies published on reducing fluoride level in the water than was the case with cessation.

To summarise the impact of cessation and reduction on each outcome measure, results were synthesised as follows:

Articles were classified into four main categories based on change in fluoride level (stopping or reduction) and outcome measure (caries or fluorosis). For caries, the outcome of interest was change in the mean DMFT/dmft/DMFS/dmfs and percentage caries free, and results were grouped into three subsets based on study designs (study with no control, study with a negative control and study with a positive control). For fluorosis, the outcome of interest was a change in fluorosis prevalence. For each of these categories, key information is presented in Tables 2.6 to 2.16 (caries) and Tables 2.17 to 2.18 (fluorosis) and results were described qualitatively. Quantitative analysis was carried out for studies that met the inclusion criteria for meta-analysis (Sections 2.6.8 and 2.6.10). Analysis was conducted separately for caries and fluorosis outcome measures.

2.6.6 Studies reporting the impact of changes in fluoride level on dental caries

Studies on the impact of changing fluoride levels are reported below in two groups: those where fluoridation ceased and those where there was a partial reduction.

Within these change levels, caries outcome is reported in terms of change in the mean DMFT/dmft/DMFS/dmfs and also the change in percentage of study participants who were caries free.

In addition, the outcome is reported according to the study design and the nature of the control group.

The only type of study that had a sufficient data to permit the conduct of a meta-analysis was those with a negative control group. Other types of study are reported qualitatively, without an attempt to combine their overall estimate of the effect of changed fluoride levels on caries prevalence.

2.6.6.1 Stopping fluoridation and caries

Fifteen publications met the inclusion criteria for caries outcome following cessation of water fluoridation. Results were grouped into three subsets based on study designs as described in the following section.

Studies with no control group

For studies without a control group, stopping water fluoridation was associated with an increased level of caries experience for studies published in the 1970s or earlier and a decreased level of caries experience for studies published from 1997 onwards. The effect of stopping water fluoridation is shown in Table 2.6. This presents the mean change in caries prevalence at tooth level (DMFT) before and after cessation. This ranged from -2.73 to 1.10. The mean change in caries prevalence at surface level (DMFS) is also shown. This indicates that mean caries prevalence decreased after fluoridation discontinued. To aid interpretation of the results, a positive difference shows caries increased after cessation. A negative difference shows that caries decreased after cessation.

Table 2.6 Summary of studies with no control group on caries outcome (permanent dentition) when fluoridation was discontinued

Authors	Country/ Area	Age	Pre-survey	Post-survey	Year change in F level	Mean caries (pre)	Mean caries (post)	Mean caries change#
Outcome: DMFT_studies with no control group								
Jordan, 1962	Austin, USA	6	1955	1959	1956	0.40	0.51	0.11
		7				1.20	1.38	0.18
		8				2.10	2.07	-0.03
Lemke et al., 1970)	Antigo, USA	8	1960	1964	1960	0.60	1.70	1.10
		10				1.70	2.40	0.70
Kunzel and Fischer, 1997	Chemnitz, Germany	8	1987	1995	1990	0.75	0.32	-0.43
		12				2.55	1.87	-0.68
		15				4.87	3.78	-1.09
	Plauen, Germany	8	1983	1995	1984	0.70	0.58	-0.12
		12				3.50	1.98	-1.52
		15				6.20	3.47	-2.73
Kunzel and Fischer, 2000	La Salud, Cuba	6/7	1982	1997	1990	0.07	0.07	0
		8/9				0.50	0.60	0.10
		10/11				1.10	0.80	-0.30
		12/13				2.10	1.10	-1.00
Kunzel et al., 2000	Spremberg, Germany	8	1993	1996	1993	0.51	0.34	-0.17
		9				0.69	0.50	-0.19
		12				2.36	1.45	-0.91
		13				2.59	1.63	-0.96
		15				4.13	3.74	-0.39
		16				5.03	3.86	-1.17
	Zittau, Germany	12	1993	1996	1993	2.47	1.96	-0.51
Wei and Wei, 2002	Gongzhou, China	15	1982	1990	1983	0.90	0.44	-0.46
Outcome: DMFS_study with no control group								
Kunzel and Fischer, 2000	La Salud, Cuba	6/7	1982	1997	1990	0.10	0.07	-0.03
		8/9				0.70	0.70	0
		10/11				1.50	1.20	-0.30
		12/13				3.10	1.50	-1.60

#Mean caries change= (PostCessation - PreCessation). A positive difference shows that the mean difference between post and pre, indicating that caries increased after cessation. A negative difference shows that caries decreased after cessation.

The impact of stopping fluoridation on the primary dentition is presented in Table 2.7. There were only two studies that contributed data to this outcome, and the change in both dmft and dmfs are shown. An increase in caries prevalence in both studies, one conducted in the USA in the early 1960s and the other in Scotland in the early 1980s, was observed.

Table 2.7 Summary of studies with a no control group on caries outcome (primary dentition) when fluoridation was discontinued

Authors	**Country/ Area**	**Age**	**Pre-survey**	**Post-survey**	**Year change in F level**	**Mean caries (pre)**	**Mean caries (post)**	**Mean caries change**[#]
Outcome: dmft_studies with a no control group								
Lemke et al., 1970	Antigo, Wisconsin, USA	5/6	1960	1964	1960	2.50	4.80	2.30
Stephen et al., 1987	Wick, Scotland	5/6	1979	1984	1979	2.60	3.92	1.32
Outcome: dmfs_study with a no control group								
Stephen et al., 1987	Wick, Scotland	5/6	1979	1984	1979	7.80	13.33	5.53

[#]Mean caries change= (PostCessation - PreCessation). A positive difference shows that the mean difference between post and pre, indicating that caries increased after cessation. A negative difference shows that caries decreased after cessation

The impact of stopping fluoridation on the change in the percentage of caries free for permanent teeth is presented in Table 2.8. The interpretation of negative or positive difference of caries free is reversed to the change in mean caries prevalence that is described above. A negative difference in the percentage caries free following water fluoridation cessation indicates a beneficial effect of water fluoridation. In the older study (Lemke et al., 1970), results show that proportion of children who were caries free reduced following fluoridation cessation. However, mixed findings were reported in the later study by Kunzel and Fischer (2000). Their results indicate that caries free proportion increased in the older children (10-14 years old) and reduced in the younger children (age 6-9 years old) after fluoridation was discontinued.

Table 2.8 Summaries of studies with no control group on percentage caries free (permanent dentition) when water fluoridation was discontinued

Authors	Country/ Area	Age	Pre-survey	Post-survey	Year change in F level	% caries free (pre)	% caries free (post)	% difference# (post-pre)
Outcome: % caries free (permanent)_studies with no control group								
Lemke et al., 1970	Antigo, Wisconsin, USA	8	1960	1966	1960	71	38.5	-32.5
		10				35	26.2	-8.8
Kunzel and Fischer, 2000	La Salud, Cuba	6/7	1982	1997	1990	95.2	93.9	-1.3
		8/9				75.6	65	-10.6
		10/11				54.8	59.6	4.8
		12/13				33.3	55.2	21.9

#Percentage (%) difference= (PostCessation - PreCessation). A positive difference shows that the % caries free increased after fluoridation cessation. A negative difference shows that % caries free decreased after fluoridation cessation.

Table 2.9 shows the change in the percentage of caries free for primary teeth. Two studies, which were published in 1970 and 1987, demonstrated that the percentage caries free reduced following fluoridation cessation. This indicates a beneficial effect of water fluoridation.

Table 2.9 Summaries of studies with no control group on percentage caries free (primary dentition) when water fluoridation discontinued

Authors	Country/ Area	Age	Pre-survey	Post-survey	Year change in F level	% caries free (pre)	% caries free (post)	% difference[#] (post-pre)
Outcome: % caries free (primary)_studies without a control group								
Stephen et al., 1987	Wick, Scotland	5/6	1979	1984	1979	27.4	24.6	-2.8
Lemke et al., 1970	Antigo, Wisconsin, USA	5/6	1960	1966	1960	39	19.8	-19.2

[#] Percentage (%) difference= (PostCessation - PreCessation). A positive difference shows that the % caries free increased after fluoridation cessation. A negative difference shows that % caries free decreased after fluoridation cessation.

Studies with a negative control group

Five publications reported on studies which contained a non-fluoridated area as a negative control. Three publications presented data on permanent teeth only (DMFT and/or DMFS, the calculation used to determine the impact of cessation differed from that where there was no control group. The calculation in this study design accounted for the change in the control groups are shown in Table 2.10 (footnote).

Two included studies for DMFT indicated a larger mean caries difference between intervention (fluoridated) and control group at baseline than the mean difference between intervention (fluoridation ceased) and control group at follow up. This implies a beneficial effect of water fluoridation. The measure of effect in DMFT ranged from 0.60 to 7.40. These results are presented in Table 2.10.

The range of measure of effect in caries change at surface level (DMFS) in permanent teeth is -0.19 to 18.80. Stopping fluoridation has resulted in a narrowing of the difference in caries prevalence between fluoridated and control areas in older children aged (9, 12 and 15 years), suggesting that fluoridation had been beneficial. However, in younger children aged 6 years, not much difference in the mean caries change was observed between intervention and control group after fluoridation cessation.

The data presented in Table 2.10, were subsequently used to inform a meta-analysis as described in Section 2.6.8.

Table 2.10 Summary of studies with a negative control group on caries outcome (permanent dentition) when fluoridation was discontinued

Authors	Country/ Area	Age	Pre-survey	Post-survey	Year change in F level	Mean caries (pre) A	Mean caries (post) B	Mean caries change$^{\#}$ (B-A)	Mean difference (pre) C	Mean difference (post) D	Difference of the difference$^{\wedge}$ (D-C)
Outcome: DMFT_studies with a negative control											
$^{+}$Atwood and Blinkhorn, 1989	UK, Stranrear	10	1980	1986	1983	1.66	1.72	0.06	-1.69	-1.09	0.60
	Annan (control)	10				3.35	2.81	-0.54			
$^{+}$Kalsbeek et al., 1993	Netherland, Tiel	15	1968	1988	1973	7.4	5.5	-1.9	-6.7	0.7	7.40
	Colemborg (control)	15				14.1	4.8	-9.3			
Outcome: DMFS_studies with a negative control											
$^{+}$Seppa et al., 1998	Finland, Kuopio	6	1992	1995	1992	0.06	0.07	0.01	0.03	-0.04	-0.07
		9				0.88	0.69	-0.19	0.18	-0.01	-0.19
		12				1.88	1.62	-0.26	-1.11	-0.01	1.10
		15				4.00	3.19	-0.81	-1.62	-0.72	0.90
	Jyvaskyla (control)	6				0.03	0.11	0.08			
		9				0.70	0.70	0			
		12				2.99	1.63	-1.36			
		15				5.62	3.91	-1.71			
$^{+}$Kalsbeek et al., 1993	Netherland, Tiel	15	1968	1988	1973	10.8	9.6	-1.2	-16.9	1.9	18.80
	Colemborg (control)	15				27.7	7.7	-20.0			

$^{+}$Studies that were included in meta-analysis.

$^{\#}$Mean caries change= (PostCessation - PreCessation). A positive difference shows caries increased after cessation. A negative difference shows that caries decreased after cessation.

Mean difference (pre)=PreCessation$_{\text{Intervention}}$ - PreCessation$_{\text{Control.}}$. Mean difference (post) =PostCessation$_{\text{Intervention}}$ - PostCessation$_{\text{Control}}$

$^{\wedge}$Difference of the difference (measure of effect)= (PostCessation$_{\text{I}}$ - PostCessation$_{\text{C}}$)- (PreCessation$_{\text{I}}$ - PreCessation$_{\text{C}}$). A positive difference shows a beneficial effect of water fluoridation (the mean caries difference between fluoridated and non-fluoridated areas was narrower after fluoridation cessation). A negative difference shows a non-beneficial effect of water fluoridation (the mean caries difference between fluoridated and non-fluoridated areas was greater after fluoridation cessation).

With regards to change in caries experience (dmft) of primary teeth, an older study reported by DHSS (1969) shows a narrower mean caries difference between fluoridated and non-fluoridated areas after fluoridation cessation (Table 2.11). This result favours the benefit of water fluoridation. A study by Atwood and Blinkhorn (1989) showed caries in primary teeth decreased after fluoridation cessation in both areas. However the magnitude of caries reduction was greater after fluoridation stopped, this implies the non-beneficial of water fluoridation.

In terms of caries change at surface level (dmfs) in the primary dentition, the measure of effect is between -0.03 to -0.66. Results were from just one study (Seppa et al., 2000a) in different age groups showed that dmfs decreased after fluoridation cessation in both areas. Findings indicate that stopping fluoridation has resulted in a narrowing of the difference in caries prevalence between fluoridated and control areas. This implies the beneficial effects of water fluoridation (Table 2.11).

Table 2.11 Summary of studies with a negative control group on caries outcome (primary dentition) when fluoridation was discontinued

Authors	Country/ Area	Age	Pre-survey	Post-survey	Year change in F level	Mean caries (pre) A	Mean caries (post) B	Mean caries change[#] (B-A)	Mean difference (pre) C	Mean difference (post) D	Difference of the difference[^] (D-C)
Outcome: dmft_studies with a negative control											
[a]DHSS, 1969	Scotland, Kilmarnock	5	1961	1968	1962	3.99	6.89	2.9	-1.82	0.91	2.73
	Ayr (control)	5				5.81	5.98	0.17			
[b]Atwood and Blinkhorn, 1989	UK, Stranrear	5	1980	1986	1983	2.48	1.17	-1.31	-1.9	-2.65	-0.75
	Annan (control)	5				4.38	3.82	-0.56			
Outcome: dmfs_study with a negative control											
[b]Seppa et al., 2000a	Finland, Kuopio	3	1992	1995	1992	0.47	0.39	-0.08	0.14	0.11	-0.03
		6				2.26	1.90	-0.36	0.94	0.64	-0.30
		9				4.90	3.55	-1.35	1.99	1.33	-0.66
	Jyvaskyla (control)	3				0.33	0.28	-0.05			
		6				1.32	1.26	-0.06			
		9				2.91	2.22	-0.69			

[a]Note included in meta-analysis (missing data on sample, size, s.e/s.d).

[b]Only one study available with complete data for the outcome measure of interest, not included in meta-analysis.

[#]Mean caries change= (PostCessation - PreCessation). A positive difference shows that caries increased after cessation. A negative difference shows that caries decreased after cessation.

Mean difference (pre)=PreCessation$_{\text{Intervention}}$ - PreCessation$_{\text{Control.}}$

Mean difference (post) =PostCessation$_{\text{Intervention}}$ - PostCessation$_{\text{Control}}$

[^]Difference of the difference (measure of effect)= (PostCessation$_{I}$ - PostCessation$_{C}$)- (PreCessation$_{I}$ - PreCessation$_{C}$). A positive difference shows a beneficial effect of water fluoridation (the mean caries difference between fluoridated and non-fluoridated areas was narrower after fluoridation cessation). A negative difference shows a counterintuitive effect of water fluoridation (the mean caries difference between fluoridated and non-fluoridated areas was greater after fluoridation cessation).

Table 2.12 shows the change in the percentage of caries-free for permanent teeth for the single study with a negative control. The proportion of children who were caries free reduced after water fluoridation stopped. In contrast, the proportion of those caries-free increased in the control group. Accounting for the change between intervention and control groups at baseline and follow up, the results show only a one percentage point difference in the proportion of children who were caries-free.

Table 2.12 Summary of studies with a negative control group on percentage caries-free (permanent dentition) when fluoridation was discontinued

Authors	Country/ Area	Age	Pre-survey	Post-survey	Year change in F level	% caries free (pre) A	% caries free (post) B	% difference[#] (B-A)	% difference (pre)	% difference (post)
Outcome: % caries free (permanent)_studies with a negative control										
[+]Seppa et al., 2000b	Finland, Kuopio	12	1992	1995	1992	44	34	-10	15	-14
		15				27	25	-2	17	-16
	Jyvaskyla (control)	12				29	48	19		
		15				10	41	31		

[#]Percentage (%) difference= (PostCessation - PreCessation). A positive difference shows that the % caries free increased after cessation (does not favours fluoridation). A negative difference shows that % caries free decreased after cessation (favours fluoridation).

Percentage (%) difference (pre)=PreCessation$_{\text{Intervention}}$ - PreCessation$_{\text{Control.}}$

Percentage (%) difference (post) =PostCessation$_{\text{Intervention}}$ - PostCessation$_{\text{Control}}$

In terms of the change in the percentage of caries free in the primary dentition, an older study (DHSS, 1969) showed a decreased in the proportion of children who were caries-free in area where fluoridation had stopped (Table 2.13).

No difference in the percentage caries-free in the control area was observed. The more recent study (Seppa et al., 2000b) showed an increased in the proportion of children who were caries-free in both areas after fluoridation stopped except for children aged 9 years old. The magnitude of the percentage difference was larger pre-cessation than the post-cessation which implies a beneficial effect of water fluoridation.

Table 2.13 Summary of studies with a negative control group on percentage caries-free (primary dentition) when fluoridation was discontinued

Authors	Country/ Area	Age	Pre-survey	Post-survey	Year change in F level	% caries free (pre) A	% caries free (post) B	% difference# (B-A)	% difference (pre)	% difference (post)
Outcome: % caries free (primary)_studies with a negative control										
+Seppa et al., 2000b	Finland, Kuopio	3	1992	1995	1992	85	98	13	-7	4
		6				44	67	23	-24	-2
		9				21	35	14	-24	-5
	Jyvaskyla (control)	3				92	94	2		
		6				68	69	1		
		9				45	40	-5		
DHSS, 1969	Kilmarnock, Scotland	5	1961	1968	1962	20	7	-13	13	0
	Ayr (control)	5				7	7	0		

#Percentage (%) difference= (PostCessation - PreCessation). A positive difference shows that the % caries free increased after fluoridation cessation (does not favours fluoridation). A negative difference shows that % caries free decreased after cessation (favours fluoridation)

Percentage (%) difference (pre)=PreCessation$_{\text{Intervention}}$ - PreCessation$_{\text{Control.}}$

Percentage (%) difference (post) =PostCessation$_{\text{Intervention}}$ - PostCessation$_{\text{Control}}$

Studies with a positive control group

There were two studies which included a positive control (i.e. a similar geographic area in which fluoridation continued). Both studies were conducted in Canada, amongst populations with a generally low caries experience, living in urban areas that had good access to dental services. The recent study (McLaren et al., 2016) was conducted following cessation in 2011. For permanent teeth, results show that there was a trend towards a decrease in DMFS in the fluoridation cessation group, which was not apparent in the control group (still fluoridated).

Findings from the McLaren et al. (2016) study were in contrast with another Canadian study (Maupome et al., 2001a), that did not observe an adverse trend in tooth decay in the cessation community, when fluoridation stopped in 1992 (Table 2.14). The Maupome study used the D1D2MFS index and reported a reduction in mean D1D2MFS score in the cessation community but no change was observed in the control (still fluoridated) community. In addition, this study also contained a prospective longitudinal investigation for recording dental caries by assessing transition in smooth and pit and fissure caries. Children were classified into three groups depending on the change in extent of their tooth surface caries: progressed, reversed and unchanged between baseline and follow-up. Among these children, the authors observed that caries progression, especially on smooth surfaces, was more frequent in the cessation community compared to the comparison community. Because Maupome and co-workers used a different approach to recording caries, this study could not be included in a meta-analysis (Maupome et al., 2001a).

Table 2.14 Summary of studies with a positive control group on caries outcome (permanent dentition) when fluoridation was discontinued

Authors	Country/ Area	Age	Pre-survey	Post-survey	Year change in F level	Mean caries (pre) A	Mean caries (post) B	Mean caries change[#] (B-A)	Mean difference (pre) C	Mean difference (post) D	Difference of the difference[^] (D-C)
Outcome: DMFS_study with positive control											
McLaren et al., 2016	Calgary	Grade 2	2004/05	2013/14	2011	0.45	0.15	-0.30	0.2	0.06	-0.26
	Edmonton (control)	Grade 2				0.25	0.21	-0.04			
Outcome: D1D2MFS_study with positive control											
Maupome et al., 2001a	Canada, Comox/ Courtney	8	93/94	96/97	1992	1.29	0.63	-0.66	0.92	0.33	-0.59
		14				4.93	3.86	-1.07	2.66	1.45	-1.21
	Canada, Kamloops (control)	8				0.37	0.30	-0.07			
		14				2.27	2.41	-0.14			

[#] Mean caries change= (PostCessation - PreCessation). A positive difference shows that caries increased after cessation. A negative difference shows that caries decreased after cessation.

Mean difference (pre)=PreCessation$_{\text{Intervention}}$ - PreCessation$_{\text{Control.}}$

Mean difference (post) =PostCessation$_{\text{Intervention}}$ - PostCessation$_{\text{Control}}$

[^] Difference of the difference (measure of effect)= (PostCessation$_{I}$ - PostCessation$_{C}$)- (PreCessation$_{I}$ - PreCessation$_{C}$). A positive difference shows a beneficial effect of water fluoridation (the mean caries difference between fluoridated and non-fluoridated areas was narrower after fluoridation cessation). A negative difference shows a counterintuitive effect of water fluoridation (the mean caries difference between fluoridated and non-fluoridated areas was greater after fluoridation cessation).

In terms of caries experience in the primary dentition, results from McLaren et al. (2016) study show there was an increased caries experience (dmfs) in both geographic areas, but the magnitude of the increase was greater in the area where fluoridation ceased (Table 2.15). This implies the beneficial effects of water fluoridation.

Table 2.15. Study with a positive control group on caries outcome (primary dentition) when fluoridation was discontinued

Authors	**Country/ Area**	**Age**	**Pre-survey**	**Post-survey**	**Year change in F level**	**Mean caries (pre) A**	**Mean caries (post) B**	**Mean caries change$^{\#}$ (B-A)**	**Mean difference (pre) D**	**Mean difference (post) E**	**Difference of the difference$^{\wedge}$ (D-E)**
Outcome: dmfs_study with positive control											
McLaren et al., 2016	Calgary, Canada	Grade 2	2004/05	2013/14	2011	2.6	6.4	3.8	-1.9	-0.2	1.7
	Edmonton (control)	Grade 2				4.5	6.6	2.1			

[#] Mean caries change= (PostCessation - PreCessation). A positive difference shows that caries increased after cessation. A negative difference shows that caries decreased after cessation.

Mean difference (pre)=PreCessation$_{\text{Intervention}}$ - PreCessation$_{\text{Control.}}$

Mean difference (post) =PostCessation$_{\text{Intervention}}$ - PostCessation$_{\text{Control}}$

[^] Difference of the difference (measure of effect)= (PostCessation$_{\text{I}}$ - PostCessation$_{\text{C}}$)- (PreCessation$_{\text{I}}$ - PreCessation$_{\text{C}}$). A positive difference shows a beneficial effect of water fluoridation (the mean caries difference between fluoridated and non-fluoridated areas was narrower after fluoridation cessation). A negative difference shows a counterintuitive effect of water fluoridation (the mean caries difference between fluoridated and non-fluoridated areas was greater after fluoridation cessation).

2.6.7 The impact of reducing fluoride content of the water supply on caries prevalence

The studies described in Section 2.6.6.1 have reported the effect of stopping fluoridation on caries prevalence. This present section reports on the impact of reduction in fluoride level on caries prevalence.

There was only one study which looked at the effect of reducing fluoride level in the water supply on caries (Kunzel, 1980). The study was conducted in Karl-Marx-Standt, Germany where a temporary reduction in the fluoride level occurred on two separate occasions, in 1970 (1 to 0.5 ppm) and in 1971 (0.5 to 0.2 ppm). These reductions occurred due to technical reasons (unintended interruption). In the present review, the 0.2 ppm was considered as sub-optimal fluoridation, because this was an unintentional interruption rather than total cessation. After the interruption, the fluoride concentration was increased again from 1972 to 1977 and the level of concentration ranged between 0.4 to 0.9 ppm.

This study reported serial surveys of caries experience among children aged 6-15 years (permanent dentition) and 3-8 years (primary dentition) from 1959 to 1977. This has produced a large amount of variable data. Therefore, to enable meaningful comparison with other studies, data were extracted only from children aged 5, 8, 12 and 15 years. Detail of fluoride concentration in the water for each survey was reported with appropriate reference.

For permanent teeth, caries prevalence continued to decrease following reduction of fluoride level in the water supply (Table 2.16). The magnitude of the decrease was greater when the first reduction occurred (1 to 0.5 ppm). However results should be treated with caution as data for pre-reduction survey were only available from 1961 (two years after water fluoridation implemented).

A similar pattern was observed in the primary teeth (dft), that caries prevalence continue to reduce following a reduction of fluoride level in the water supply.

However, results should be treated with caution because of a lack of blind outcome measurement and absence of a comparison community.

Table 2.16 Summary of mean caries data (permanent and primary) dentition when fluoride level was reduced

Age	Year of survey and fluoride levels		
	1961 1.0 ppm	1970 0.5 ppm	1971 0.2 ppm
	Mean DMFT (permanent)		
8	1.7	0.4	0.2
12	4.5	1.7	1.7
15	7.1	3.6	3.1
	Mean dft (primary)		
5	3.9	1.3	0.9
6	4.0	1.9	1.6

2.6.8 A meta-analysis to examine the relationship between change in fluoride level and caries

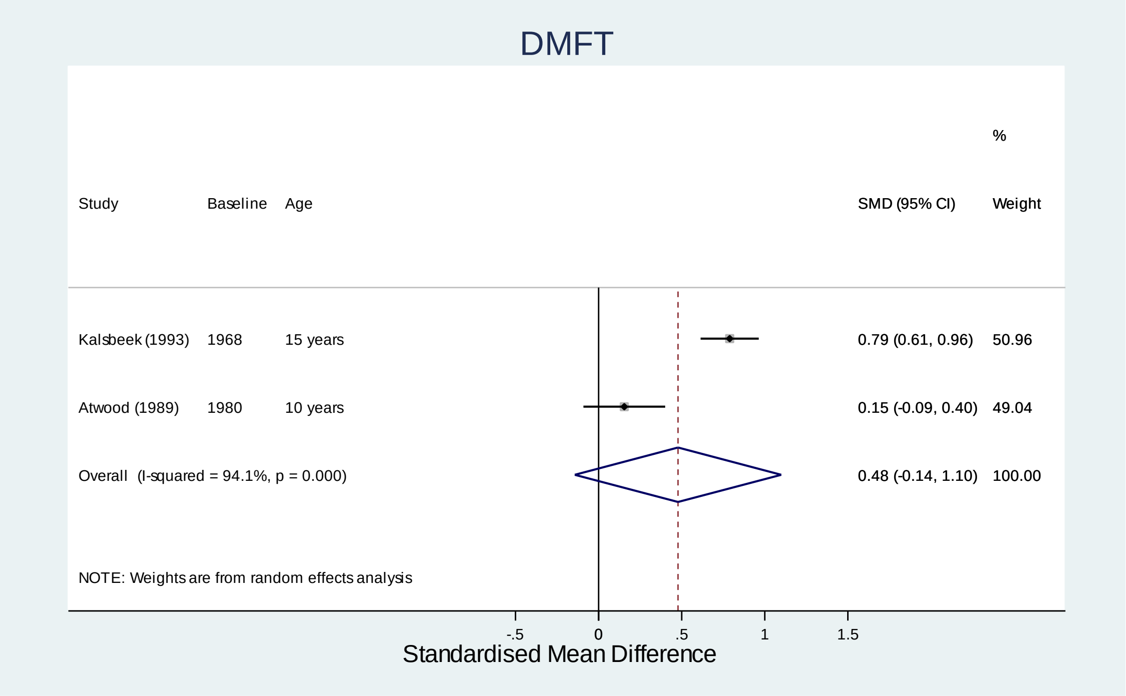
In order to summate the findings for the individual studies identified by this review, it was thought appropriate to undertake a meta-analysis.

It was not appropriate to combine the three types of study design (no control, negative control, positive control) into one meta-analysis. Quantitative analysis using meta-analysis on caries outcome focused on studies with a control group (positive or negative control). However, as explained in Section 2.6.6.1 only one study with a positive control and using the DMFT index reported on caries as an outcome. It was not therefore possible to undertake a meta-analysis for this study type. Only studies with a negative control were identified in sufficient number to permit the conduct of a meta-analysis.

Three publications with a negative control presented data on DMFT or/and DMFS and the data were included in the meta-analysis. Details of the included studies are summarized in Table 2.10 as described earlier in section 2.6.6.1.

Figure 2.2 shows that stopping fluoridation resulted in a narrowing of the difference in caries prevalence between fluoridated and control areas. This indicates a beneficial effect of water fluoridation. A statistically significant difference was found in one study. The range of measures of effect on DMFT score was 0.15 to 0.79. However, the measure of study variance (heterogeneity) was large and statistically significant ($p<0.001$), therefore the results should be interpreted with caution.

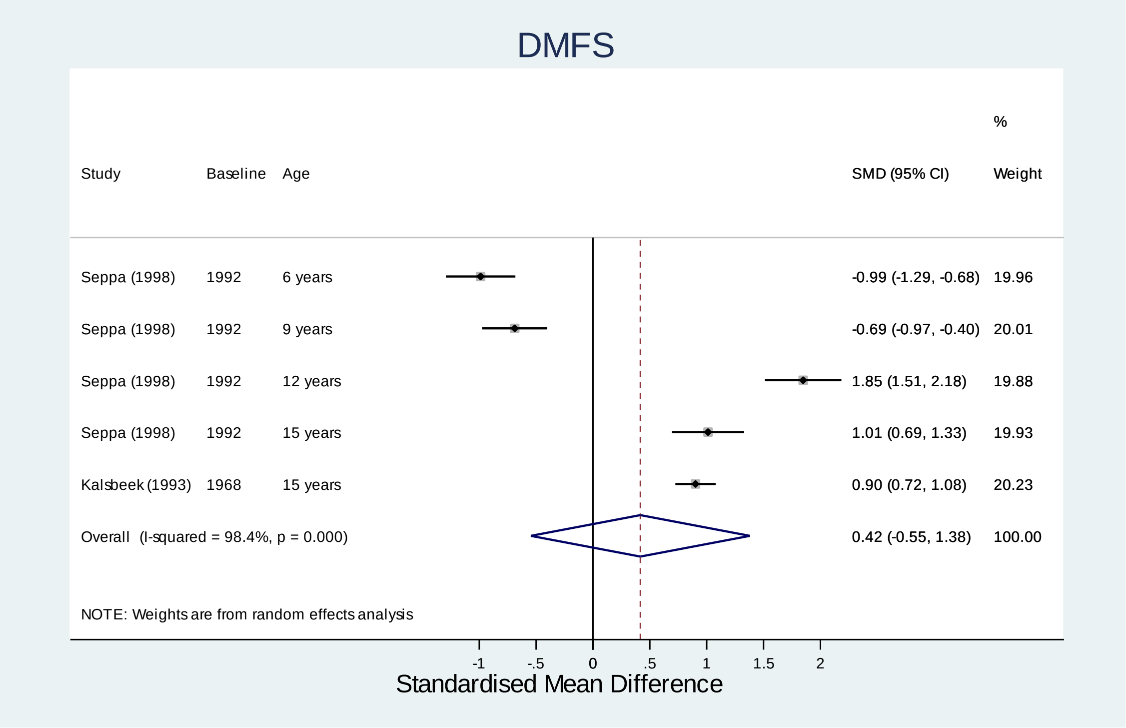
Figure 2.2 Meta-analysis for caries outcome (DMFT)



For DMFS outcome, three of five analyses showed a statistically significant difference that favoured fluoridation (Figure 2.3). The two analyses that did not find a statistically significant effect were from the same study in different age groups (Seppa et al. 1998). The range in measures of effect for DMFS score was -0.99 to 1.85.

However, the results should be treated with caution because the study variance (heterogeneity) was large and statistically significant (p<0.001).

Figure 2.3 Meta-analysis for caries outcome (DMFS)



2.6.9 Studies reporting the impact on change in fluoridation status on the prevalence of fluorosis

The following sections report on the impact of changed fluoride levels in the public water supply on the prevalence of fluorosis. There were no studies which included either a negative or positive control group. The studies encountered either measured fluorosis pre and post change on fluoride level, or were studies which compared different age groups exposed to different levels of fluoride.

Seven publications met the inclusion criteria for fluorosis outcome. Two publications reported on cessation and seven publications were from areas where the level of fluoride in the water supply had been reduction. Of these, six publications were included in the meta-analysis.

2.6.9.1 Stopping fluoridation and the prevalence of dental fluorosis

Two publications, reported data on cessation of water fluoridation on fluorosis (Wei and Wei, 2002, Clark et al., 2006). These studies were cross-sectional in nature with historical controls conducted in China and Canada. Both studies reported a decrease in fluorosis prevalence following the cessation of water fluoridation. The results are summarised in Table 2.17.

Table 2.17 Summary of studies after stopping fluoride level in the water on dental fluorosis

Authors	Country/ Area	Age	Pre-survey	Post-survey	Year change in F level	% prevalence (pre)	% prevalence (post)	Index
Wei and Wei, 2002	Gongzhou, China	15	1982	1990	1983	85.3	21.0	Deans
Clark et al., 2006	Comox/Courtney & Campbell River, British Columbia, Canada	6-9	93/94	2002/03	1992	58.6	24.4	TF

The study in Canada used the TF index and reported the presence of fluorosis on (a) all teeth and (b) maxillary anterior teeth alone. For both of these outcome measures, fluorosis prevalence decreased following the cessation of water fluoridation. When all teeth are included the reported decrease was greater than when considering only the anterior teeth.

The Chinese study reported on fluorosis prevalence using Dean’s Index. Results demonstrated a decrease in fluorosis prevalence following the cessation of water fluoridation (85.3% to 21.0%). However, these findings should be interpreted with caution because while the number of affected individuals was described by the separate categories in Dean’s Index in the pre-cessation survey, for the post-cessation survey, results were not broken down by level of severity. It is therefore unclear if the fluorosis prevalence reported post-cessation included the questionable category.

2.6.9.2 The impact of reducing fluoride level on the prevalence of fluorosis

Five publications which were scored by the author as of low quality, reported data on the effect of reducing the fluoride level in the water on fluorosis. None of these studies had a control group. Four publications used Dean’s index and one study used the DDE index to report fluorosis prevalence. Three publications reported on studies conducted in Hong Kong and another two publications related to the United States. The Hong Kong studies were conducted post-1975, and assessed minor reductions in fluoride level (range from 0.15 to 0.27 ppm). The US studies were conducted pre-1975 and assessed much wider reductions in the level of fluoride as they related to naturally fluoridated communities (range from 5 to 7 ppm). The type of teeth examined for fluorosis varied across studies. Two publications reported prevalence on the upper right central incisor (Evans 1989, Evans and Stamm, 1991b), one publication on maxillary incisors (Wong et al., 2014) and two publications on all permanent teeth (Horowitz and Heifetz 1972, Horowitz et al., 1972). A recent study examined fluorosis with photographs for blind outcome assessment (Wong et al. 2014). The earlier studies were solely based on clinical examination, which lacks blinding of outcome assessment (Evans 1989, Evans and Stamm, 1991b, Horowitz and Heifetz, 1972, Horowitz et al., 1972). The results are summarised in Table 2.18.

Findings indicated that as fluoride levels decrease, so does the prevalence of fluorosis. The decreased was greater for studies with major a reduction in fluoride level (5 to 7 ppm).

Table 2.18 Summary of studies after reducing fluoride level in the water on dental fluorosis

Authors	**Country/ Area**	**Age**	**Pre-survey**	**Post-survey**	**Year change in F level**	**% prevalence (pre)**	**% prevalence (post)**	**Index**
Horowitz and Heifetz, 1972	Bartlett Texas, USA	8-11	1954	1969	1952	97.7	51.0	Deans
Horowitz et al.,1972	Britton, USA	8	1948	1970	1954	100	79.2	Deans
++Evans and Stamm, 1991b	Hong Kong	7-12	n/a	1986	1978	88.0	77.0	Deans
Wong et al., 2014	Hong Kong	12	1983	2010	1988	89.3	42.1	DDE

++Study that compared different age groups exposed to different levels of fluoride during development of enamel.

Two publications of the Hong Kong studies (Evans, 1989, Evans and Stamm, 1991b) were linked papers whereby the first publication reported prevalence of fluorosis among four districts and the later publication reported overall fluorosis data of the studied sample. To avoid data duplication, only overall fluorosis data were used in the analysis. These studies were a single point study that compared children in different age groups that were exposed to different fluoride levels, where change in fluoride level occurred during the period of enamel development. The baseline prevalence data were extracted from the groups that were exposed to the old fluoride concentration and the 'after' prevalence data were extracted from the group that were exposed to the new fluoride level after reduction occurred.

2.6.10 A meta-analysis to examine the relationship between change fluoride level and fluorosis

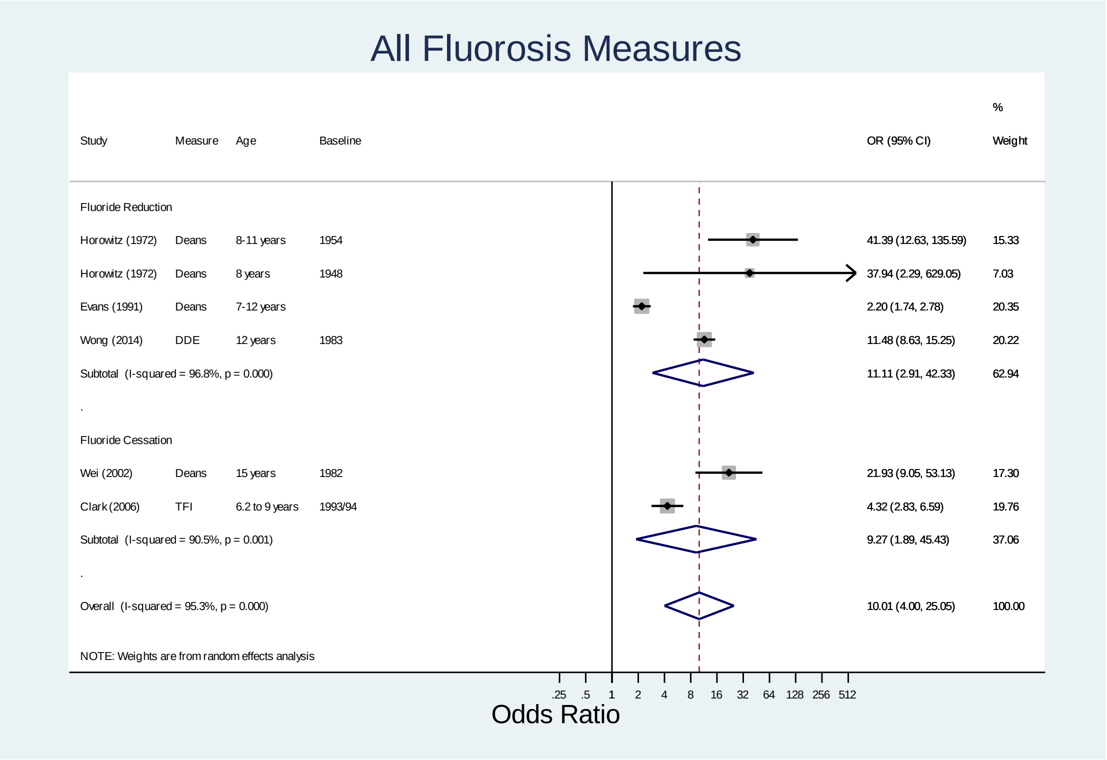
A meta-analysis was performed to summate the findings for the individual fluorosis studies identified in this review.

The meta-analysis for fluorosis outcome is presented into three analyses on one forest plot (Figure 2.4). The first analysis combined individual studies on the effect of reducing fluoride level and fluorosis. The second analysis combined individual studies on the effect of stopping fluoridation and fluorosis. The third analysis combined studies from both interventions (stopping or reducing) fluoride level for pooled estimates of effects across time points. Details of included studies are summarised in Tables 2.17 to 2.18 as described earlier in Sections 2.6.9.1 to 2.6.9.2 Effort has been made to avoid data duplication in the meta-analysis. For example, in the study by Clark et al. (2006) that provided data for anterior teeth only and for all teeth, only fluorosis prevalence for all teeth was included. In addition, for publications that refer to the same intervention

(Evans, 1989, Evans and Stamm, 1991b), only one study with overall fluorosis prevalence was included (Evans and Stamm, 1991b). In total, six publications were suitable for inclusion in the meta-analysis.

Figure 2.4 shows that fluorosis prevalence decreased following reduction of fluoride levels in the water. The decrease was greater for studies with a major reduction of fluoride level (5 to 7 ppm) with odds ratio range between 37.94 to 41.39. However results should be treated with caution because heterogeneity is high and statistically significant (p<0.0001). Also there was lack of examiner blinding and small sample sizes.

Figure 2.4 Meta-analysis for fluorosis outcome



Similarly, fluorosis prevalence was significantly decreased after water fluoridation cessation in both studies. The decrease was greater in the Chinese study (OR: 21.93) in comparison to the Canadian study (OR: 4.32).

When all studies were combined, the pooled estimate of effect indicated a statistically significant difference of fluorosis reduction (OR: 10.01, 95%CI: 4.00-25.05). However, as already discussed, these results should be treated with caution because of high heterogeneity, lack of blinding and small sample size.

2.7 Discussion

This section highlights the key findings of the review, strengths and limitations and research implications. Whether the findings are in agreement or disagreement with other published work are also discussed. How the findings from this review link with the main study and overall implications of the PhD project are discussed in Chapter 6.

2.7.1 Quality of evidence

All available reviews acknowledge that a randomised controlled trial is not feasible as a study design in evaluating the effectiveness of water fluoridation. This explains the complexity of assessing such an intervention and why the majority of water fluoridation studies were mostly cross sectional in nature. Taking into consideration the methodological limitations in assessing fluoridation, this review also includes additional studies with no concurrent control group. The aim was to appraise the available literature with a wider range of study designs.

This study adapted criteria used in the York Review (NHS Centre for Reviews and Dissemination, 1996) with some modification for study validity assessment. Similar to

York, some of the included cross sectional studies were ‘upgraded’ as moderate quality (moderate risk of bias) when they had concurrent control group, blinding of outcome assessment, and address and control confounding factors in the analysis. Taking these factors into account, sixteen studies were rated as low quality and six studies are moderate quality. McLaren and Singhal (2016) reported more studies with moderate quality of evidence in their water fluoridation cessation review when they used the Cochrane Collaboration’s tool with some modification to assess risk bias.

In contrast, the Cochrane review introduced a new inclusion criteria requirement when assessing water fluoridation cessation, namely, studies with a positive reference (control group remained fluoridated) (Iheozor-Ejiofor et al., 2015). Unlike the York Review, the Cochrane work disregarded studies with a negative control (non-fluoridated as control), which led to only one study available for data synthesis. This strict criteria in assessing water fluoridation cessation has been challenged by a group of researchers with the basis of complexity of evaluating population based public health interventions and difficulty in having a community with a positive reference population (Rugg-Gunn et al., 2016). These additional study designs have also been agreed as relevant by a recent publication which sets out recommendations for designing a community fluoridation cessation study (Singhal et al., 2017).

2.7.2 Agreements and disagreements with other reviews and published work

The York review concluded that caries increased following the cessation of water fluoridation. Results from our meta-analysis (that only include studies with a negative control) confirm this finding. However from a qualitative analysis, results were rather mixed. Studies that were published before the 1990s (four studies) indicate an increased caries prevalence following cessation (Jordan, 1962, DHSS, 1969, Lemke et al., 1970, Stephen et al., 1987). The majority of studies published from the 1990s onwards (nine studies) indicate a decrease in caries prevalence (Kalsbeek et al., 1993, Kunzel and Fischer, 1997, Maupome et al., 2001a, Kunzel and Fischer, 2000, Kunzel et al., 2000, Seppa et al., 2000a, Seppa et al., 2000b, Wei and Wei, 2002). Factors reported to explain this were attributed to the availability of fluoridated toothpaste and other caries preventive programmes (such as fluoride varnish, fissure sealants) post-cessation. Another three studies reported mixed results for different age groups in primary and permanent dentitions. (Attwood and Blinkhorn, 1989, Seppa et al., 1998, McLaren et al., 2016). Cessation on dental caries support findings in a recent review by McLaren and Singhal (2016), that also reported on the mixed results of the effect of stopping water fluoridation on subsequent caries prevalence.

There is insufficient evidence to determine the effect of lowering fluoride level in the water supply on dental caries. Only one study with low methodological quality was included in this review. Data from this study indicate caries in permanent and primary teeth continue to decrease following reduction of fluoride level in the water supply. This study rated as at a high risk of bias, reported a series of data in Karl-Marx-Standt, Germany from 1959 to 1977 (Kunzel, 1980). There was no blinding assessment and data did not address or control for confounding factors. The reduction occurred twice, in 1970 (1 to 0.5 ppm) and in 1971 (0.5 to 0.2 ppm) due to technical reasons. Some may argue the inclusion of this study under reduction of fluoride level as opposed to cessation. Change of the fluoride level was unintentional and not due to change in fluoridation policy. After the interruption the level was increased again from 1972 to 1977 when the level of fluoride in the public water supply ranged between 0.4 to 0.9 ppm. In additional, there was no intention of stopping water fluoridation at the time the study was conducted.

A series of epidemiological studies from Hong Kong have reported on the prevalence of fluorosis after reduction of fluoride level in the water supply. However only very limited data from Hong Kong have considered the impact of changing fluoride levels on caries. A work published in 2014, cited unpublished data from the Hong Kong Department of Health that reported no concurrent increase in caries following a change in fluoride concentration in the water supply (Wong et al., 2014). The exact figure of caries reduction cannot be extracted because data were presented using a graph.

Spencer and Do (2016) have argued that the traditional method of assessing the effectiveness of a lower level of fluoride in the water on dental caries has limitations. These authors questioned what the caries levels would have been if the concentration remained at the one of the higher levels (i.e. a concurrent control). This argument reflects similar requirement (concurrent positive control) addressed by Cochrane when assessing fluoridation cessation. However, the possibility of having a comparable positive reference community is very challenging if not unfeasible. This is because changes to fluoridation level are affected by regional and national policy (Rugg-Gunn et al., 2016). This means that a change in one area is likely to result in a change in all neighbouring areas, such as when changes have occurred in Hong Kong and Singapore where there is 100% water fluoridation coverage (Petersen et al., 2012).

There are more studies that have reported on the impact of change in the fluoride level in the water supply on dental fluorosis. This could be due the different role that fluoride plays in the development of caries and fluorosis, and the time scale involved. Fluorosis occurs due to excessive exposure to fluoride during tooth development and the risk period is between birth to three years of life. The use of multiple birth cohorts exposed to different fluoride level during tooth development means that less time and effort is required to study fluorosis than caries, when a number of years have to pass, post the change in fluoride level(s) for the impact on caries to become clinically evident. Taking into account the value of birth cohort studies, it was deemed relevant to include this study design in the review. However, these cross-sectional birth cohort studies were rated as lower quality in comparison to caries studies that fulfilled the ‘at least two point in time’ requirement.

This design (i.e. birth cohort) was also used in several studies that assessed short term unintended cessation of water fluoridation on fluorosis where the interruption of the fluoride provision usually occurred due to technical issues (Burt et al., 2000, Burt et al., 2003). These studies were identified during data searching but excluded from this review as not meeting the inclusion criteria (Section 2.6.3). The trend of reducing the optimal fluoride level in other countries occurred in early 2000s except for Hong Kong and Singapore. Therefore an appropriate time frame is needed in order to evaluate the impact of such intervention on dental caries prevalence. The effect of fluoride in caries prevention is mainly post-eruptive and systemic effect of fluoride during tooth development for caries prevention is questionable (Featherstone, 2000). This may explain that full effect of changing the level of fluoride in the water will take longer to become evidence on the case of dental caries compared with the development of fluorosis.

Another important factor is the time between baseline and follow-up survey. The variation in survey time points may affect the estimation of effect size. In addition, an equally important factor is the time lapse between change in fluoride level and subsequent clinical examination with regards to age, the type of teeth examined and stage of dental development. Changes of water fluoridation status may affect the primary and permanent dentitions differently. For example, Attwood and Blinkhorn (1989) reported caries decreased in permanent teeth but increased in primary teeth after cessation. As for fluorosis evidence suggests that childhood fluorosis can diminish over time (Do et al., 2016). This may be due to potential effect by external factors after eruption such as wear or erosion that may reduce the appearance of fluorosis by adolescence (Do et al., 2016).

In terms of the meta-analysis for fluorosis studies, the results obtained in this work (Section 2.6.10) indicate a decrease in the prevalence of fluorosis after reducing or stopping fluoridation. A pooled estimate effect indicated a statistically significant difference in fluorosis reduction (OR: 10.01, 95% CI: 4.00- 25.05). This is as expected, the dose-response relationship in terms of fluoride level and fluorosis has been established for decades (Dean, 1938, Dean, 1942) and confirmed by the York Report (McDonagh et al., 2000) and the Cochrane review (Iheozor-Ejiofor et al., 2015). However these results should be treated with caution because of significant heterogeneity across studies, lack of examiner blinding, small sample sizes and different indices used to measure fluorosis. The results are mainly derived from low quality primary studies in which none of the fluorosis studies had concurrent control group. Only newer studies tend to control for confounders (Clark et al., 2006) and used blind photographic assessment to score fluorosis (Wong et al., 2014).

2.7.3 Implications for future research and recommendations

Study design and confounders

Of all the included studies, ten studies (Evans and Stamm, 1991b, Kalsbeek et al., 1993, Kunzel and Fischer, 1997, Seppa et al., 1998, Seppa et al., 2000a, Seppa et al., 2000b, Maupome et al., 2001a, Clark et al., 2006, Wong et al., 2014, McLaren et al., 2016) mentioned potential confounding factors, only four studies (Seppa et al., 2000a, Maupome et al., 2001a, Clark et al., 2006, McLaren et al., 2016) used analysis to control for them. Future research should consider appropriate study design and better handling of confounders. If possible, a longitudinal study design is the ideal method to assess the effects of change of fluoride level in the water supply. If resources are limited, a study design with concurrent controls (positive or negative control) is desirable. If this design is not possible, a repeated cross sectional survey is preferable than a single point survey (Singhal et al., 2017).

Confounding factors such as exposure to other sources of fluoride (e.g. fluoridated toothpaste) diet (e.g sugar consumption) and social economic status should be measured and adjusted in the analysis.

Other possible confounders of particular relevance to fluorosis are temperature and altitude. People living in climates with a higher mean temperature drink more water, thus may be exposed to more total fluoride. Higher altitude has also been reported to be associated with the development of fluorosis, however the mechanism for this is unclear. Future studies should consider this factor. More research is also needed to measure consumption of tap water within a population and how it is associated with fluorosis development.

Outcome assessment

All of the included studies reported outcomes in child populations only. In caries related studies ages ranged form 3 to 6 years in the primary dentition and 6 to 15 years in the permanent dentition. All fluorosis studies measured permanent teeth with ages ranging from 6 to 12 years. Evaluation amongst older age groups is recommended in future research.

In terms of outcome measurement, the DMF Index and its variation (tooth level or surface level) is the most commonly used in caries assessment. One study used a modified DMFS index (D1D2MFS), which aimed to distinguish different caries level (Maupome et al., 2001a). Data with different severity of caries are important not only for monitoring the disease prevalence but also helps in providing effective treatment and prevention. A new caries index that allows identification of cavitated and non-cavitated lesion is the International Caries Detection and Assessment System (ICDAS) (Ismail et al., 2007). This index allows modification for epidemiology survey and data can be re-coded to match with the traditional DMFT scoring system at the “into dentine” level, enabling comparison across studies. Therefore, future research is recommended to look into effect of water fluoridation on different caries severity.

In terms of fluorosis assessment, Dean’s, TF and DDE index was the most commonly reported index reported in the primary studies. Blinding of fluorosis assessment can be achieved using standardized photographs. This method also allows archiving, remote assessment and data comparison across different time points.

Uniform diagnostic criteria and reporting techniques for caries and fluorosis may improve the comparability of results across studies and aid in meta-analysis. Future research should consider this factor for high quality data.

2.7.4 Strengths and limitations

Study strengths

This is the first review that synthesizes evidence on both, stopping or reducing fluoride level in the water supply on dental caries and fluorosis. This review includes multiple studies with different designs for comprehensive evidence appraisal. The findings can be useful for authorities that revisit their fluoridation policy. Gaps in knowledge have been identified in and the methodological considerations discussed may be valuable future research on this topic.

Study limitations

Only four major electronic databases were used. Relevant work from non-English publications and some grey literature such as local reports may have been missed. For example two non-English articles (Gu and Shen, 1989, Lekesova, 1998) which were not identified in the original search and analysis were subsequently identified in work published recently by McLaren and Singhal (2016). These papers did not report on fluorosis, only on caries which showed an increased in caries prevalence following cessation of fluoridation.

The main limitations of the meta-analysis is the lack of data and different outcome assessment across studies. Although several studies with concurrent control group were identified that looked into the effect of water fluoridation cessation on dental caries, not all can be included in the meta-analysis because of missing information (sample size, standard deviation). This reflects lack of standard in reporting caries data. Similar issue were encountered in relation to the meta-analysis for fluorosis. An analysis on the dose-response relationship between fluoride in the water and fluorosis could not be undertaken because of lack of data.

2.8 Conclusions

Twenty-two studies were included in the review. There is limited evidence with low methodologically quality to determine the effect of stopping or reducing fluoride level in the public water supply on dental caries and fluorosis. The majority of the studies were of cross-sectional design and the quality of studies was assessed as low. Stopping water fluoridation was associated with an increased caries experience for studies published up to 1989. A decrease in caries experience post cessation / reduction was reported from 1990 onwards. There is insufficient information to determine the impact of reducing the fluoride level in the water supply on dental caries prevalence. Stopping or reducing fluoride levels in the water is associated with a decrease in fluorosis prevalence. Future studies in this area are recommended with appropriate study design and better handling of confounding factors.

2.9 Chapter summary

There is limited evidence with low to moderate methodological quality to determine the effect of reducing or stopping fluoride level in the water supply on dental caries and fluorosis. A summary of the review key findings are as follows:

- The available data indicated mixed results on stopping fluoridation and subsequent prevalence of dental caries.
- There is insufficient information to determine the impact of stopping fluoridation on the subsequent prevalence of dental fluorosis.
- There is insufficient information to determine the impact of reducing the fluoride level in water supply on dental caries prevalence.
- Five studies published on reducing the fluoride level in the water supply on fluorosis. This is associated with a decrease in fluorosis prevalence.

In response to the gaps in knowledge highlighted in this review, the main study of this PhD project aimed to evaluate the effect of reducing the level of fluoride in the Malaysian water supply on caries and fluorosis. The rationale and objectives of the study are described in Chapter 3.

3 Study rationale, research questions, aims and objectives

3.1 Introduction

This chapter describes the study rationale, research questions and the aims and objectives of the study.

3.2 Study rationale

In Malaysia, as a public health measure to control caries, the public water supply has been artificially fluoridated since 1972 at a concentration of 0.7ppm (Oral Health Division Ministry of Health Malaysia, 2006). However, concern arose that a fluoride concentration at 0.7 ppm maybe too high given increasing exposure to other sources of fluoride, leading to an increased prevalence of dental fluorosis (Oral Health Division Ministry of Health Malaysia, 2001, Tan et al., 2005). This prompted a downward adjustment of fluoride concentration from 0.7 to 0.5 ppm in December 2005 (Oral Health Division Ministry of Health Malaysia, 2006).

In addition to Malaysia, other countries have reviewed their fluoridation policy in light of alternative means of fluoride delivery. For example the US Public Health Services recommended lowering fluoride concentration in the public water supply from the range of 0.7 to 1.2 ppm to a level of 0.7 ppm (Federal Panel on Community Water Fluoridation, 2015). In Europe, Ireland has lowered the fluoride concentration in the water from to 1.0 ppm to a new range 0.6 - 0.8 ppm, with a target concentration of 0.7 ppm in 2007 (Parnell et al., 2009, Whelton and O'Mullane, 2012). In Asia, authorities in Hong Kong have reduced the fluoride concentration in their public water supply twice, from 1 ppm to 0.7 ppm in 1978 and then a further reduction to 0.5 ppm in 1988 (Wong et al., 2014). In

Southeast Asia, Singapore has taken similar action by reducing the concentration of fluoride in drinking water twice from 0.7 to 0.6 ppm in 1992 and further to 0.5 ppm in 2008 (Petersen et al., 2012). However, despite the substantial evidence of the effectiveness of water fluoridation, evidence relating to minor changes of fluoride concentration of public water supply has seldom been investigated.

Based on the systematic review conducted as part of this PhD (Chapter 2), there have only been six studies that assessed the effect of reducing fluoride level in the water on caries (one study) (Kunzel, 1980) and fluorosis (five studies) (Horowitz and Heifetz, 1972, Horowitz et al., 1972, Evans, 1989, Evans and Stamm, 1991b, Wong et al., 2014). The available studies indicated that reducing fluoride level is associated with a decrease in fluorosis prevalence. The only caries study reported that caries prevalence continues to decrease following reduction of fluoride level in the water supply in permanent and primary dentitions (Künzel, 1980). It can be concluded that there is insufficient evidence to determine the effect of lowering fluoride level in the water supply on dental caries. In terms of fluorosis outcome, results mainly derived from low quality primary studies which none of the fluorosis studies has concurrent control group. Only a newer study tends to control for confounders (Clark et al., 2006) and used blind photographs assessment for fluorosis score (Wong et al., 2014).

The situation in Malaysia offers a unique opportunity to evaluate the outcome of the 0.2 ppm adjustment of fluoride concentration in public water supply on both dental caries and fluorosis. In addition, there is also a need to assess the relationship between exposure to other fluoride sources such as infant feeding practices, oral hygiene habits and exposure to fluoride varnish/gel with dental caries and fluorosis.

Apart from generating evidence on the effectiveness of the policy initiative, information about fluoride exposure is useful for policy makers, public health planners and health care professionals when planning effective community-based fluoride therapy for the prevention of dental caries, while limiting dental fluorosis. Data can also be used to address public concerns, propose any adjustment to the policies concerning water fluoridation, control of dental products and oral health awareness programmes. The evidence from the study would serve as a guide for improving the monitoring system, and justifying monetary spending and allocations of oral health prevention programmes.

3.3 Research questions

The following questions were addressed by the research undertaken:

1. What is the prevalence and severity of dental fluorosis following a 0.2 ppm reduction of fluoride level in the public water supply?
2. What is the prevalence and severity of dental caries following a 0.2 ppm reduction of fluoride level in the public water supply?
3. Has the policy measure to reduce the fluoride level in the water supply maintained the preventive effect of dental caries and reduced the prevalence of fluorosis?
4. Are there any other risk factors (in particular exposure to difference sources of fluoride) associated with dental fluorosis?
5. Are there any other risk factors (in particular exposure to difference sources of fluoride) associated with dental caries?

3.4 Aims

The aims for the clinical study were to evaluate the outcomes of the downward adjustment of fluoride concentration in the community water supply from 0.7 ppm to 0.5 ppm

a. in relation to the prevalence of dental fluorosis
b. in relation to the prevalence of dental caries

3.5 Objectives

These aims were broken down into five specific objectives as follows:

1. To determine the prevalence and severity of dental fluorosis among 9 and 12-year-old Malaysian children living in fluoridated and non-fluoridated areas.
2. To evaluate the prevalence and severity of dental caries among 9 and 12-year-old Malaysian children living in fluoridated and non-fluoridated areas.
3. To explore associations between changes in fluoride level in the water supply and dental caries and fluorosis among Malaysian children.
4. To explore risk factors associated with fluorosis, in particular water use, infant feeding patterns and oral hygiene practices.
5. To explore risk factors associated with caries, in particular water use, infant feeding patterns and oral hygiene practices.

4 Materials and methods

This chapter describes details of the study design, sampling method, sample size calculation, data collection procedure, study instruments and approach to data analysis.

4.1 Study design

This study had both a cross sectional and retrospective design. Two types of data were collected:

- Clinical data: on dental fluorosis (including intra-oral photographs) and dental caries status.
- Questionnaire data: retrospective fluoride history, infant feeding practice, oral hygiene practice and current socio economic status.

4.2 Study population

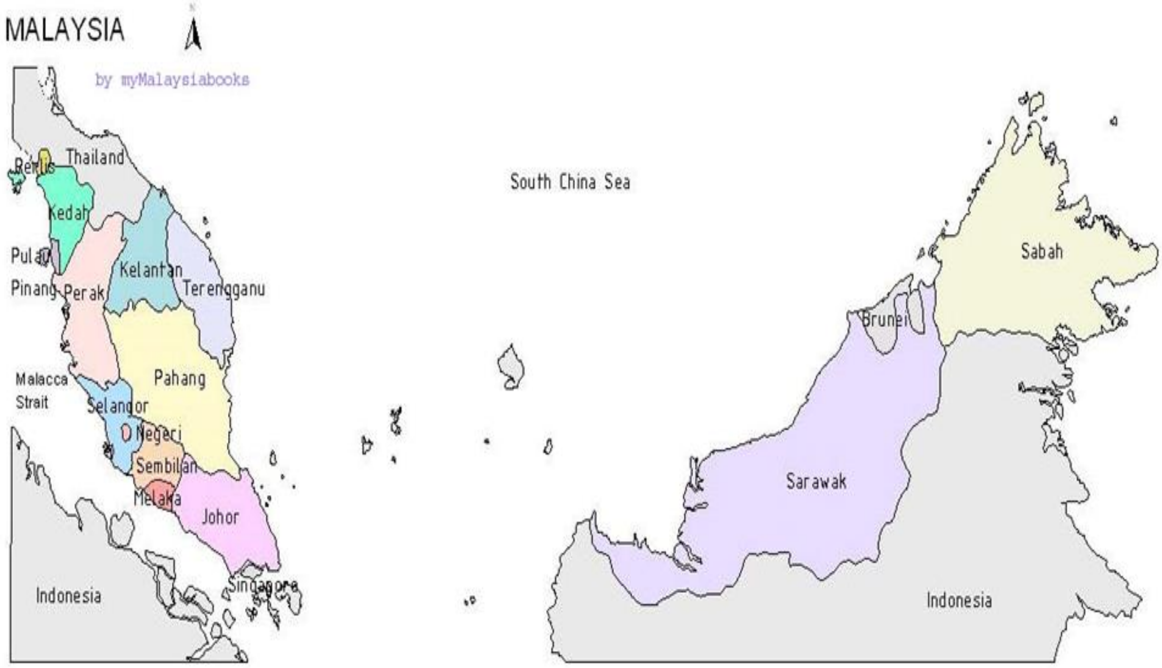
A representative sample (n =1155) of 9 and 12-year-old primary school children in Malaysia. Data were collected over a five month period from the beginning of January 2015 until the end of May 2015.

4.3 Research site

Malaysia is located in the South-East Asia region. The federation of Malaysia comprises of the Peninsular Malaysia and the East Malaysia which are situated in two different geographic areas. These are separated by the South China Sea. Peninsular Malaysia consists of ten states and two Federal Territories which are Perlis, Kedah, Penang, Perak, Negeri Sembilan, Malacca, Selangor, Johor, Pahang, Kelantan, Terengganu, Federal Territory of Kuala Lumpur and Federal Territory of Putrajaya. The East of Malaysia consists of Sabah, Sarawak and Federal Territory of Labuan on the islands of Borneo (Department of Survey and Mapping Malaysia, 2013).

Research in the thesis was carried out in Negeri Sembilan (non-fluoridated) and Kelantan (fluoridated) states which are located in the Peninsular Malaysia (**Figure 4.1**) (myMalaysiabooks, n.d).

Figure 4.1 Map of Malaysia



4.4 Sample population, sample size and method of sample selection

4.4.1 Sample population

The sample population was selected from two states, one fluoridated and the other non-fluoridated. In Malaysia, more than 95% of the population receive a piped water supply, however, only 76.7% of the population receive a fluoridated water supply (Oral Health Division Ministry of Health Malaysia, 2011). In most of the wealthier states more than 90% of the population benefit from fluoridated water, however this public health measure provides lower coverage in less affluent states such as in Pahang (82.5%), Sarawak (66.4%), Terengganu (62.8%), Kelantan (14.5%) and Sabah (0.4%) (Oral Health Division Ministry of Health Malaysia, 2011). The two states with the least fluoridation coverage are Kelantan located in Peninsular region (West Malaysia), and Sabah, located in the Borneo region (East Malaysia).

For logistical and financial reasons, Kelantan was selected to represent an area without water fluoridation. Those districts in which the water was fluoridated in Kelantan state were excluded from the study. Of the fluoridated states, Negeri Sembilan was selected based on the following reasons; firstly, the state was the most similar to non-fluoridated Kelantan state in term of population density and ethnic composition (Department of Statistics Malaysia, 2016). Secondly, based on technical reports, Negeri Sembilan was among the fluoridated sates that were reported to be very consistent in maintaining fluoride levels as recommended by Ministry of Health, Malaysia (Oral Health Division Ministry of Health Malaysia, 2011). Thirdly, the state is logistically feasible for the research purposes.

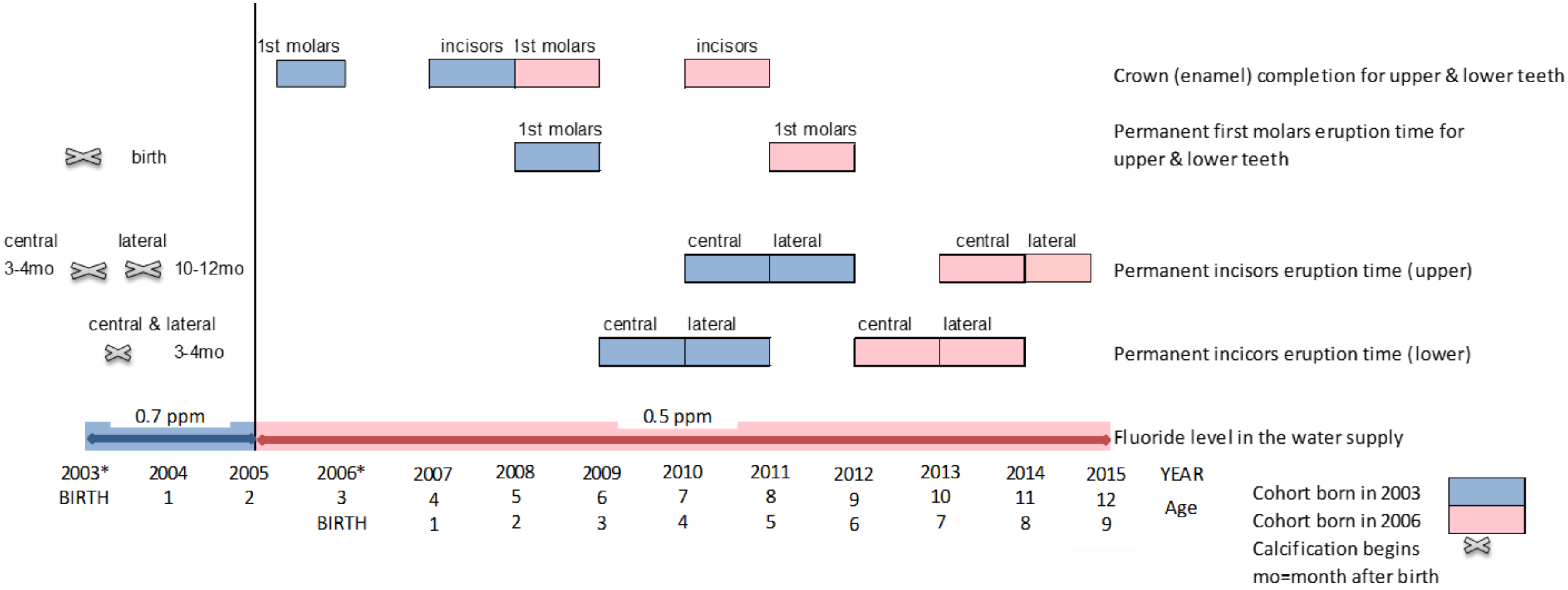
The Malaysian Ministry of Health has made a downward adjustment of the level of fluoride in the community water supply from 0.7 ppm to an optimal level of 0.5 ppm on 22nd December 2005 (Oral Health Division Ministry of Health Malaysia 2006, 2011). The policy measure would have affected Malaysian children born after its introduction because fluorosis is a product of fluoride intake in early childhood. The outcomes were assessed by comparing children who were likely to be affected by the policy measures (test cohort) and children whose teeth developed before the adjustment in fluoride level (comparison cohort). At the time of the clinical examination in this study, children born after the policy change were 9 years of age and children born before the policy change were 12 years of age. The 9 year-old children were born between 1st January to 31st December 2006 and the 12 year-old children were born between 1st January to 31st December 2003. The period between the cohorts had been chosen taking into account, critical fluoride exposure from water fluoridation during maxillary central incisor development, which is between 16 to 36 months of age (Evans and Stamm 1991a; Levy et al. 2001; Hong et al. 2006b; Buzalaf and Levy 2011). In this study, the 9 year-old children in the test cohort had been exposed to 0.5 ppm fluoridated water throughout their life. Children in the comparison cohort have had mixed exposure to fluoridated water during the development of their permanent teeth. The oldest children (born 1.1.2003) in this birth cohort were exposed to 0.7 ppm fluoridated water from birth until 2 years of age followed by 0.5 ppm fluoridated water from age 2 to 12. The youngest children in this birth cohort (born 31.12.2003) were exposed to 0.7 ppm fluoridated water from birth until 1 year of age followed by 0.5 ppm fluoridated water from age 1 to 12. Years of fluoride exposure was calculated based on the date of birth and the commencement of school term in January.

(Refer Figure 4.2 for developmental period of central incisors and first molars).

Inclusion criteria for children in this study were as follows:

1. Children who were born between (01.01.2006 to 31.12.2006); after the policy change to lower the level for fluoride in the public water supply from 0.7 ppm to 0.5 ppm and children who were born between (01.01.2003 to 31.12.2003); before this policy change.
2. Lifelong residents - Born and raised within the boundary of the selected fluoridated (Negeri Sembilan) and non-fluoridated (Kelantan) states.
3. Provision of informed written consent by the child’s parent or guardian.
4. No medical contraindication to undergoing a clinical dental examination.
5. Fully erupted permanent maxillary central incisors (at least half of the tooth surface is visible for clinical examination).

Figure 4.2 Developmental period of central incisors and first molars of children born in 2003 and 2006, and mean fluoride concentration in Malaysian's drinking water supply



Note: The diagram is produced based on developmental period of permanent dentition (Berkowitz et al., 1992)

4.4.2 Sample size estimation

The sample size was calculated to achieve the main study objectives which were to evaluate the outcomes of the downward adjustment of fluoride level in the community water supply from 0.7ppmF to 0.5ppmF in relation to the prevalence of dental fluorosis and dental caries.

The subjects of the study were divided into four groups: 9 and 12 years-old children in fluoridated and non-fluoridated area.

The sample size estimation was as follows:

4.4.2.1 Fluorosis

For the sample size calculation of this study, the prevalence of 'mild fluorosis' in fluoridated and non-fluoridated communities was considered as several studies have shown that the increase in fluorosis in areas subject to artificial water fluoridation occurs mainly in the 'mild' categories (Clark, 1994, Mascarenhas, 2000). In the previous Malaysian national survey of enamel opacities in children aged 16 years-old, the prevalence of 'mild fluorosis' was reported as 17.8% in a fluoridated area and 0.4% in the non-fluoridated areas, with a corresponding difference between the two areas of 17.4% (Oral Health Division Ministry of Health Malaysia, 2001). Based on clinical judgment, it is important to clinically detect the percentage difference between the two areas at 10% and at the same time avoiding a type II error (false negative) in the findings. The estimated sample size that was required to detect a difference in the prevalence of fluorosis among children of each group with a statistical significance level of 0.05, a confidence interval level of 95%, a power of 90%, calculated on the prevalence of mild fluorosis at 17.8%; results in an estimated minimum sample size of 227 per each cell in each of the two age groups in communities with fluoridated and non-fluoridated water. The estimated total sample required for four groups was therefore 908.

4.4.2.2 Caries

According to the national survey of school children's oral health status, caries prevalence (DMFT) in 12 years-old in Malaysia was 39.0% (Oral Health Division Ministry of Health Malaysia, 2010) and the mean dmft was 2.6 (S.D: 4.1). A previous international study study has used a 25% difference as a clinically significant effect (Do, 2004). The sample size required to detect a 25% difference in population mean decayed, missing and filled permanent teeth with 90% power and significance level of 0.05 was calculated as a minimum sample size of 116 per group. The estimated total sample for four groups was therefore 464 children.

4.4.2.3 Final sample size estimation

Based on the highest sample size estimation, inflated by an additional 30% to account for non-respondents, 15% non-consenting and 15% mobility rate yields 1453 children required for this study [227 x 2 age groups x 2 areas + (30% non-respondents + 15% non-consented + 15% mobility rate) =1453]. Rounded to 400 children per cell, a total of 1600 children aged 8 and 12-years-old were estimated for this study.

4.4.3 Method of sample recruitment

Sampling of the subjects was conducted according to a two-stage sampling method based on guidance for child dental health surveys by the British Association for the Study of Community Dentistry (Pine et al. 1997).

The first stage is the selection of schools and second stage is selection of children. Two states in Peninsular Malaysia were selected to represent fluoridated (Negeri Sembilan) and non-fluoridated (Kelantan) areas. Overall, there were 356 primary schools (Grade 3=16,821 pupils; Grade 6=17,291 pupils) in Negeri Sembilan State Education Department and 418 primary schools (Grade 3=29, 676 pupils; Grade 6=34,350 pupils) in Kelantan State Education Department, of which 162 schools (Grade 3=9223 pupils; Grade 6=10,263 pupils) were located in non-fluoridated districts; only the latter schools which were included. Therefore the final sampling frame consisted of 518 public primary schools. Only public schools under coverage of School Dental Services, Ministry of Health were selected. Private and special schools were excluded from the sampling frame.

4.4.3.1 Sampling schools

Schools lists and student enrolment data were obtained from the Ministry of Education, Malaysia. Schools were divided according to school size (small schools $\leq$50 children aged 9-years / large schools >50 children aged 9 years). Each school was allocated a number and a random number generator used to select the survey schools.

4.4.3.2 Selecting schools

The proportions of the total school population of 9-year-old and 12-year-old children attending each group of the schools were calculated (Appendix 13). The minimum sample size was 227 per group and 330 children were selected for inclusion in the survey, so that substitution was not required for absentees.

Assuming a minimum of 50 children examined from each school for each age group, eight schools were required per state. In addition, three reserve schools were selected for each school size in case a school declined to participate. A proportionate sample of schools based on the ratio of large to small schools was selected.

4.4.3.3 Sampling individuals

In terms of sampling individuals from each school, the following method was used:

For small schools, every child was selected. For large schools, systematic sampling was used, when every second child on the class list was selected. All the class lists from a school were collated and treated as a single list.

- Small schools: every child was selected
- Large schools: every second child was selected

Based on the Ministry of Education, Malaysia records, there was minimal variation in relation to student enrolment between different age groups in the same schools, therefore the same schools were selected for both age groups (9 year-old and 12 year-old). Therefore a similar sampling process was used for both 9 and 12 year-old children.

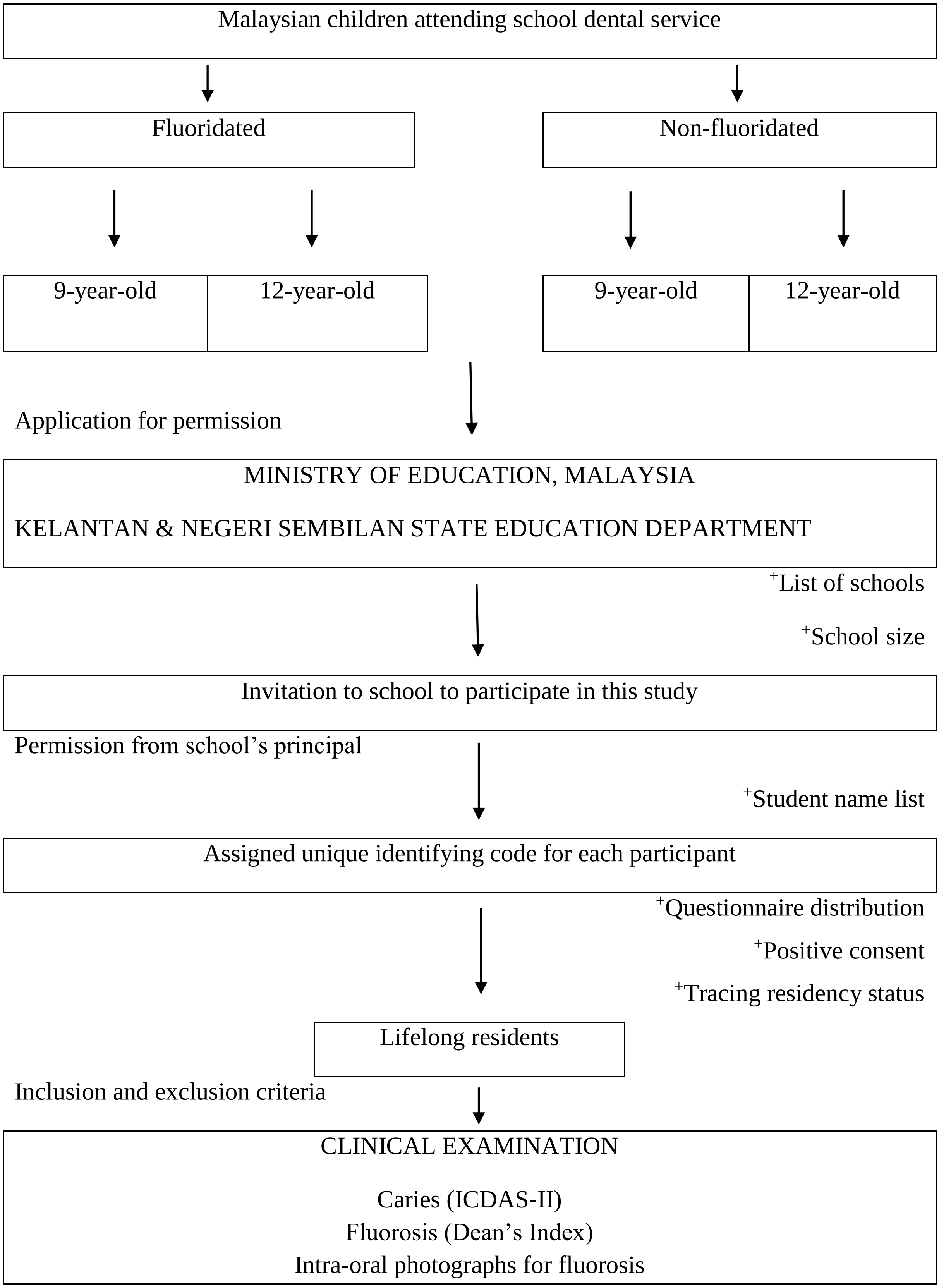
4.5 Conduct of study

The fieldwork involved two stages, which were pilot study and main study. A pilot study was conducted prior to the main data collection. The pilot study involved development of the questionnaire and the conduct of the pilot study followed the same protocol as described for main study. Details of the pilot study are described in Sections 4.8 and 4.9. The main study was conducted as follows:

- administration of the questionnaire and obtaining positive consent
- a clinical examination of dental caries and fluorosis between two birth cohorts in selected schoolchildren in fluoridated and non-fluoridated areas and
- an intra-oral photograph of the anterior teeth

An overview of the conduct of study is shown in Figure 4.3.

Figure 4.3 An overview of the overall conduct of study



4.6 Data collection instrument and methods of execution

4.6.1 Child identification code

After receiving name list of participants from each school, each child was assigned eight digits identification code. The first two digits are refer to school code, the next two digits are refer to age group and the last four digits are unique identifying code for each individual. The school code and the last four digits code were generated randomly using excel.

This code was then used as identification number for questionnaire, clinical examination form, photographic log, photographic fluorosis scoring and data entry procedure.

4.6.2 Final questionnaire

The final version of the parental questionnaire consisted of 29 questions, divided into the following sections: residency status, demographic characteristics, infant feeding practices, oral hygiene practices (which sub-divided into previous practice [age less than 6 years old] and current practice [in 2015]), exposure to fluoride varnish/gel and sources of water at home. A copy of the questionnaire is presented as Appendix 14. Details of questionnaire development are described in Section 4.8.1.

4.6.3 Questionnaire distribution

After obtaining approval from the selected schools, a set of survey forms (including consent form, patient information sheet, parental questionnaire) was delivered by hand to the head teacher or representative teacher of the school. Detailed written (Appendix 15) and verbal instructions were given to the teachers concerning the purpose of the study and questionnaire content. The pupils selected to participate in the study were given a copy of the questionnaire by their teachers. Pupils were advised to deliver the questionnaire to their parents for completion and return to school on the following day. Those pupils whose parents had failed to return the questionnaire were given a reminder one week after the initial distribution. The completed questionnaires and consent form were then collected by the teachers and passed to the investigator during visits to each school.

4.6.4 Consent

Alongside with the questionnaire, parents were given an information sheet (Appendix 16) and consent form (Appendix 17). The information sheet provided clear information explaining the nature and purpose of the research. Consent form refer to provision of a form which parents can report consent or refusal for the survey (which include taking intra-oral photograph of their children), indication that parents have read and understood the information sheet and includes a signature and a date.

The information sheet and consent form were translated to the Malay language and reported in Appendix 18 and Appendix 19 respectively. An example of an original signed consent form by parents also enclosed in Appendix 20.

On examination day, children were also asked verbally their willingness to be examined and photographed.

4.6.5 Assessment of subject eligibility

Upon receiving all the survey forms from the teachers, the investigator identified consented children and their lifelong residency status. The subjects were subsequently assessed for their eligibility for clinical examination based on inclusion criteria described in Section 4.4.1.

4.6.6 Clinical examination

All examinations were performed by a single examiner, Nor Azlida Mohd Nor (NAMN). The clinical assessment index is discussed in Sections 4.6.6.1 and 4.6.6.2. Details of examiner training and calibration exercise are discussed in Section 4.6.6.3. The examinations were conducted during school hours either in the classroom or first aid room (Appendix 21). Clinical examinations form for caries and fluorosis are reported in Appendix 22.

4.6.6.1 Fluorosis assessment

Children were examined for dental fluorosis on index teeth (maxillary central permanent incisors) using the Dean's Index (Dean, 1942). Only the maxillary central incisors were examined because they are the most aesthetically important. Dean's Index was chosen because it is a valid and reliable index and it enables comparison with existing national data. Dean's Index is comprised of six categories in an ordinal scale (0=normal, 1 =questionable, 2=very mild, 3=mild, 4=moderate, 5=severe). The criteria for Dean's Index Criteria are described in Appendix 23. The advantages and disadvantages of Dean's index were discussed in Section 1.2.2.4.

4.6.6.2 Caries assessment

Caries status was examined on all erupted teeth using ICDAS-II criteria (ICDAS Coordinating Committee, 2009). ICDAS-II index was chosen because of the ability of the system to detect cavitated and non-cavitated lesions making it possible to compare differences of caries severity between the two populations studied. In addition, it allows data comparison with local and international studies.

The ICDAS-II index is a two-digit scoring method, where the first digit represents restorations and sealant codes. The second digit relates to a dental caries code. The caries code consisted of seven scores (code 0 is sound, codes 1-6 classified as caries). This study used the epidemiology modification, which allows the use of gauze for drying. The details of the index are discussed in Section 1.2.3.2. The ICDAS-II criteria are reported in Appendix 1.

4.6.6.3 Training of examiner and intra-examiner reproducibility

The examiner was trained by Prof Barbara Chadwick (BLC) and Prof Ivor Chestnutt (IGC), who are experienced in conducting caries and fluorosis assessment using ICDAS and Dean’s index.

For caries assessment, the examiner underwent the ICDAS online training module (International Caries Detection and Assessment System, n.d) followed by a six-hour ICDAS workshop at the Dental School, Cardiff University in September 2014. The training workshop involved theoretical explanation and clinical photograph case scenarios. The training exercise was followed by a calibration exercise using 40 clinical slides. The diagnoses were compared with the score recorded by the reference examiner followed by group discussion for every case. In order to test the consistency of the examiner in the field, twenty children were re-examined during clinical examination at random two weeks after the first examination. The level of agreement and intra-examiner reliability were assessed using the Kappa Statistic. Results of intra and inter-examiner reliability for caries examination are described in Section 4.9.2.

For fluorosis assessment, the examiner was also previously trained by the Ministry of Health, Malaysia and international expert (Prof Helen Whelton from the University of Leeds) as a national examiner for Malaysian National Fluoride Enamel Opacities Survey in February-March 2013. The comprehensive training involved a combination of theoretical information, seminar, preliminary diagnostic training, examination of patients and a calibration exercise. The examiner repeated the same online module slides training of fluorosis assessment with Dean's Index used in previous training (Whelton et al., n.d) in September 2014. The online training consists of four modules, the last of which generates a kappa value for a calibration exercise using 40 clinical images. The online training was repeated until the examiner achieved good to excellent kappa score.

The online module training for ICDAS and Dean' Index was also repeated just before the commencement of data collection as a refresher session.

For intra-oral photographic training, the examiner was trained by the chief clinical photographer, Samuel Evans from the Dental School, Cardiff University. The calibration exercise for fluorosis scoring using photographic methods is described in Sections 4.6.7.2 and 4.8.1.5. Results of intra and inter-examiner reliability for fluorosis scoring are described in section 4.9.3.

4.6.6.4 Training of the recorder

The recorder was trained by the examiner (NAMN) in Malaysia before embarking on data collection. This training was to ensure that the recorder was familiarised with the survey forms, field work procedures and equipment to be used.

4.6.6.5 Method of examination

Fluorosis

Children were examined sitting on a chair in the upright position, with the examiner (NAMN) facing them with her back to the light (window). Teeth were not cleaned prior to the examination except for the removal of food debris with gauze or a WHO periodontal probe if necessary. The distribution pattern of any defects was noted and the presence or absence of fluorosis recorded in natural light, with the teeth wet. Children were asked to moisten their teeth .If this not possible, damp cotton wool was used to keep the teeth moist. If fluorosis was present, diagnosis was based on the condition of the maxillary central incisors. If the two teeth were not equally affected, the score on the least affected of the pair was recorded.

Caries

Immediately after fluorosis examination, children were examined for caries on a mobile dental chair in a supine position. Dental caries was diagnosed by visual examination with the aid of a portable light (Halogen bulb, Daray light x100, 12 Volt and 20 Watt) disposable mouth mirror using and a WHO periodontal probe (if necessary) using ICDAS-II criteria (with epidemiology modification). The teeth were dried and cleaned with gauze if the presence of debris interfered with the examination of the tooth surface.

All clinical procedures followed standard infection control guidelines from the Ministry of Health, Malaysia.

4.6.7 Photographic examination

4.6.7.1 Standardized photographic method of recording dental fluorosis

Digital images of the maxillary incisors were taken to enable blind scoring of dental fluorosis. Intraoral photographs were taken using standardized methods described in previous studies (Cochran et al., 2004a). Standardized images were taken using a digital SLR camera, Nikon D3300 body, Sigma 105mm f/2.8 macro lense, Sigma ring flash EM 140DG.

Prior to photograph taking, the child was asked to keep their head still and placed their teeth edge to edge if possible. If it was not possible to maintain edge to edge incisal contact, the child was instructed to bring their upper and lower central incisors into the same vertical plane as far as possible. The child was asked to maintain the position for photography and cheek retractors were inserted to reflect the soft tissues. Sunglasses were used to protect participants' eyes during photography. When necessary, teeth were cleaned with gauze or the periodontal probe if the presence of debris interfered with the examination. Children were asked to moisten their teeth before the photograph was taken. If this was not possible, damp cotton wool was used to keep the teeth moist and the photographs were taken after eight seconds while the teeth were still wet. An assistant verbally counted down the eight seconds.

Most of the photographs only involved one exposure per child. However on occasion, where the examiner was not satisfied with the first photograph (such as issues with specular reflection), further exposures were attempted.

None of the images contained any identifying aspects of the subject's face. A photograph of the children identifying code and their clinical examination form were captured first followed by the images of their teeth. This process enables the digital images link to a subject identity.

4.6.7.2 Blind scoring of fluorosis status

The primary outcome measure for fluorosis was the consensus score from the digital photographs. This method was used with the aim of minimising bias during clinical scoring. The final score used was based on agreement from three examiners as described below.

All digital images were transferred to a computer and transported to the School of Dentistry, Cardiff University. The best quality image representing each participant was chosen and later the photographs were mixed randomly for blind fluorosis scoring. All images (n=1155) were included for assessment and projected onto a white screen using Microsoft Power Point in a darkened room. Two trained examiners (IGC, BLC) who were not involved in the clinical examination, scored these photographs together with the clinical examiner (NAMN). All examiners were blinded to the subject fluoride exposure and each photographic slide was assigned a unique code number. The three scorers (NAMN, IGC and BLC) were seated approximately three meters from the screen and scored the photographs at the same time under identical lighting conditions. Following individual assessment, all examiners re-examined all photographs and discussed thoroughly for consensus agreement of final photographic score. Any problems with the images such as presence of light reflection and flash of the camera were noted during evaluation of each photograph. A calibration exercise was carried out using 111 images following the pilot study and inter-examiner reliability was determined using the kappa test statistic as described in Section 4.8.1.5. Results of intra and inter-examiner reliability for fluorosis scoring are described in Section 4.9.3.

The primary outcome measure for fluorosis was based on the consensus score from the digital photographs. This method was used with aim of minimising bias during clinical scoring. The score used was based on agreement from the three examiners. Example of the intra-oral photographs are presented in Appendix 24.

4.7 Data management and statistical analysis

4.7.1 Data management

4.7.1.1 Data entry and processing

There were three individual data sets in this study which refer to questionnaire, photographic fluorosis score and caries data. For questionnaire and photographic fluorosis score, the data were entered directly to SPSS software version 21 for statistical analysis by the examiner (NAMN).

For caries data, a Visual Basic for Windows (Version 10) data entry programme was specifically designed by a statistician (Damian J Farnell [DJF]) based on examination record forms that were used in the clinical examination (Appendix 25). Data were entered using this interface by a research assistant. On completion of caries data entry, the interface data were converted to SPSS. This data set was than merged with questionnaire and fluorosis data sets using a unique identifier to form a complete data set of study participants for analysis. Cross-checking was performed to ensure no data duplication and other error during merging. Only complete data with clinical and photographic data were included in the analysis.

A number of steps were taken throughout data entry of all data sets to ensure data quality. In order to reduce the chance of human error by transferring data from the questionnaire and charting sheets into the computer, 50 cases were selected randomly and re-entered separately after 30 days of first entry by the examiner. The data were then analysed and verified if differences found in the two entries. Only a very minimal data entry error was found and therefore it was decided that no duplicate data entry was needed.

4.7.1.2 Syntax development for dental caries using ICDAS code

In this study, a new caries index namely ICDAS was used for caries measurement. Since this is an index with a two-digit scoring method, a new syntax for caries outcome variables was developed by a statistician (DJF) from scratch. Caries was calculated at three different ICDAS cut-off points; (D_{1-3}) for enamel caries, (D_{4-6}) for dentine caries, and (D_{1-6}) caries at all level. For teeth surfaces with codes representing both restoration and caries [i.e. any caries score 4 or greater was (dentine caries supersedes restoration), the surface was counted as decayed; and in the absence of any caries score 4 or greater (restoration score supersedes enamel caries) the surface was counted as a restoration]. Fissure sealant codes (code 10 and code 20) were counted as a sound surface. However if the sealant was associated with caries, the surface was recorded as caries at different ICDAS cut-off points as mentioned earlier. A summary of the ICDAS and syntax coding are described in Table 4.1 and Table 4.2.

To ensure the accuracy of the newly developed syntax calculation for caries experience, data were analysed and compared with a manual caries experience (dmft and DMFT) calculation. Manual caries calculation was conducted by the investigator (NAMN) and her supervisor (IGC) using Microsoft Excel independently. Any disagreements were resolved by discussion for an agreed manual caries score. This agreed score was then compared with the syntax caries score. The syntax and manual caries calculation was piloted on 20 cases. Findings from the pilot test resulted in minor modification to the syntax. The final syntax was re-tested on an additional 20 cases against manual calculation and no further amendment was required. The final version of the syntax was used for data analysis using SPSS.

Table 4.1 Syntax coding for ICDAS

ICDAS Code	**Code**	**Variable description**
97	M	Missing due to caries
98	ignore	Missing due to other reason
00	Sound	Sound
01, 02, 03	D_{1-3}	Enamel caries
04, 05, 06	D_{4-6}	Dentine caries
10, 20	Sound	Sound sealant
11, 12, 13, 21, 22, 23	D_{1-3}	Sealant with enamel caries
14, 15, 16, 24, 25,26	D_{4-6}	Sealant with dentine caries
30, 40, 50, 60, 70, 80	F	Sound filling
31, 41, 51, 61, 71, 81 32, 42, 52, 62, 72, 82 33, 43, 53, 63, 73, 83	Restoration supersedes enamel caries = F	Filling with enamel caries (count as filling)
34, 44, 54, 64, 74, 84 35, 45, 55, 65, 75, 85 36, 46, 56, 66, 76, 86	Dentine caries supersedes restoration = D_{4-6}	Filling with dentine caries (count as caries)
99	ignore	Unerupted teeth (ignore)
All other codes		*Ignore*

Note: similar principles apply for primary teeth, only difference is use of lower case dmft and dmfs.

Table 4.2 Syntax coding for DMFT calculation

ICDAS Code	Description	Variable code	Variable description
97	If any surface =97	M	Missing
04, 05, 06, 14, 15, 16, 24, 25,26, 34, 44, 54, 64, 74, 84 35, 45, 55, 65, 75, 85 36, 46, 56, 66, 76, 86	Any caries score on any surfaces 4 or greater	D_{4-6}	Dentine caries
01, 02, 03, 11, 12, 13, 21, 22, 23	In the absence of any F or caries score 4 or greater	D_{1-3}	Enamel Caries
30, 40, 50, 60, 70, 80, 31, 41, 51, 61, 71, 81 32, 42, 52, 62, 72, 82 33, 43, 53, 63, 73, 83	In the absence of any caries score 4 or greater counts	F	Filling

Note: similar principles apply for primary teeth, only difference is use of lower case dmft and dmfs.

4.7.2 Statistical analysis

This section describes the statistical approach used in this study, which includes: measurement of independent variables prior to statistical analysis and specific approach to answering the study objectives.

4.7.2.1 Measurement of independent variables

Variables from the questionnaire include: socio-demographic characteristics, exposure to fluoride from the water, fluoride varnish/gel, infant feeding patterns and oral hygiene practices.

To have a meaningful explanation of each answer options, the data were re-categorised as follows:

i) Exposure to fluoride from the water was categorised into three categories: 0.5 ppmF lifetime, 0.7+0.5 ppmF lifetime and 0 ppmF lifetime.

ii) Demographic characteristic: parents' education level were categorised into three categories: ≤primary school (low education level), high school (moderate education level) and College/University[1] level (high education level); parents' monthly income were categorised as <MYR[2] 1000 (low income), MYR 1000-3999 (moderate income), ≥MYR 4000 (high income).

The majority of the respondents were Malay. There were only a small number of other ethnic groups, which restrict further analysis to compare differences across ethnicity. Therefore ethnicity was excluded from further analysis.

iii) Oral hygiene practices: age started toothbrushing was categorised as before 2 years and after 2 years; age started toothbrushing with toothpaste was categorised as before 2 years and after 2 years; frequency toothbrushing was categorised as once per day or less and twice per day or more; supervised toothbrushing was categorised as never and yes (those answering everyday and sometimes); habits after toothbrushing was categorised as swallowed (for those answering 'swallow/ rinse and swallow') and spat (for those answering 'spit/rinse and spit'); habits of eating and licking toothpaste was categorised as never and yes (those who answered often and sometimes); amount of toothpaste used was categorised as small (pea to smear) and large (moderate to full length brush head); type of toothpaste was categorised as fluoridated (adult and children fluoridated toothpaste) and non-fluoridated toothpaste. The same questions were asked for two time frames of oral hygiene practices: previous practices (aged less than 6 years old) and

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[1] This category includes Malaysian education qualification known as 'Sijil Tinggi Pelajaran Malaysia' (STPM), which is equivalent to Pre-University certificate.

[2] MYR 4.40 (Malaysian Ringgit) equivalent to 1 USD (United States Dollars)

current practices (in 2015), therefore the same categorisation were used for these variables.

iv) Infant feeding practices: infant formula use was categorised as yes and no; aged finished breast feeding was categorised as finished breast feeding at ≤12 months and after 12 months; aged started infant formula was categorised as at ≤12 months and after 12 months; aged finished breast feeding was categorised as at ≤48 months and after 48 months; duration of infant formula feeding was categorised as at ≤48 months and after 48 months. Information relating to methods of feeding practices was also converted into a categorical variable: breast feeding only, formula feeding only and a combination of breast and formula feeding. Respondents who answered 'yes' to breast feeding question and 'yes' to infant formula question were categorised as 'combination of breast and formula feeding'. Respondents who answered 'yes' to breast feeding question and 'no' to infant formula question were categorised as 'breast feeding only' and those who gave the opposite response were categorised as 'formula feeding only'. Respondents with missing or conflicting information were excluded from further analysis. An example of conflicting information is when a respondent reported 'never fed with infant formula' (Question 9) but answering the following question on infant formula feeding time period (Question 10 and Question 11).

When evaluating the questionnaire, it was decided that 'don't know and not sure' answers were excluded from bivariate analysis, which refer to the following questions: Question 18 (supervise toothbrushing), Question 19 (habits after toothbrushing), Question 22 (type of toothpaste) and Question 24 (exposure to fluoride varnish/gel).

4.7.2.2 Statistical analysis plan to address specific objectives of the study

Descriptive analysis was used to explain sample characteristics including frequency and percentage distribution of gender, parents' education levels and parents' socio-economic status. The data were stratified by age group and fluoridation status.

The overall data in the present study was not normally distributed, therefore non-parametric tests was employed for association analysis.

Data were analysed using SPSS Version 21 and STATA Version 13 where indicated.

Objective 1

A descriptive analysis was used to describe the prevalence and severity of dental fluorosis by Dean's Index. The prevalence of dental fluorosis was based on the percentage of children having fluorosis on maxillary central incisors by consensus digital photographs score. The cases for fluorosis were defined as any fluorosis by Dean's score>0, which include questionable or greater and fluorosis at Dean's score≥2 which indicate very mild or greater. The data were stratified by age group and fluoridation status. Chi square test was used to compare association between fluorosis prevalence by age group and fluoridation status. The significance level was set at $p<0.05$.

Objective 2

Descriptive analysis was used to describe caries experience using ICDAS score (DMFT/dmft and DMFS/dmfs) by birth cohorts and fluoridation status. To establish how the decay component using ICDAS-II correlated with the DMF caries classification scores, the DMFT/dmft and DMFS/dmfs scores were calculated at three cut off points: scores D_{1-3}/ d_{1-3} classified as enamel caries, score D_{4-6}/ d_{4-6} classified as dentine caries and D_{1-6}/ d_{1-6} classified as caries at all levels. A comparison of the ICDAS scoring system with the DMF index is shown in Appendix 2. In terms of caries prevalence, the dentine caries prevalence (D_{4-6}MFT>0/ d_{4-6}mft>0) was dichotomized into absence and presence of the disease. A comparison was made between enamel caries and dentine caries, and the data were stratified by age group and fluoridation status. To compare the mean caries scores of the subgroups, non-parametric test was performed (Mann Whitney test). The significance level was set at $p<0.05$.

Objective 3

Fluorosis

The differences between birth cohorts was the key factor in comparing prevalence of fluorosis. In order to detect differences following fluoride level adjustment, the change in fluorosis prevalence in the fluoridated community was compared to the change in non-fluoridated community. The 'baseline' data were extracted from the groups that were exposed to the old fluoride level (0.7 ppm) and the 'after' prevalence data were extracted from the group that were exposed to the new fluoride level (0.5 ppm) after the reduction occurred. Both definitions of fluorosis prevalence (Deans>0, Deans≥2) were analysed. In addition, the association between the prevalence of aesthetic fluorosis (Deans≥ 2) and different levels of fluoride exposure in the water were analysed using binary logistic regression and odds ratio. The non-fluoridated group was used as reference category.

Caries

Caries experience cannot be compared directly across different age groups. To determine differences in caries experience following the reduction of fluoride level in the public water supply, two types of multivariate analyses (namely zero-inflated negative binomial and generalised linear model) were conducted. These analyses were performed using two caries outcome measures (mean D_{4-6}MFT and D_{4-6}MFT>0) to generate two caries models to evaluate the caries preventive effect after the change of fluoride level in the water. The zero-inflated negative binomial analysis was performed using STATA Version 13 for caries model 1. The generalised linear model analysis was performed using SPSS Version 21 for caries model 2. The zero-inflated negative binomial was analysed using mean caries experience into dentine (D_{4-6}MFT) with different fluoridation status and age groups. Meanwhile, the generalised linear model was analysed using percentage caries prevalence into dentine (D_{4-6}MFT>0) with different fluoridation status and age groups. In both models, data were presented by age, fluoridation status and when interaction between age and fluoridation were included in the analysis.

Objective 4

Bivariate analysis was used to determine the association between fluorosis and independent variables from the questionnaire using Chi Square test and odds ratio. Independent variables were dichotomised prior to bivariate analysis as described in Section 4.7.2.1. The selection of variables to test for association with fluorosis was based on the exposure to fluoride during the developmental stages of the central incisors.

Analysis was conducted to explore associations between fluorosis (Deans≥2) as dependent variable and other factors such as: oral hygiene practices (during the first six years of life), infant feeding practices and demographic characteristics as independent variables. Data on independent variables were obtained from the questionnaire which included factors such as exposure to fluoride in the water supply; fluoride gel/varnish; infant feeding patterns (method of feeding, age at which breast-feeding terminated, age started and finished formula and type of water use to reconstitute formula when it was used); and oral hygiene practices (the age started toothbrushing, the age at which toothbrushing with toothpaste started, the frequency of toothbrushing, toothbrushing supervision, habits after toothbrushing, type of toothpaste and amount of toothpaste used). Other demographic variables such as gender, age, parents' education levels, and parents' socio-economic status were also tested for association with fluorosis prevalence. The outcome was reported as unadjusted odds ratios, confidence intervals and *p* value. The significant variables were entered into a multivariate logistic regression analysis as independent predictors as described below.

Multivariate models for fluorosis

Variables with significant association ($p<0.05$) at bivariate analysis were further analysed using multivariate logistic regression to develop a model for dental fluorosis using binary logistic regression. These variables were entered in one block using the Enter method. Interaction was also tested between inter-dependent factors to test their contribution to a model. If any interaction were contributory, they were retained and reported. The outcome was reported as adjusted odds ratios, confidence intervals and *p* values.

Objective 5

Similarly, bivariate analysis was conducted between caries prevalence at dentine level ($D_{4-6}MFT>0$) as dependent variables and exposure to fluoride from the water, fluoride varnish/gel, oral hygiene practices, infant feeding practices and demographic characteristics as independent variables. The same analysis as described above was conducted for caries prevalence at all levels ($D_{1-6}MFT>0$). The outcome was reported as unadjusted odds ratios, confidence intervals and *p* value. The significant variables were entered into a multivariate logistic regression analysis as independent predictors as described below.

Multivariate models for caries

Variables with significant association ($p<0.05$) and approaching significant ($p<0.10$) at bivariate analysis were further analysed using multiple logistic regression to develop a model for caries at dentine level ($D_{4-6}MFT>0$) and caries at both enamel and dentine levels ($D_{1-6}MFT>0$) using binary logistic regression. These variables were entered in one block using the Enter method. Interaction was also tested between inter-dependent factors to test their contribution to a model. If any interaction were contributory, they were retained and reported. The outcome was reported as adjusted odds ratios, confidence intervals and *p* values.

4.8 Pilot study

The purpose of the pilot study was to test the methods and logistics before the subsequent conduct of main study.

4.8.1 Questionnaire

4.8.1.1 Development of draft questionnaire

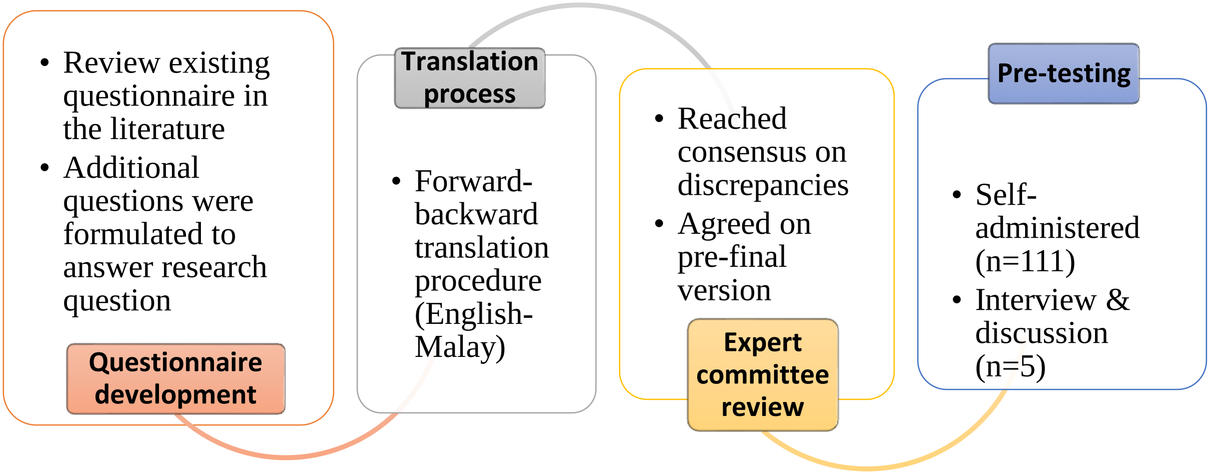
Specific questions were adapted from a National Survey of Fluoride Enamel Opacities (NSFEO), Malaysia 2013 (Oral Health Division Ministry of Health, 2013) and the Child Oral Health Study, Australia 2002/2003 (Do, 2004). Additional questions were formulated based on literature reviews and group discussion between the author and her supervisors in order to answer the research objectives. The questionnaire from NSFEO was available in Malay and English versions. Other questions were underwent translation process as described in section 4.8.1.2. The English version of the questionnaire draft was underwent face validation by two dental experts in Cardiff University and required minor amendments.

4.8.1.2 The translation process

Following face validation, the original English questionnaire was translated into the Malay language by a bilingual translator and investigator. Following forward translation, discussion were carried out to achieve a single Malay version of the questionnaire. A bilingual expert committee consisting of three dental experts (two dental academics from the University of Malaya and a dental public health specialist from Ministry of Health, Malaysia) reviewed both the Malay version and the English version of the questionnaire independently. The committee reviewed the questionnaire with regards to the wording used, structure, content and semantic equivalence with the original questionnaire. Any discrepancies from independent reviews were discussed thoroughly until consensus was achieved. Following expert committee discussion, both Malay and English versions required some modifications as listed in Appendix 26. The English pre-final version of the questionnaire was assessed by two dental specialists who were native English speakers. The Malay pre-final version was assessed by a linguistic expert in the University of Malaya. Following the assessment, no further changes were needed and the draft underwent pre-testing among a group of Malaysian parents.

The development stage of the parental questionnaire is illustrated in Figure 4.4.

Figure 4.4 Development of the questionnaire



4.8.1.3 Pre-test of the questionnaire

A pre-final draft of the questionnaire was pre-tested among selected parents (n=111) in one of the school (Sekolah Kebangsaaan Padang Jawa) in a fluoridated area located in Shah Alam, Selangor between (20th September to 30th October 2014). Method of the questionnaire distribution was similar to the method used in the main study as described in Section 4.6.3. Twenty parents were randomly invited to answer the questionnaire twice after one week interval for internal reliability test. Results of internal reliability test of the questionnaire are described in Section 4.9.1. The same parents were also invited for qualitative interviews to give their feedback on the questionnaire, however only five parents agreed to participate. During telephone interviews, parents were asked to give feedback on the clarity of the questionnaire instruction, language, its content and the times taken to answer overall questions. All telephone interviews were tape-recorded and transcribed. Findings from the pilot study and interviews resulted in minor amendments to the questionnaire which was removal of Question 9 “at what age did your child begin breast feeding?” as this was deemed confusing to the parents.

The final version of the parental questionnaire consisted of 29 questions and divided into the following sections: residency status, demographic characteristics, infant feeding practices, oral hygiene practices (which sub-divided into previous practice [age less than 6 years old] and current practice [in 2015]), exposure to fluoride varnish/gel and sources of water at home.

4.8.1.4 Clinical examination for pilot study

The clinical assessment index was discussed earlier in Sections 4.6.6.1 and 4.6.6.2. Details of examiner training and calibration exercise were discussed earlier in Section

4.6.6.3. During the pilot study, children (n=111) were examined clinically for dental caries and fluorosis by a single examiner (NAMN) in Malaysia. Twenty children were re-examined after a two-week interval for intra-examiner reliability. Results of intra-examiner reliability were described in section 4.9.2.

4.8.1.5 Photographic assessment of dental fluorosis for pilot study

Two independent photographic examiners (IGC, BLC) and the clinical examiner (NAMN) scored 111 photographic images of the same children in a standardized manner as described in Section 4.6.7.2. The blinded fluorosis scores were compared individually between examiners for both clinical and photographic scoring. Results of intra and inter-examiner reliability were described in Section 4.9.3.

4.9 Results of the pilot study

4.9.1 Internal reliability of the questionnaire

Internal reliability of the questionnaire was assessed using Cronbach's alpha coefficient. Twenty parents answered the questionnaire twice after one-week interval. Twelve questions of oral hygiene practices were used to for test-retest analysis. Internal consistency was good with Cronbach alpha ($\alpha > 0.80$).

4.9.2 Examiner reliability for caries assessment

Results of inter-examiner reliability of calibration exercise using 40 clinical slides were substantial (0.61). The kappa score for intra-examiner reliability for the duplicate clinical examination of caries assessment was excellent (0.81).

4.9.3 Examiner reliability for fluorosis assessment

The overall findings of examiner reliability for fluorosis assessment using clinical and photographic methods was published in the Community Dental Health Journal (2016). The full text article is presented in (Appendix 27). The results described in this section are the key findings from the publication.

The kappa score for intra-examiner reliability for the duplicate clinical examination of fluorosis assessment was excellent (0.89).

In terms of weighted kappa statistics, a weight of 1 was given for exact agreement, a weight of 0.5 was given when examiner disagreed by only one severity level and a weight of 0 was given when examiners disagreed by more than one severity level.

Table 4.3 shows inter-examiner reliability between clinical and photographic methods. Inter-examiner reliability between photographic examiners (Examiner 2 and Examiner 3) versus clinical examiner (Examiner 1) was found to have substantial agreement using both weighted and simple kappa statistics.

Table 4.3 Inter-examiner agreement of dental fluorosis by clinical and photographic examination

	Unweighted data		*Weighted data*	
Clinicians	***Kappa***	***Agreement (%)***	***Kappa***	***Agreement (%)***
Examiner 1 clinical versus Examiner 2 photographs	0.82	92.8%	0.77	89.6%
Examiner 1 clinical versus Examiner 3 photographs	0.72	89.2%	0.74	86.5%

Table 4.4 shows all examiners demonstrated substantial to excellent inter-examiner reliability for photographic scoring (either when they were compared against each other or when comparison were made with consensus photographs score) with weighted kappa values ranging from 0.72 to 0.91. There was little difference found between weighted and simple kappa analysis.

Table 4.4 Inter-examiner agreement of dental fluorosis between individual photographic score and consensus photographic score

	Unweighted data		***Weighted data***	
Clinicians	***Kappa***	***Agreement (%)***	***Kappa***	***Agreement (%)***
Examiner 1 vs Examiner 2	0.78	91.9%	0.80	94.8%
Examiner 1 vs Examiner 3	0.72	90.1%	0.85	96.2%
Examiner 2 vs Examiner 3	0.85	94.6%	0.75	89.2%
Examiner 1 vs Consensus	0.83	93.7%	0.91	95.9%
Examiner 2 vs Consensus	0.91	96.4%	0.87	94.4%
Examiner 3 vs Consensus	0.90	96.4%	0.82	92.3%

Note: Consensus photographic score based on the agreement of at least two of the three examiners. (Examiner 1=clinical and photographic examiner, Examiner 2 and Examiner 3=photographic examiner only).

4.10 Ethical considerations

Ethical approval to conduct this study was obtained from the Research Ethics Committee, School of Dentistry, Cardiff University (Appendix 28). Permission to conduct this study on Malaysian school children was obtained from Malaysian Ministry of Health (Appendix 29), Ministry of Education (Appendix 30), State Education Department (Appendix 31). Informed signed consent was obtained from the children's parents or guardians. An example of informed signed consent is presented in Appendix 20.

4.10.1 Data confidentially and security

All research data were treated in strict confidence and stored under secure conditions, in line with Cardiff University data security requirements. To maintain anonymity, all participants were given a unique individual code in all recorded measurements and files as a replacement for the subject's name.

Following field work, questionnaire data were entered straight away into SPSS software. The original copies of the questionnaire were kept securely at the Department of Community Oral Health & Clinical Prevention, Faculty of Dentistry, University of Malaya, Malaysia. Data files which include intra-oral images and soft copies of the questionnaire data were transferred on a password protected external hard drive from fieldwork to Cardiff University. In terms of clinical examination forms, original charts were transferred to Cardiff University by NAMN directly in her personal hand luggage on a flight from Kuala Lumpur to Cardiff. The original copies of the clinical examination forms were kept securely at the Dental Research Unit, Cardiff University. Duplicates of clinical examination forms were held in the Department of Community Oral Health & Clinical Prevention, Faculty of Dentistry, University of Malaya, Malaysia.

Where data are recorded directly onto computers software a back-up copy was made everyday and stored separately on a password protected external hard disc.

Names and other basic information and their corresponding codes were stored in a safe place, and locked cabinet and only accessible to researchers.

4.10.2 Token of appreciation for participant

As a token of appreciation for participation in the study, children were provided with a toothbrush and toothpaste. In line with local practice a Certificate of Participation was given to the Schools involved. In addition to maximise response rate we offered parents an incentive of entry to a prize draw for one of twenty MYR100 (23 USD) shopping vouchers.

5 Results

This results chapter consists of four main sections, each divided into sub-sections. Each section will report descriptive, bivariate and multivariate analyses which address the research questions. The data are based on information collected from the parental questionnaire, photographic examination of dental fluorosis and the clinical examination for caries. The main sections are divided as follows.

The chapter begins by describing the response rate and description of the study participants (Section 5.1). Next, the descriptive analysis of the questionnaire data with regards to exposure to difference sources of fluoride are presented in Section 5.2. These independent variables were sub-divided into fluoride exposures from water, oral hygiene habits, infant feeding practices and fluoride gel/varnish (Sections 5.2.1. to 5.2.7).

The following sections are divided into the main outcome measures which were dental fluorosis (Section 5.3) and caries (Section 5.4). In the dental fluorosis section, results were presented in the following order: prevalence and severity of dental fluorosis, association between changes in fluoride level in the water supply and fluorosis prevalence, as well as association of the other risk factors and fluorosis prevalence (Sections 5.3.1 to 5.3.2). A multivariable model for dental fluorosis is presented in Section 5.3.3. In the caries section, results are presented in a similar fashion as fluorosis section: prevalence and severity of dental caries, association between changes in fluoride level in the water supply and caries prevalence, as well as association of the other risk factors and dental caries (Sections 5.4.1 to 5.4.3) and a multivariable model for dental caries (Section 5.4.4). The last section summarises the key findings of this study (Section 5.5).

5.1 Response rate and description of study participants

This section describes the participant response rate followed by a description of study participants based on information derived from the parental questionnaire.

5.1.1 Response rate

Initially 20 public schools in Malaysia were invited to participate in the study. Of these 16 schools accepted. Eight schools were from a fluoridated state (Negeri Sembilan) and another eight schools were from a non-fluoridated state (Kelantan). A total of 1,600 children were approached to participate in this study. Following questionnaire distribution, 1,298 returned the questionnaire giving an 81.1% overall response rate. The response rate was higher among children in the fluoridated areas (83.9%) in comparison to those living in non-fluoridated areas (79.3%). The difference in response rate between the two areas was statistically significant among the 9 year-old cohort (p=0.038). Table 5.1 presents the number of participants invited and the response rate by age group and area of residence.

Table 5.1 Response rate by age group and area of residence

Age/area	Number of participants invited (questionnaire distribution)^A	Questionnaire returned^B	Response rate (%) [$^{B/A}$ x 100]
Fluoridated area (F)			
9	400	343	85.8*
12	400	321	80.3^{NS}
Total F	800	664	83.0 ^{NS}
Non-fluoridated area (NF)			
9	400	291	72.3
12	400	343	85.8
Total NF	800	634	79.3
Overall response for both areas and age groups			
F & NF	1600	1298	81.1

*p=0.038 (statistically significant between two areas)

^{NS}=not significant

Of those who responded, 1291 provided signed parental consent. All consented participants were further assessed for their residency status. At this stage fifty children were excluded as non-lifelong residents.

Lifelong residents children with parental consent were further assessed for other inclusion criteria as described in Section 4.4.1 and Section 4.6.5 during the clinical examination. Reasons for exclusion during this stage of eligibility assessment are presented in Table 5.2. In total 57 children were absent on clinical examination day. Of those who attended the examination, 21 children were excluded because of unerupted upper central incisor/s, followed by fractured incisor(s) (n=4), partially erupted incisor(s) (n=3) and presence of a fixed orthodontic appliance (n=1). The number of children excluded across age groups and in fluoridated and non-fluoridated areas was broadly similar.

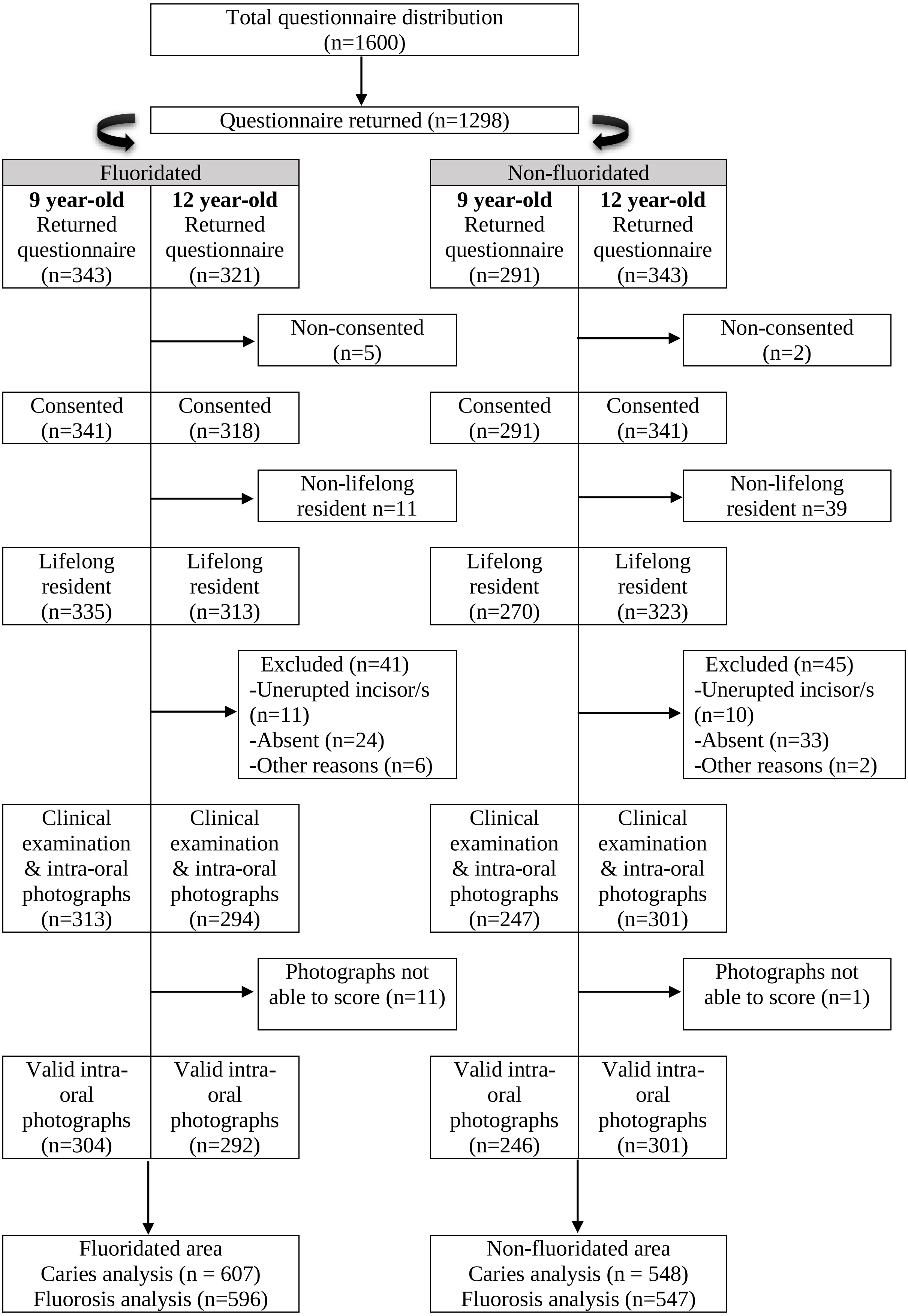
Table 5.2 Reasons of exclusion by age group and residency area

	Reasons for exclusion					
Age/ Area	Absent	Unerupted incisor(s)	Partial erupt incisor(s)	Fixed orthodontic appliance	Facture incisor	Total
Fluoridated (F)						
9	9	10	3	0	0	22
12	15	1	0	1	2	19
Total F	24	11	3	1	2	41
Non-Fluoridated (NF)						
9	13	10	0	0	0	23
12	20	0	0	0	2	22
Total NF	33	10	0	0	2	45
Overall	57	21	3	1	4	86

In total (n=1155) were clinically examined and photographed. After clinical examination, all photographs were blind scored for fluorosis scoring (Section 4.6.7). Out of 1155 photographs available for scoring, 12 photographs were not able to be scored because of poor quality photographs. This resulted in 1143 children for whom both a valid photograph and questionnaire data were available for analysis. In terms of caries analysis, all clinical and questionnaire data (n=1155) were analysed.

Participant flow through each stage of the study is presented in Figure 5.1.

Figure 5.1 Participants eligibility through each stage of the study



5.1.2 Description of study participants

A description of the demographic characteristics of study participants by age and residency area is presented in (Table 5.3 to Table 5.5).

Participants were similar in terms of gender distribution and ethnicity in both birth cohorts and geographic areas. The majority of the respondents were Malays and there were more girls than boys were recruited to the study (Table 5.3).

Table 5.3 Demographic characteristics of the study participants

Variables	Fluoridated (F) n (%)			Non-fluoridated (NF) n (%)		
	9 n=313	12 n=294	Total F n=607	9 n=247	12 n=301	Total NF n=548
Gender (n=1155)						
Male	134 (42.8)	131 (44.6)	265 (43.7)	106 (42.9)	125 (41.5)	231 (42.2)
Female	179 (57.2)	163 (55.4)	342 (56.3)	141 (57.1)	176 (58.5)	317 (57.8)
Ethnicity (n=1155)						
Malay	303 (96.8)	283 (96.3)	586 (96.5)	246 (99.6)	298 (99.0)	544 (99.3)
Chinese	0	0	0	0	1 (0.3)	1 (0.2)
Indian	10 (3.2)	10 (3.4)	20 (3.3)	0	0	0
Others	0	1 (0.3)	1 (0.2)	1 (0.4)	2 (0.7)	3 (0.5)

Socio-economic status of the respondents were based on parents' education level and parents' monthly income. For descriptive analysis, parents' education levels are presented in five categories (never been to school, primary school, high school, College and University level). Overall, two thirds of children had parents with education at high school level, followed by College level, University level and primary school level (Table 5.4). The patterns were slightly different among fathers' educational level in the non-fluoridated area. The proportion of fathers with University level education (11.7%) was double the proportion of those educated to primary school level (6.4%) among the 9 year-old children in the non-fluoridated area. The opposite distribution was observed among 12 year-old children in the same area. Only a small proportion (less than 3%) of parents had not received any formal education in both areas.

Table 5.4 Parents' education level by age group and residency area

Variables	Fluoridated (F) n (%)			Non-fluoridated (NF) n (%)		
	9 n=313	12 n=294	Total F n=607	9 n=247	12 n=301	Total NF n=548
Father education level (n=1061)						
Never been to school	4 (1.3)	2 (0.7)	6 (1.0)	4 (1.6)	3 (1.0)	7 (1.3)
Primary school	19 (6.0)	15 (3.3)	34 (5.6)	16 (6.4)	39 (13.0)	55 (10.1)
High school	189 (60.3)	181 (61.5)	370 (60.9)	153 (61.9)	182 (60.4)	335 (61.1)
College	46 (14.7)	45 (15.3)	91 (15.0)	23 (9.3)	26 (8.6)	49 (8.9)
University	30 (9.6)	29 (9.8)	59 (9.7)	29 (11.7)	26 (8.7)	55 (10.0)
Mother education level (n=1092)						
Never been to school	2 (0.6)	5 (1.7)	7 (1.2)	7 (2.8)	8 (2.7)	15 (2.7)
Primary school	21 (6.7)	20 (6.8)	41 (6.7)	21 (8.5)	25 (8.3)	46 (8.4)
High school	163 (52.1)	175 (59.5)	338 (55.7)	147 (59.5)	200 (66.5)	347 (63.3)
College	69 (22.0)	47 (16.0)	116 (19.1)	37 (15.0)	29 (9.6)	66 (12.0)
University	33 (10.5)	36 (12.3)	69 (11.3)	25 (10.1)	22 (7.3)	47 (8.6)

Sums may not total 1155 due to missing response variables

Parents' monthly income in (Malaysian Ringgit-MYR) was used as a measure of economic status. One United States Dollars (USD) is equivalent to 4.40 MYR. The distribution of the father's monthly income was different among children living in both areas studied (Table 5.5). Most fathers in the fluoridated area had a higher income in comparison to those in the non-fluoridated area. More than half of the fathers in the non-fluoridated area (58.4%) had income less than MYR 1999 in comparison to those in fluoridated area (25.6%).

In terms of mother's monthly income, most mothers reported a low income compared with that reported by the participants' fathers. In both areas studied, the majority of mother's earned less than MYR 1999. A greater proportion of mothers were in the higher income bands in the fluoridated when compared with the non-fluoridated states.

Table 5.5 Parents' monthly income by age group and area of residence

	Fluoridated (F) n (%)			**Non-fluoridated (NF) n (%)**		
Variables	**9 n=313**	**12 n=294**	**Total F n=607**	**9 n=247**	**12 n=301**	**Total NF n=548**
Father income (n=1061)						
No income	0	1 (0.3)	1 (0.2)	0	1 (0.3)	1 (0.2)
< MYR 1000	8 (2.6)	10 (3.4)	18 (3.0)	5 (2.0)	2 (0.7)	7 (1.3)
MYR 1001- 1999	75 (24.0)	61 (20.7)	136 (22.4)	133 (53.8)	179 (59.5)	312 (56.9)
MYR 2000- 3999	90 (28.8)	89 (30.3)	179 (29.5)	38 (15.4)	42 (14.0)	80 (14.6)
MYR 4000- 4999	77 (24.6)	65 (22.1)	142 (23.4)	23 (9.3)	26 (8.6)	49 (8.9)
>MYR 5000	40 (12.8)	44 (15.0)	84 (13.8)	26 (10.5)	26 (8.6)	52 (9.5)
Mother income (n=1061)						
No income	0	0	0	0	1 (0.3)	1 (0.2)
< MYR 1000	131 (41.9)	144 (49.0)	275 (45.3)	148 (59.9)	189 (62.8)	337 (61.5)
MYR 1001- 1999	40 (12.8)	42 (14.3)	82 (13.5)	37 (15.0)	59 (19.6)	96 (17.5)
MYR 2000- 3999	47 (15.0)	25 (8.5)	72 (11.9)	17 (6.9)	8 (2.7)	25 (4.6)
MYR 4000- 4999	41 (13.1)	38 (12.9)	79 (13.0)	21 (8.5)	20 (6.6)	41 (7.5)
>MYR 5000	27 (8.6)	36 (12.2)	63 (10.4)	16 (6.5)	15 (5.0)	31 (5.7)

Sums may not total 1155 due to missing response variables

5.2 Descriptive analysis on fluoride exposure from water, infant feeding practices, oral hygiene habits and fluoride varnish/gel in study participants

This section provides a descriptive analysis of fluoride exposure from multiple sources among study participants. The fluoride history data were obtained from the parental questionnaire. The fluoride exposure was divided into four main sub-sections; exposure to fluoride from water, infant feeding practices, oral-hygiene habits and exposure to fluoride varnish/gel. Further analysis on how these factors associated to fluorosis and caries prevalence are described in Sections 5.3.2 and 5.4.3.

5.2.1 Exposure to fluoride from water

Table 5.6 shows exposure to fluoride from drinking water in the participants recruited. There was a slightly higher proportion of participants resident in the fluoridated area (52.6%) than in the non-fluoridated area (47.4%). The highest number of participants was among 9 year-old children in the fluoridated area and the lowest number of participants was among the 9 year-old children in non-fluoridated area. Of those living in the fluoridated area, the 9 year-old children were exposed to 0.5 ppmF throughout life (27.1%) and the older age group were exposed to 0.7 ppmF in the first 2 years of life followed by 0.5 ppmF (25.5%).

Table 5.6 Frequency table of exposure to fluoride from water among study participants

Exposure to fluoride from water	n (%)
By age group	
Fluoridated (F)	
9 year-old F	313 (27.1)
12 year-old F	294 (25.5)
Total F	607 (52.6)
Non-fluoridated (NF)	
9 year-old NF	247 (21.4)
12 year-old NF	301 (26.1)
Total NF	548 (47.4)
By different level of fluoride exposure	
0.5 ppmF lifetime (9 year-old)	313 (27.1)
0.7 ppmF at first 2 years of life & 0.5ppmF lifetime (12 year-old)	294 (25.5)
0 ppmF lifetime (9 and 12 year-old)	548 (47.4)

5.2.2 Sources of drinking water and use of water filters at home

A question was asked about the usage of water filters at home because there is a tendency towards use of domestic water filtration systems among in Malaysia due to concerns over polluted water. Whether fluoride concentred in public water supply is affected by the filter or not is discussed in Section 6.3.5.

Table 5.7 presents sources of drinking water and the use of a water filter at home. The majority of respondents reported tap water as the main source of water at home. However about 11% of children in the non-fluoridated area reported that they had other than tap water as the source of water at home.

Higher water filter use was reported among those living in the fluoridated area (60%) as compared to those in non-fluoridated area (42.9%).

Table 5.7 Sources of drinking water and use of a water filter at home among study participants

	Fluoridated (F) n (%)			Non-fluoridated (NF) n (%)		
	9 (n=313)	**12 (n=294)**	**Total F (n=607)**	**9 (n=247)**	**12 (n=301)**	**Total NF (n=548)**
Source of drinking water at home (n=1142)						
Tap water	300 (95.8)	286 (97.3)	586 (96.5)	218 (88.3)	259 (86.0)	477 (87.0)
River/stream/well water	3 (1.0)	2 (0.7)	5 (0.8)	23 (9.3)	37 (12.3)	60 (10.9)
Bottled water	8 (2.6)	5 (1.7)	13 (2.1)	5 (2.0)	5 (1.7)	1 (0.2)
Others	0	0	0	1 (0.4)	0	0
Filtered tap water (n=1128)						
Yes	185 (59.1)	179 (60.9)	364 (60.0)	99 (40.1)	136 (45.2)	235 (42.9)
No	126 (40.3)	113 (38.4)	239 (39.4)	139 (56.3)	151 (4.3)	290 (52.9)

Sums may not total 1155 due to missing response variables

5.2.3 Breast-feeding and infant formula practices in study participants

Infant feeding practices in particular the use of infant formula are potential risk factors to the development of dental fluorosis. A descriptive analysis of these factors are presented in this section.

Breast-feeding practices

Table 5.8 shows reported breast-feeding among the study participants. Parents reported that almost all children had been breast-fed during infancy. However the duration of breast-feeding varied across age groups and residency area. A higher proportion of children in the non-fluoridated area were breast-fed up to 24 months in comparison to those in the fluoridated area.

Table 5.8 Self-reported breast-feeding practices among study participants

	Fluoridated n (%)			**Non-fluoridated n (%)**		
	9 (n=313)	**12 (n=294)**	**Total F (n=607)**	**9 (n=247)**	**12 (n=301)**	**Total NF (n=548)**
Breast feeding (n=1155)						
Yes	305 (97.4)	287 (97.6)	592 (97.5)	238 (96.4)	298 (99.0)	536 (97.8)
No	8 (2.6)	7 (2.4)	15 (2.5)	9 (3.6)	3 (1.0)	12 (2.2)
Age finished breast feeding (n=1131)						
Before 6 months	60 (19.2)	62 (21.1)	122 (20.1)	19 (7.7)	30 (10.0)	49 (8.9)
6 months to 12 months	88 (28.1)	89 (30.3)	177 (29.2)	42 (17.0)	57 (18.9)	99 (18.1)
13 months to 24 months	98 (31.1)	91 (31.0)	189 (31.1)	144 (58.3)	175 (58.1)	319 (58.2)
After 24 months	61 (9.5)	46 (15.6)	107 (17.6)	33 (13.4)	36 (12.0)	69 (12.6)

Sums may not total 1155 due to missing response variables

Infant formula practices

Table 5.9 shows infant formula practices among study participants. There was greater proportion of children in fluoridated area (83.9%) who had been given infant formula compared to 60.4% of children in the non-fluoridated area who were fed infant formula. Most of the children in fluoridated area (81.4%) had a combination of breast-feeding and formula feeding in comparison to only 58.2% of children in the non-fluoridated area who had combined feeding methods.

Children in the fluoridated area were more likely to have been fed infant formula earlier (before 12 months of age) than those in the non-fluoridated area. Children resident in the fluoridated area were older when feeding with infant formula ceased. Half (50.7%) of parents reported using infant formula beyond 48 months. This contrasts with 22.8% of parents in the non-fluoridated area who similarly reported that their child stopped using infant formula beyond 48 months.

The most common means of reconstituting infant formula was to use unfiltered tap water (59.3%) in the fluoridated area and (50.5%) in the non-fluoridated communities. Infant formula made-up with filtered tap water was three times more common among children in the fluoridated area (22%) than those in the non-fluoridated area (7%). Only a small proportion of respondents reported using bottled water to reconstitute infant formula.

Table 5.9 Infant formula practices among study participants

	Fluoridated n (%)			**Non-fluoridated n (%)**		
Variables	**9 (n=313)**	**12 (n=294)**	**Total F (n=607)**	**9 (n=247)**	**12 (n=301)**	**Total NF (n=548)**
Infant formula user (n=1152)						
Yes	265 (84.7)	244 (83.0)	509 (83.9)	157 (63.6)	174 (57.8)	331 (60.4)
No	48 (15.3)	49 (16.7)	97 (16.0)	88 (35.6)	127 (42.2)	215 (39.2)
Infant feeding practice (n=1152)						
Infant formula only	8 (2.6)	7 (2.4)	15 (2.5)	9 (3.6)	3 (1.0)	12 (2.2)
Breast feeding only	48 (15.3)	49 (16.7)	97 (16.0)	88 (35.6)	127 (42.2)	215 (39.2)
Combination of breast feeding and infant formula	257 (82.1)	237 (80.6)	494 (81.4)	148 (59.9)	171 (56.8)	319 (58.2)
Age started infant formula (n=850)						
Before 6 months	87 (27.8)	80 (27.2)	167 (27.5)	58 (23.5)	57 (18.9)	115 (21.0)
6 months to 12 months	89 (28.4)	72 (24.5)	161 (26.5)	35 (14.2)	31 (10.3)	66 (12.0)
13 months to 24 months	56 (17.9)	55 (18.7)	111 (18.3)	40 (16.2)	58 (19.3)	98 (17.9)
After 24 months	35 (11.2)	39 (13.3)	74 (12.2)	28 (11.3)	30 (10.0)	58 (10.6)
Age finished infant formula (n=845)						
Before 6 months	0	0	0	1 (0.4)	5 (1.7)	6 (1.1)
6 months to 12 months	3 (1.0)	1 (0.3)	4 (0.7)	2 (0.8)	1 (0.3)	3 (0.5)
13 months to 24 months	20 (6.4)	22 (7.5)	42 (6.9)	11 (4.5)	26 (8.6)	37 (6.8)
25-48 months	83 (26.5)	75 (25.5)	158 (26.0)	84 (34.0)	78 (25.9)	162 (29.6)
>48 months	160 (51.1)	148 (50.3)	308 (50.7)	59 (23.9)	66 (21.9)	125 (22.8)
Type of water use to prepare infant formula (n=839)						
Formula user with tap water	176 (56.2)	184 (62.6)	360 (59.3)	132 (53.4)	145 (48.2)	277 (50.5)
Formula user with filtered tap water	77 (24.6)	54 (18.4)	131 (21.6)	18 (7.3)	21 (7.0)	39 (7.1)
Formula user with bottled water	11 (3.5)	7 (2.4)	18 (3.0)	6 (2.4)	8 (2.7)	14 (2.6)
Duration of infant formula (n=836)						
Before 6 months	4 (1.3)	5 (1.7)	9 (1.5)	3 (1.2)	10 (3.3)	13 (2.4)
6 to 12 months	14 (4.5)	14 (4.8)	28 (4.6)	16 (6.5)	23 (7.6)	39 (7.1)
13 to 24 months	42 (13.4)	38 (12.9)	80 (13.2)	43 (17.4)	53 (17.6)	96 (17.5)
25 to 48 months	94 (30.0)	101 (34.4)	195 (32.1)	62 (25.1)	54 (17.9)	116 (21.2)
>48 months	110 (35.1)	85 (28.9)	195 (32.1)	34 (13.8)	31 (10.3)	65 (11.9)

Sums may not total 1155 due to missing response variable

5.2.4 Age started toothbrushing and age exposed to fluoridated toothpaste in the study participants

The age at which participants started toothbrushing and using a fluoridated toothpaste are potential contributing factors to the development of dental fluorosis. The continuous variable (age started toothbrushing) was dichotomised as before or after 24 months old for ease of interpretation.

Table 5.10 shows the age at which parents reported that their child started toothbrushing and the age at which children were exposed to fluoridated toothpaste. Almost all children brushed their teeth. Two thirds of children in the fluoridated area and over 70% children in non-fluoridated area started toothbrushing practice after 24 months of age. When they were asked when toothbrushing with toothpaste commenced, more than one third of parents answered between 24 to 48 months of age. More children in fluoridated areas started toothbrushing with toothpaste earlier (before 24 months of age). In contrast children in the non-fluoridated area tended to brush teeth with toothpaste after 48 months of age.

Table 5.10 Age started toothbrushing and age exposed to fluoridated toothpaste among study participants

	Fluoridated (F) n (%)			Non-fluoridated (NF) n (%)		
	9 (n=313)	**12 (n=294)**	**Total F (n=607)**	**9 (n=247)**	**12 (n=301)**	**Total NF (n=548)**
Does your child brush their teeth (n=1155)						
Yes	312 (99.7)	294 (100)	606 (99.8)	246 (99.6)	301 (100)	547 (99.8)
No	1 (0.3)	0	1 (0.2)	1 (0.4)	0	1 (0.2)
Age started tooth brushing (n=1144)						
Before 24 months	116 (37.1)	98 (33.3)	214 (35.3)	65 (26.3)	88 (29.2)	153 (27.9)
After 24 months	197 (62.9)	196 (66.7)	393 (64.7)	181 (73.3)	213 (70.8)	394 (71.9)
Age when toothbrushing with toothpaste started (n=1151)						
Before 24 months	97 (31.0)	88 (29.9)	185 (30.5)	58 (23.5)	67 (22.3)	125 (22.8)
Between 24-48 months	147 (47.0)	138 (46.9)	285 (47.0)	98 (39.7)	134 (44.5)	232 (42.3)
After 48 months	64 (20.4)	66 (22.4)	130 (21.4)	88 (35.6)	100 (33.2)	188 (34.3)
Does not use toothpaste	3 (1.0)	2 (0.7)	5 (0.8)	1 (0.4)	0	1 (0.2)

Sums may not total 1155 due to missing response variables

5.2.5 Oral hygiene habits when were aged less than six years-old

Parents were asked about their child's oral hygiene habits using the same questions at two time periods. The first period was when their child was aged less than six years-old. These oral hygiene variables were potential contributing factors to the development of dental fluorosis. The second period was their child oral hygiene habits at the time the questionnaire was distributed (in 2015). These variables were potential factors associated with caries prevention. Descriptive analysis of oral hygiene habits were described in this Section 5.2.5 and also the following Section 5.2.6. The test of association between the outcome measure and oral hygiene variables were described further in bivariate analyes (Section 5.3.2.4).

Table 5.11 shows descriptive analysis on participants' oral hygiene habits when they were aged less than six years old. More than half of the children in the fluoridated area were reported as having their teeth brushed twice a day. In comparison 35.6% to 45.5% of children in the non-fluoridated area reported toothbrushing frequency twice a day among both age groups respectively. Similar patterns of parental supervision in toothbrushing activity were reported across birth cohorts and residency area. More than half of parents reported daily tooth brushing supervision, with a slightly higher proportion among parents in the fluoridated area.

A similar distribution of after toothbrushing routine was observed among both age groups and residency area. Only a small proportion practiced the recommended routine (spit after toothbrushing). The majority rinsed and spat after toothbrushing. About half of the respondents reported that they sometimes had habits of eating/licking toothpaste. About 39.1% to 41.5% of the respondents used a moderate amount of toothpaste when brushing followed by pea size, smear size and large size. The pattern of such practice was similar across age groups and residency areas. Over 70% of respondents reported using children’s fluoridated toothpaste for toothbrushing in their early life. About 10-17% reported using non-fluoridated toothpaste, the highest proportion being 17.3% in the younger age cohort in the fluoridated area.

Table 5.11 Oral hygiene habits at age less than six years old among study participants

	Oral hygiene habits at age less than 6 years old					
	Fluoridated (F) n (%)			Non-fluoridated (NF) n (%)		
	9 (n=313)	12 (n=294)	Total F (n=607)	9 (n=247)	12 (n=301)	Total NF (n=548)
Frequency of toothbrushing (n=1149)						
Less than once a day	13 (4.2)	7 (2.4)	20 (3.3)	23 (9.3)	16 (5.3)	39 (7.1)
Once a day	111 (35.5)	96 (32.7)	207 (34.1)	112 (45.3)	113 (37.5)	225 (41.1)
Twice a day	166 (53.0)	163 (55.4)	329 (54.2)	88 (35.6)	137 (45.5)	225 (41.1)
More than twice a day	22 (7.0)	26 (8.8)	48 (7.9)	22 (8.9)	34 (11.3)	56 (10.2)
Frequency of supervise toothbrushing (n=1148)						
Everyday	192 (61.3)	195 (66.3)	387 (63.8)	132 (53.4)	178 (59.1)	310 (56.6)
Sometimes	109 (34.8)	89 (30.3)	198 (32.6)	92 (37.2)	100 (33.2)	192 (35.0)
Never	2 (0.6)	5 (1.7)	7 (1.2)	6 (2.4)	7 (2.3)	13 (2.4)
Not sure	8 (2.6)	3 (1.0)	11 (1.8)	15 (6.1)	15 (5.0)	30 (5.5)
After brushing routine (n=1148)						
Just swallow	5 (1.6)	3 (1.0)	8 (1.3)	8 (3.2)	5 (1.7)	13 (2.4)
Rinse and swallow	15 (4.8)	11 (3.7)	26 (4.3)	10 (4.0)	11 (3.7)	21 (3.8)
Rinse and spit	273 (87.2)	263 (89.5)	536 (88.3)	210 (85.0)	261 (86.7)	471 (85.9)
Just spit	16 (5.1)	12 (4.1)	28 (4.6)	15 (6.1)	20 (6.6)	35 (6.4)
Don’t know	2 (0.6)	3 (1.0)	5 (0.8)	2 (0.8)	3 (1.0)	5 (0.9)
Eating/licking toothpaste (n=1146)						
Often	19 (6.1)	16 (5.4)	35 (5.8)	15 (6.1)	22 (7.3)	37 (6.8)
Sometimes	165 (52.7)	152 (51.7)	317 (52.2)	129 (52.2)	143 (47.5)	272 (49.6)
Never	126 (40.3)	123 (41.8)	249 (41.0)	101 (40.9)	135 (44.9)	236 (43.1)
Amount of toothpaste used when brushing (n=1146)						
Smear	65 (20.8)	44 (15.0)	109 (18.0)	58 (23.5)	47 (15.6)	105 (19.2)
Pea size	82 (26.2)	64 (21.8)	146 (24.1)	64 (25.9)	87 (28.9)	151 (27.6)
Moderate	120 (38.3)	132 (44.9)	252 (41.5)	94 (38.1)	120 (39.9)	214 (39.1)
Large (all bristles)	44 (14.1)	51 (17.3)	95 (15.7)	29 (11.7)	45 (15.0)	74 (13.5)
Type of toothpaste used when brushing (n=1147)						
Fluoridated adult toothpaste	22 (7.0)	29 (9.9)	51 (8.4)	18 (7.3)	40 (13.3)	58 (10.6)
Fluoridated children toothpaste	231 (73.8)	227 (77.2)	458 (75.5)	193 (78.1)	223 (74.1)	416 (75.9)
Non-fluoridated toothpaste	54 (17.3)	30 (10.2)	84 (13.8)	28 (11.3)	29 (9.6)	57 (10.4)
Don’t know	3 (1.0)	6 (2.0)	9 (1.5)	6 (2.4)	8 (2.7)	14 (2.6)

Sums may not total 1155 due to missing response variables

5.2.6 Oral hygiene habits at the time of study (in year 2015) among study participants

Table 5.12 shows oral hygiene habits at the time of study in 2015. About 49% of children in the non-fluoridated area and 60% of children in the fluoridated area reported that they brushed their teeth twice a day.

Over 90% of children were reported as rinsing and spitting after toothbrushing. Only a small proportion (2%) reported practiced the recommended routine, which to spit after toothbrushing. The majority of them reported that they did not have a habit of eating/licking tooothpaste. About 54.4% to 58.1% of 12 year-old children in both areas were more likely to use a large amount of toothpaste in comparison to children in younger age group who were more likely to use a moderate amount of toothpaste (49.4% to 50.8%).

The majority of children in both areas were more likely to use fluoridated adult toothpaste in comparison to fluoridated children’s toothpaste.

Table 5.12 Oral hygiene habits at the time of study (in 2015) among study participants

	Oral hygiene habits at the time of study (in 2015)					
	Fluoridated (F) **n (%)**			**Non-fluoridated (NF)** **n (%)**		
	9 (n=313)	**12 (n=294)**	**Total F (n=607)**	**9 (n=247)**	**12 (n=301)**	**Total NF (n=548)**
Frequency of toothbrushing (n=1149)						
Less than once a day	4 (1.3)	3 (1.0)	7 (1.2)	7 (2.8)	2 (0.7)	9 (1.6)
Once a day	57 (18.2)	41 (13.9)	98 (16.1)	60 (24.3)	43 (14.3)	103 (18.8)
Twice a day	192 (61.3)	171 (58.2)	363 (59.8)	120 (48.6)	146 (48.5)	266 (48.5)
More than twice a day	57 (18.2)	77 (26.2)	134 (22.1)	59 (23.9)	110 (36.5)	169 (30.8)
Frequency of supervised toothbrushing (n=1149)						
Everyday	152 (48.6)	119 (40.5)	271 (44.6)	107 (43.3)	102 (33.9)	209 (38.1)
Sometimes	140 (44.7)	130 (44.2)	270 (44.5)	120 (48.6)	128 (42.5)	248 (45.3)
Never	11 (3.5)	33 (11.2)	44 (7.2)	11 (4.5)	49 (16.3)	60 (10.9)
Not sure	7 (2.2)	10 (3.4)	17 (2.8)	8 (3.2)	22 (7.3)	30 (5.5)
After brushing routine (n=1148)						
Just swallow	1 (0.3)	0	1 (0.2)	0	1 (0.3)	1 (0.2)
Rinse and swallow	3 (1.0)	1 (0.3)	4 (0.7)	4 (1.6)	3 (1.0)	7 (1.3)
Rinse and spit	301 (96.2)	283 (96.3)	584 (96.2)	235 (95.1)	284 (94.4)	519 (94.7)
Just spit	4 (1.3)	7 (2.4)	11 (1.8)	4 (1.6)	6 (2.0)	10 (1.8)
Don’t know	1 (0.3)	1 (0.3)	2 (0.3)	2 (0.8)	7 (2.3)	9 (1.6)
Eating/licking toothpaste (n=1146)						
Often	3 (1.0)	3 (1.0)	6 (1.0)	2 (0.8)	6 (2.0)	8 (1.5)
Sometimes	55 (17.6)	28 (9.5)	83 (13.7)	31 (12.6)	27 (9.0)	58 (10.6)
Never	250 (79.9)	260 (88.4)	510 (84.0)	213 (86.2)	268 (89.0)	481 (87.8)
Amount of toothpaste used when brushing (n=1144)						
Smear	6 (1.9)	4 (1.4)	10 (1.6)	3 (1.2)	3 (1.0)	6 (1.1)
Pea size	19 (6.1)	9 (3.1)	28 (4.6)	27 (10.9)	12 (4.0)	39 (7.1)
Moderate	159 (50.8)	119 (40.5)	278 (45.8)	122 (49.4)	110 (36.5)	232 (42.3)
Large (all bristles)	122 (39.0)	160 (54.4)	282 (46.5)	94 (38.1)	175 (58.1)	269 (49.1)
Type of toothpaste used when brushing (n=1148)						
Fluoridated adult toothpaste	175 (55.9)	256 (87.1)	431 (71.0)	145 (58.7)	254 (84.4)	399 (72.8)
Fluoridated children toothpaste	113 (36.1)	22 (7.5)	135 (22.2)	87 (35.2)	32 (10.6)	119 (21.7)
Non-fluoridated toothpaste	17 (5.4)	11 (3.7)	28 (4.6)	9 (3.6)	10 (3.3)	19 (3.5)
Don’t know	4 (1.3)	3 (1.0)	7 (1.2)	5 (2.0)	5 (1.7)	10 (1.8)

Sums may not total 1155 due to missing response variables

5.2.7 Exposure to fluoride varnish/gel

Parents were asked whether their children were exposed to fluoride varnish/gel by a health professional before age six (Table 5.13). Only a small proportion of children were reported as having received fluoride varnish/gel with a slightly higher percentage among the 9 year-old children in the non-fluoridated area (17%). Almost one third of the parents reported they didn't know whether their children had such exposure.

Table 5.13 Exposure to fluoride varnish/gel before age six, among study participants

	Fluoridated (F) n(%)			**Non-fluoridated (NF) n (%)**		
	9 (n=313)	**12 (n=294)**	**Total F (n=607)**	**9 (n=247)**	**12 (n=301)**	**Total NF (n=548)**
Exposure to fluoride varnish (n=1154)						
Yes	35 (11.2)	38 (12.9)	73 (12.0)	42 (17.0)	39 (13.0)	81 (14.8)
No	202 (64.5)	168 (57.1)	370 (61.0)	134 (54.3)	156 (51.8)	290 (52.9)
Don't know	76 (24.3)	87 (29.6)	163 (26.9)	71 (28.7)	106 (35.2)	177 (32.3)

Sums may not total 1155 due to missing response variables

5.3 Dental fluorosis

The prevalence of dental fluorosis based on the percentage of children having fluorosis on their maxillary central incisors was determined. This was recorded by consensus scoring of digital photographs by three examiners (Section 4.6.7.2). Fluorosis cases were defined by two cut off points. Dean's score equals "questionable or greater" is reported as any fluorosis (Dean's>0). Dean's score of very mild or greater" is reported as fluorosis (Dean's ≥ 2). The fluorosis (Dean's ≥ 2) case definition was used for bivariate analysis and multivariate logistic regression.

When testing for association between independent variables and fluorosis, data were combined for both age groups in both areas. Separate bivariate analysis between independent variables and fluorosis was also performed for each area, however limited difference was observed. These data are presented in Appendix 32 and are not reported in the main results section.

5.3.1 The prevalence and severity of dental fluorosis among study participants

Table 5.14 shows the distribution of dental fluorosis in fluoridated and non-fluoridated areas. A clear difference in the proportion of children affected between the fluoridated and non-fluoridated communities is apparent. In the fluoridated area, the most common type of fluorosis severity was "very mild" followed by mild, moderate and questionable categories for both age groups. In the non-fluoridated area, the most common level of fluorosis severity was "very mild" for the 9 year-old and "questionable" for the 12 year-old. None of the participants had severe fluorosis.

Table 5.14 Fluorosis distribution among study participants based on the consensus photographic score on maxillary central incisors

	Fluoridated n (%)			**Non-fluoridated n (%)**		
Fluorosis Dean's Score	**12**	**9**	**Total**	**12**	**9**	**Total**
(0) Normal	161 (54.8)	181 (57.8)	342 (56.3)	271 (89.7)	224 (90.7)	494 (90.1)
(1) Questionable	18 (6.1)	23 (7.3)	41 (6.8)	17 (5.6)	6 (2.4)	23 (4.2)
(2) Very mild	48 (16.3)	47 (15.0)	95 (15.7)	10 (3.3)	13 (5.3)	23 (4.2)
(3) Mild	33 (11.2)	32 (10.2)	65 (10.7)	3 (1.0)	2 (0.8)	5 (0.9)
(4) Moderate	32 (10.9)	21 (6.7)	53 (8.7)	1 (0.3)	1 (0.4)	2 (0.4)
(5) Severe	0	0	0	0	0	0
Not able to score[a]	2 (0.7)	9 (2.9)	11 (1.8)	0	1 (0.4)	1 (0.2)
Total	294 (100)	313 (100)	607 (100)	301 (100)	247 (100)	548 (100)

[a] 'Not able to score' photos were excluded from further analysis

Table 5.15 shows the prevalence of fluorosis defined by Dean's>0 and Dean's≥ 2 in fluoridated and non-fluoridated areas. When both age groups were combined, fluorosis prevalence was significantly higher in fluoridated area than non-fluoridated area (p<0.001). Similar results were observed for both fluorosis case definitions.

Table 5.15 The prevalence of fluorosis by area of residence

Area	**Any fluorosis (Dean's > 0) n (%)**	**Normal (Dean's=0) n (%)**	**p value[a]**	**Fluorosis (Dean's ≥ 2) n (%)**	**Normal Dean's=0) n (%)**	**p value[a]**
Fluoridated	254 (42.6)	342 (56.3)	**0.001**	213 (35.7)	383 (64.3)	**0.001**
Non-fluoridated	53 (9.7)	494 (90.3)		30 (5.5)	519 (94.5)	

[a] Chi square analysis between children living in different areas.

Table 5.16 shows the prevalence of fluorosis by fluoridation status and age group. In the fluoridated area, regardless of which outcome measure is used (Deans>0, Deans≥2), the prevalence of fluorosis was higher among the 12 year-old cohort (38.4% to 44.6%) compared to the 9 year-old cohort (31.9% to 39.3%). However, the difference was not statistically significant. In the non-fluoridated area, fluorosis prevalence was higher in the older age group when case was defined by any fluorosis but the difference was not statistically significant.

Table 5.16 The prevalence of fluorosis by age group and area of residence

	Any fluorosis (Dean's > 0) n (%)		**p value[a]**	**Fluorosis (Dean's ≥ 2) n (%)**		**p value[a]**
	12 Born 2003	9 Born 2006		12 Born 2003	9 Born 2006	
Fluoridated[b]	131 (44.6)	123 (39.3)	0.277	113 (38.4)	100 (31.9)	0.139
Non-fluoridated	3 (10.3)	22 (8.9)	0.594	14 (4.7)	16 (6.5)	0.344

[a] Chi square analysis between 9 and 12 year old children living in the same area.

[b] The 12 year-old cohort were exposed to 0.7ppmF in the first two years of life and 0.5ppmF lifetime. The 9 year-old cohort were exposed to 0.5ppmF lifetime.

5.3.2 The relationship between risk factors and dental fluorosis

Bivariate analysis was conducted to assess risk factors (as described in earlier Sections 4.7.2.2) and dental fluorosis (Deans≥2). A relationship between a change in fluoride concentration in the water and fluorosis is described first, followed by association between other risk factors and fluorosis. When reporting results from bivariate analysis on fluorosis outcome, the other risk factors were divided into four sub-sections (demographic characteristics, infant feeding practices oral hygiene practices at aged less than six years-old, exposure to fluoride varnish/gel). Independent variables were di or trichotomised prior to bivariate analysis as described in Section 4.7.2.1 Significant variables from the bivariate analysis were subsequently used in the multivariate analysis as described in Section 4.7.2.2.

5.3.2.1 Relationship between a change in the concentration of fluoride in the water supply and the prevalence of fluorosis

Results in this section aim to answer the primary research question of this work, whether a change in fluoride level of the public water supply has an impact on the prevalence of dental fluorosis.

This study was a single point study that compared children in two age groups that were exposed to different fluoride levels, where a change in the fluoride level occurred during the period of enamel development. A non-fluoridated area was used as a control group. The ‘baseline’ prevalence data were extracted from the groups that were exposed to the old fluoride concentration and the ‘after’ prevalence data were extracted from the group that were exposed to the new fluoride level after reduction occurred. Both definitions of fluorosis prevalence (Deans>0, Deans≥2) were analysed.

Table 5.17 shows that any fluorosis prevalence decreased following reduction of fluoride level in the water in fluoridated and non-fluoridated area. Reducing fluoride level in the water has resulted in a narrowing of the any fluorosis prevalence between fluoridated and control areas. This implies that the decrease in fluorosis prevalence corresponds with the reduction (0.2 ppm) of fluoride in the drinking water during the time of enamel development.

Table 5.17 Proportion of any fluorosis prevalence (Deans>0) after fluoride concentration in the water supply was reduced

	% prevalence 12 year-old (Pre_reduction)	**% prevalence 9 year-old (Post_reduction)**	**% difference (post-pre)[#]**	**% difference (pre)**	**% difference (post)**
Outcome: Any fluorosis (Deans>0)					
Fluoridated	44.6	39.3	-5.3	34.3	30.4
Non-fluoridated (control)	10.3	8.9	-1.4		

[#] Percentage (%) difference= (PostReduction - PreReduction). A negative difference shows that the % fluorosis prevalence decreased after reduction of fluoride level in the water.
Percentage (%) difference (pre)=$\text{PreReduction}_{\text{Intervention}}$ - $\text{PreReduction}_{\text{Control.}}$
Percentage (%) difference (post) =$\text{PostReduction}_{\text{Intervention}}$ – $\text{PostReduction}_{\text{Control.}}$

A similar trend was observed when fluorosis case was defined by (Deans≥2). Accounting for the change between intervention and control groups at baseline and follow up, the magnitude of the percentage difference was larger pre-reduction than the post-reduction group (Table 5.18). This implies a beneficial effect of lowering the fluoride level in the water in reducing fluorosis prevalence.

Table 5.18 Proportion of fluorosis prevalence (Deans≥2) after fluoride concentration in the water supply was reduced

	% prevalence 12 year-old (Pre_reduction)	**% prevalence 9 year-old (Post_reduction)**	**% difference (post-pre)[#]**	**% difference (pre)**	**% difference (post)**
Outcome: Fluorosis (Deans≥2)					
Fluoridated	38.4	31.9	-6.5	33.7	25.4
Non-fluoridated (control)	4.7	6.5	1.8		

[#] Percentage (%) difference= (PostReduction - PreReduction). A negative difference shows that the % fluorosis prevalence decreased after reduction of fluoride level in the water.
Percentage (%) difference (pre)=$\text{PreReduction}_{\text{Intervention}}$ - $\text{PreReduction}_{\text{Control.}}$
Percentage (%) difference (post) =$\text{PostReduction}_{\text{Intervention}}$ – $\text{PostReduction}_{\text{Control.}}$

Table 5.19 shows the bivariate analysis between the prevalence of fluorosis and different fluoride exposures from the water in the study participants. For both outcome measures, children who were exposed to 0.7 ppmF in the first two years of life and then 0.5 ppmF thereafter were 8 to 11 times more likely to develop fluorosis than those who did not have any exposure. Those who had been exposed to 0.5 ppmF in the local water supply throughout life were 6 to 8 times more likely to have in fluorosis compared to the non-fluoridated reference group. Among those living in the fluoridated area, children who had been exposed to 0.7 ppmF in the first two years of life and then 0.5 ppmF thereafter had a higher fluorosis prevalence than those exposed to 0.5 ppmF throughout life but the difference was not statistically significant.

Table 5.19 Bivariate analysis of fluorosis prevalence with fluoride exposure from the water in the study participants

Exposure to fluoride in the water supply	**Fluorosis Deans≥ 2 n (%)**		**Unadjusted Odds ratio 95% CI**	**p value**	**Any fluorosis Deans>0 n (%)**		**Unadjusted Odds ratio 95% CI**	**p value**
	Yes	**No**			**Yes**	**No**		
0 lifetime	30 (12.30	517 (57.4)	Ref		53 (9.7)	494 (90.3)	Ref	
0.5ppmF lifetime	100 (41.2)	204 (22.7)	8.45 (5.45-13.10)	**0.001**	123 (40.5)	181 (59.5)	6.33 (4.40-9.12)	**0.001**
0.7ppmF for first 2 years and then 0.5ppmF	113 (46.5)	179 (19.9)	10.88 (7.03-16.84)	**0.001**	131 (44.9)	161 (55.1)	7.58 (5.26-10.93)	**0.001**

Ref: reference group

5.3.2.2 Relationship between the prevalence of fluorosis and demographic characteristics of study participants

Table 5.20 presents a bivariate analysis of the prevalence of fluorosis and the demographic characteristics of the study participants. Girls had a marginally higher prevalence of fluorosis (22.2%) compared to boys (20%), however the difference was not statistically significant. Children whose parents had only primary school education or lower had significantly lower fluorosis prevalence than those whose parents had a college/university education. Children whose parents had a low monthly income had significantly lower fluorosis prevalence than those whose parents had high monthly income.

Table 5.20 Bivariate analysis of fluorosis prevalence and demographic characteristics of study participants

Variables	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
	Yes	No		
Gender				
Boys	98 (20.0)	393 (80.0)	Ref	
Girls	145 (22.2)	507 (77.8)	1.15 (0.86-1.53)	0.351
Age				
12 year-old	127 (21.4)	466 (78.6)	Ref	
9 year-old	116 (21.1)	434 (78.9)	0.98 (0.74-1.30)	0.893
Father Education				
College/University	60 (24.0)	190 (76.0)	Ref	
High school	151 (21.7)	546 (78.3)	0.88 (0.62-1.23)	0.447
≤Primary school	12 (11.8)	90 (88.2)	0.42 (0.22-0.82)	**0.011**
Mother Education				
College/University	68 (23.4)	223 (76.6)	Ref	
High school	145 (21.3)	535 (78.7)	0.49 (0.64-1.23)	0.481
≤Primary school	14 (12.8)	95 (87.2)	0.48 (0.26-0.90)	**0.022**
Father monthly income				
≥ MYR 4000	88 (27.2)	235 (72.8)	Ref	
MYR 1000-3999	131 (18.7)	569 (81.3)	0.62 (0.45-0.84)	**0.002**
<MYR 1000	2 (7.4)	25 (92.6)	0.21 (0.05-0.92)	**0.038**
Mother monthly income				
≥ MYR 4000	61 (29.0)	149 (71.0)	Ref	
MYR 1000-3999	60 (22.1)	212 (77.9)	0.69 (0.46-1.05)	0.080
<MYR 1000	104 (17.1)	504 (82.9)	0.50 (0.35-0.73)	**0.000**

Ref: reference group

5.3.2.3 Relationship between the prevalence of fluorosis and infant feeding practices

Table 5.21 shows the bivariate association between the prevalence of fluorosis and infant feeding practices. Children who used infant formula were 2.93 times more likely to have fluorosis (p<0.001). Children who started formula at an earlier age (12 months or less), finished formula at a later age (after 48 months) and had longer duration of formula use (more than 48 months) were significantly associated with a higher fluorosis prevalence. Children who were breast-fed only had a significantly lower fluorosis prevalence than those who used formula only. Infant formula reconstituted with tap water or filtered tap water were significantly associated with high fluorosis prevalence compared to those who used non-tap water (bottle and other sources). In terms of type of water used to prepare infant formula, further inspection on separate bivariate analysis between this independent variables and fluorosis for each area were performed. Data were presented in Appendix 32. Results shows that residents in fluoridated area who prepared infant formula with tap water or filtered tap water had significantly higher fluorosis prevalence compared to those who use non-tap water. No statistical significant difference observed among residents in non-fluoridated area.

Table 5.21 Bivariate analysis of fluorosis prevalence and infant feeding practices in the study participants

Variables (Infant feeding practices)	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
	Yes	No		
Use of infant formula				
No	32 (10.4)	277 (89.6)	Ref	
Yes	210 (25.3)	621 (74.7)	2.93 (1.97-4.36)	< **0.001**
Breast feeding				
No	8 (29.6)	19 (70.4)	Ref	
Yes	235 (21.1)	881 (78.9)	0.63 (0.27-1.47)	0.286
Age finished breast feeding				
>12 months	104 (15.3)	574 (84.7)	Ref	
≤12 months	132 (29.9)	309 (70.1)	2.36 (1.76-3.16)	< **0.001**
Age started formula				
>12 months	70 (20.8)	267 (79.2)	Ref	
≤12 months	145 (28.8)	359 (71.2)	1.54 (1.11-2.14)	**0.009**
Age finished formula				
>48 months	125 (29.2)	303 (70.8)	Ref	
≤48 months	89 (21.8)	319 (78.2)	0.68 (0.49-0.93)	**0.014**
Type of water used to prepare formula				
Bottled water	3 (9.4)	29 (90.6)	Ref	
Tap water	162 (25.7)	469 (74.3)	3.34 (1.0-11.11)	**0.049**
Filtered tap water	47 (28.1)	120 (71.9)	3.79 (1.1-13.03)	**0.035**
Duration of formula use				
>48 months	85 (32.8)	174 (67.2)	Ref	
≤48 months	125 (22.0)	443 (78.0)	0.58 (0.42-0.80)	**0.001**
Feeding method				
Formula only	8 (29.6)	19 (70.4)	Ref	
Combine breast & formula	202 (25.1)	602 (74.9)	0.80 (0.34-1.85)	0.597
Breast only	32 (10.4)	277 (89.6)	0.27 (0.11-0.68)	**0.005**

Ref: reference group

5.3.2.4 Relationship between the prevalence of fluorosis and oral hygiene habits at age less than six years

The association between fluorosis prevalence and oral hygiene habits at age less than six years were chosen (over oral hygiene habits at the time of study) based on the exposure to fluoride during developmental stages of central incisors. There was some variation in terms of early childhood oral hygiene practices with fluorosis status, however the associations were not statistically significant (Table 5.22).

Table 5.22 Bivariate analysis of fluorosis prevalence and oral hygiene habits at age less than six years among study participants

Variables (Oral hygiene habits at age less than 6 years)	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
	Yes	No		
Frequency of toothbrushing				
Twice/day or more	138 (21.1)	516 (78.9)	Ref	
Once /day or less	104 (21.5)	379 (78.5)	1.03 (0.77-1.37)	0.861
Supervised toothbrushing				
Never	4 (20.0)	16 (80.0)	Ref	
Yes	234 (21.8)	841 (78.2)	1.11 (0.37-3.36)	0.849
Habits after toothbrushing				
Spat	227 (21.5)	831 (78.5)	Ref	
Swallowed	13 (19.1)	55 (80.9)	0.87 (0.47-1.61)	0.648
Eating/ licking toothpaste				
Never	110 (22.8)	372 (77.2)	Ref	
Yes	131 (20.1)	521 (79.9)	0.85 (0.64-1.13)	0.267
Amount of toothpaste used				
Medium to large	134 (21.2)	497 (78.8)	Ref	
Small	107 (21.3)	396 (78.7)	1.00 (0.75-1.33)	0.988
Type of toothpaste used				
Non-fluoridated toothpaste	28 (20.1)	111 (79.9)	Ref	
Fluoridated toothpaste	210 (21.6)	763 (78.4)	1.09 (0.70-1.70)	0.700
Age started toothbrushing				
After 2 years	161 (20.7)	618 (79.3)	Ref	
Before 2 years	82 (22.6)	281 (77.4)	1.12 (0.83-1.51)	0.460
Age started toothbrushing with toothpaste				
After 2 years	172 (20.7)	657 (79.3)	Ref	
Before 2 years	68 (22.3)	237 (77.7)	1.10 (0.80-1.51)	0.572

Ref: reference group

5.3.2.5 Relationship between the prevalence of fluorosis and exposure to fluoride varnish/gel

Table 5.23 shows the bivariate association between the prevalence of fluorosis and exposure to fluoride varnish or gel before age six years old. Results indicated that those who did not receive fluoride varnish/gel had slightly higher fluorosis. However the difference was not significant.

Table 5.23 Bivariate analysis of fluorosis prevalence and exposure to fluoride gel/varnish among study participants

Variable	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
	Yes	No		
Exposure to fluoride varnish/gel				
No	147 (22.6)	503 (77.4)	Ref	
Yes	28 (18.2)	126 (81.8)	0.76 (0.49-1.19)	0.231

Ref: reference group

5.3.3 Multivariate logistic regression models for having fluorosis (Deans≥2)

Binary logistic regression model using the Enter method was generated for the prevalence of fluorosis defined by Deans score ≥ 2. Significant variables from the bivariate analysis were entered into the model as a block. Results were presented as adjusted odds ratios and 95% confidence intervals.

Table 5.24 shows the multivariate logistic regression model for the prevalence of fluorosis. Father's and mother's education level, father's and mother's monthly income, fluoride exposure from the water, use of infant formula, age finished breast feeding, age started formula, age finished formula, type of water used to reconstitute formula, duration of formula use and type of feeding method were contributing factors to the model.

After controlling other factors in the model, exposure to fluoride from the water and type of water used to reconstitute the infant formula remained significantly associated with having higher fluorosis prevalence. Children who had been exposed to fluoridated water had 6 to 9 times the prevalence of dental fluorosis compared to those who did not have any exposure. Children who had exposed to (0.7ppmF in the first two years of life then 0.5ppmF lifetime) had higher odds of having fluorosis than those who had exposed to lower fluoride level (0.5ppmF) throughout life. Infant formula reconstituted with tap water or filtered tap water had 8.78 to 9.90 times the prevalence of fluorosis compared to those who used non-tap water. Other factors were not significantly associated with fluorosis in the model.

Table 5.24 Multivariate logistic regression model for having fluorosis (Deans≥2)

Explanatory variable	Adjusted Odds ratio (95% CI)	p value
Fluoride level in the water		
0 lifetime	Ref	
0.5 ppmF lifetime	5.97 (3.32-10.72)	**0.000**
0.7 ppmF in the first two years of life, then 0.5 ppmF	9.12 (5.15-16.14)	**0.000**
Father education		
College/ University	Ref	
High school	0.85 (0.50-1.43)	0.532
≤Primary school	0.74 (0.27-2.04)	0.565
Mother Education		
College/ University	Ref	
High school	1.44 (0.83-2.53)	0.198
≤Primary school	1.09 (0.37-3.19)	0.872
Father income		
≥ RM 4000	Ref	
RM1000-3999	0.93 (0.57-1.51)	0.766
<RM 1000	0.29 (0.06-1.54)	0.147
Mother income		
≥ RM 4000	Ref	
RM1000-3999	0.91 (0.48-1.71)	0.763
<RM 1000	0.84 (0.47-1.51)	0.558
Infant formula		
No	Ref	
Yes	0.68 (0.02-23.14)	0.831
Age finished breast feeding		
>12 months	Ref	
≤12 months	1.40 (0.85-2.32)	0.188
Age started formula		
>12 months	Ref	
≤12 months	1.10 (0.63-1.92)	0.726
Age finished formula		
>48 months	Ref	
≤48 months	1.00 (0.57-1.75)	0.998
Type of water used to prepare formula		
Bottled water	Ref	
Tap water	9.90 (1.28-76.38)	**0.028**
Filtered tap water	8.78 (1.11-69.71)	**0.040**
Duration of formula use		
>48 months	Ref	
≤48 months	0.98 (0.54-1.78)	0.955
Feeding method		
Formula only	Ref	
Combine breast & formula	0.26 (0.01-5.32)	0.378
Breast only	-	-

Ref: reference group

5.4 Dental Caries

Caries experience was analysed at tooth level and surface level for both primary and permanent dentitions. The results were also analysed by age group and area of residence. Mean caries experience was calculated at three different ICDAS cut-off points; (d/D_{1-3}) for enamel caries, (d/D_{4-6}) for dentine caries, and (d/D_{1-6}) caries at all levels (that is both enamel and dentine caries). A comparison of the ICDAS scoring system with the conventional caries index is shown in Appendix 2. The caries experience of the permanent dentition at dentine level (D_{4-6}MFT) and caries at all levels (D_{1-6}MFT) were used for bivariate and multivariate logistic regression.

Caries results are reported in the following sections as caries prevalence, followed by bivariate and multivariate analyses between risk factors and dental caries.

5.4.1 Prevalence and severity of dental caries in study participants

This section addresses the prevalence and severity of dental caries among study participants. Results for caries experience are presented based on different level of caries severity by age group and area of residence. Prevalence of fissure sealants is also described in the subsequent section (5.4.2.3).

The use of ICDAS as a caries index allowed comparison of enamel and dentine caries between areas. To ease interpretation between enamel and dentine caries, a ratio calculation was also used. The ratio between enamel and dentine caries was calculated by dividing the mean enamel caries (d/D_{1-3}) by the mean dentine caries (d/D_{4-6}) scores.

5.4.1.1 Mean caries experience in the permanent dentition

Mean caries experience in the permanent dentition at tooth level

At tooth level, regardless of which level of diagnosis is used, the mean caries experience in the permanent dentition was significantly lower in the fluoridated area than the non-fluoridated area for both age groups ($p<0.05$) (Table 5.25). The enamel caries prevalence was higher than the dentine caries prevalence for both age groups and area of residence. When enamel caries lesions were included, the mean DMFT score increased by 2 to 4 times more than when only dentine caries lesions were included among all study participants. The prevalence of filled teeth was three times higher in non-fluoridated areas for both age groups and the differences were significant ($p<0.001$). Missing teeth due to extraction was also higher among children in the non-fluoridated area and the difference was significant in 12 year-old children.

Table 5.25 Mean caries experience of permanent dentition at tooth level and at different severity of caries for 9 and 12 years-old Malaysian children in fluoridated and non-fluoridated areas

Age/ Area	D_{1-3} Mean (SD)	D_{4-6} Mean (SD)	D_{1-6} Mean (SD)	M Mean (SD)	F Mean (SD)	D_{1-3}MFT Mean (SD)	D_{4-6}MFT Mean (SD)	D_{1-6}MFT Mean (SD)
9 year-old cohort								
9 F	0.75	0.22	0.97	0.01	0.17	0.93	0.40	1.15
(n=313)	(1.08)	(0.75)	(1.42)	(0.08)	(0.57)	(1.24)	(0.96)	(1.55)
9 NF	0.71	0.24	0.96	0.03	0.45	1.20	0.73	1.44
(n=247)	(1.10)	(0.63)	(1.37)	(0.25)	(0.88)	(1.46)	(1.17)	(1.70)
p value[a]	0.646	0.319	0.980	0.142	**<0.001**	**0.043**	**<0.001**	**0.021**
12 year-old cohort								
12 F	1.54	0.13	1.67	0	0.34	1.88	0.47	2.01
(n=294)	(1.92)	(0.47)	(2.04)		(0.80)	(2.07)	(0.97)	(2.19)
12 NF	1.52	0.26	1.78	0.02	1.03	2.57	1.31	2.83
(n=301)	(1.62)	(0.70)	(1.90)	(0.16)	(1.52)	(2.47)	(1.81)	(2.74)
p value[a]	0.506	**0.006**	0.175	**0.027**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

[a] Mann Whitney test, association between mean caries by age group and residency area.

F: fluoridated area, NF: non-fluoridated area

D_{1-3}=enamel caries; D_{4-6}=dentine caries; D_{1-6}= caries at all levels of severity.

Table 5.26 shows ratio of enamel to dentine caries at tooth level in permanent dentition by age group and area of residence. In both age groups, the ratio of enamel to dentine caries was higher in the fluoridated than non-fluoridated areas [9 year-old: 3.41 (F) vs 2.96 (NF) and 12-year old: 11.85 (F) vs 5.85 (NF)].

Table 5.26 Ratio of enamel (D_{1-3}) to dentine (D_{4-6}) caries in permanent dentition at tooth level by age group and area of residence

Age/Area	Mean D_{1-3}/ Mean D_{4-6}	Ratio enamel to dentine caries
9 year-old cohort		
9F	0.75/0.22	3.41
9NF	0.71/0.24	2.96
12 year-old cohort		
12F	1.54/0.13	11.85
12NF	1.52/0.26	5.85

Mean caries experience in the permanent dentition at surface level

A similar caries pattern was observed for caries experience in the permanent dentition at surface level as shown in Table 5.27. The mean caries experience was significantly higher in children in the non-fluoridated area than children in the fluoridated area for both age groups (p<0.05). When enamel caries were included, the mean DMFS score increased by 1.75 to 4 times more than when only dentine lesions were included. The prevalence of filled surfaces was higher in the non-fluoridated area for the 12 year-old cohort and the differences were statistically significant (p<0.001). In contrast, the prevalence of filled surfaces was higher in the fluoridated area among the 9 year-old cohort and the differences were statistically significant (p<0.001). In terms of missing teeth due to caries, the prevalence was higher among children in the non-fluoridated area and the difference was significant in 12 year-old cohort.

Table 5.27 Mean caries experience of permanent dentition at surface level and at different degree of caries for 9 and 12 years-old Malaysian children in fluoridated and non-fluoridated areas

Age/ Area	D_{1-3} (SD)	D_{4-6} (SD)	D_{1-6} (SD)	M (SD)	F (SD)	D_{1-3}MFS (SD)	D_{4-6}MFS (SD)	D_{1-6}MFS (SD)
9 year-old cohort								
9F	0.88	0.44	1.32	0.03	0.22	1.13	0.68	1.57
(n=313)	(1.42)	(1.67)	(2.27)	(0.32)	(0.72)	(1.66)	(1.86)	(2.43)
9 NF	0.89	0.43	1.32	0.55	0.17	1.61	1.14	2.03
(n=247)	(1.48)	(1.24)	(2.07)	(1.16)	(1.22)	(2.41)	(2.11)	(2.81)
p value[a]	0.975	0.341	0.805	0.075	**<0.001**	**0.032**	**<0.001**	**0.022**
12 year-old cohort								
12 F	1.90	0.22	2.12	0	0.38	2.28	0.61	2.50
(n=294)	(2.37)	(0.98)	(2.67)		(0.95)	(2.63)	(1.46)	(2.95)
12 NF	1.92	0.31	2.23	0.10	1.37	3.40	1.78	3.70
(n=341)	(2.17)	(0.87)	(2.52)	(0.81)	(2.27)	(3.73)	(2.91)	(4.12)
p value[a]	0.416	**0.007**	0.159	**0.027**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

[a] Mann Whitney test, association between mean caries by age group and residency area.

F: fluoridated area, NF: non-fluoridated area

$D_{1-3=}$enamel caries; $D_{4-6=}$dentine caries; $D_{1-6=}$ caries at all levels of severity.

Table 5.28 shows ratio of enamel to dentine caries at surface level in permanent dentition by age group and area of residence. In the 9 year-old cohort, the ratio of enamel to dentine caries was higher in the non-fluoridated areas [2.00 (F) vs 2.07 (NF)]. However, in the 12 year-old cohort, the ratio enamel to dentine caries was higher in the fluoridated area [8.64 (F) vs 6.20 (NF)].

Table 5.28 Ratio of enamel ($D_{1\text{-}3}$) to dentine ($D_{4\text{-}6}$) caries in permanent dentition at surface level by age group and area of residence

Age/Area	Mean $D_{1\text{-}3}$/ Mean $D_{4\text{-}6}$	Ratio enamel to dentine caries
9 year-old cohort		
9F	0.88/0.44	2.00
9NF	0.89/0.43	2.07
12 year-old cohort		
12F	1.90/0.22	8.64
12NF	1.92/0.31	6.20

5.4.2 Mean caries experience in the primary dentition of study participants

Mean caries experience in the primary dentition at tooth level

Table 5.29 shows the mean caries experience in the primary dentition of study participants. At tooth level, the mean caries experience was lower in the fluoridated area than the non-fluoridated area except for mean caries experience at enamel level ($d_{1\text{-}3}$mft) in 12 year-olds. Although the mean caries experience was higher among children in the non-fluoridated area, the statistical significant association was only observed between mean caries at dentine level for both age groups and mean caries at all levels of severity for the 9 year-old cohort. The number of teeth missing due to caries was 3 times higher among 9 year-old children in the non-fluoridated area than those in the fluoridated area and the difference was significant ($p<0.001$). In both age groups, the mean number of filled teeth was slightly higher among children in the fluoridated area than the non-fluoridated area but the difference was not statistically significant.

Table 5.29 Mean caries experience of primary dentition at tooth level and at different severity of caries for 9 and 12 years-old Malaysian children in fluoridated and non-fluoridated areas

Age/ Area	d_{1-3} (SD)	d_{4-6} (SD)	d_{1-6} (SD)	m (SD)	f (SD)	d_{1-3}mft (SD)	d_{4-6}mft (SD)	d_{1-6}mft (SD)
9 year-old cohort								
9 F	0.92	2.49	3.42	0.24	0.77	1.93	3.50	4.42
(n=313)	(1.26)	(2.69)	(2.81)	(0.72)	(1.17)	(1.78)	(2.99)	(3.06)
9 NF	0.77	4.51	5.29	0.70	0.65	2.12	5.86	6.63
(n=247)	(1.05)	(3.17)	(3.15)	(1.32)	(1.03)	(1.77)	(3.32)	(3.24)
p value[a]	0.338	**<0.001**	**<0.001**	**<0.001**	0.387	0.152	**<0.001**	**<0.001**
12 year-old cohort								
12 F	0.54	0.85	1.40	0	0.19	0.73	1.04	1.58
(n=294)	(0.99)	(1.19)	(1.49)		(0.44)	(1.17)	(1.30)	(1.65)
12 NF	0.42	1.26	1.68	0	0.15	0.56	1.41	1.82
(n=301)	(0.79)	(1.48)	(1.64)		(0.41)	(0.87)	(1.48)	(1.62)
p value[a]	0.585	**0.023**	0.127	-	0.404	0.653	**0.036**	0.126

[a]Mann Whitney test, association between mean caries among children in fluoridated and non fluoridated area.

F: fluoridated area, NF: non-fluoridated area

$d_{1-3=}$enamel caries; $d_{4-6=}$dentine caries; $d_{1-6=}$ caries at all levels of severity

In contrast to the permanent dentition, the ratio of enamel to dentine caries was lower in non-fluoridated than fluoridated area for both age groups [9 year-olds:0.37 (F) vs 0.17 (NF) and 12 year-olds: 0.64 (F) vs 0.33 (NF)]. Results are presented in

Table 5.30.

Table 5.30 Ratio of enamel (d_{1-3}) to dentine (d_{4-6}) caries in primary dentition at tooth level

Age/Area	Mean d_{1-3}/ Mean d_{4-6}	Ratio enamel to dentine caries
9 year-old cohort		
9F	0.92/2.49	0.37
9NF	0.77/4.51	0.17
12 year-old cohort		
12F	0.54/0.85	0.64
12NF	0.42/1.26	0.33

Mean caries experience in the primary dentition at surface level

Table 5.31 shows the mean caries experience in the primary dentition of study participants at surface level. In contrast to the permanent dentition, the dentine caries prevalence was higher than the enamel caries prevalence among children in both age groups and area of residence. Missing surfaces due to caries were higher among 9 year-old children in the non-fluoridated area and the difference was statistically significant (p<0.001).

Table 5.31 Mean caries experience of primary dentition at surface level and at different degree of severity caries for 9 and 12 years-old Malaysian children in fluoridated and non-fluoridated areas

Age/ Area	d_{1-3} (SD)	d_{4-6} (SD)	d_{1-6} (SD)	m (SD)	f (SD)	d_{1-3}mfs (SD)	d_{4-6}mfs (SD)	d_{1-6}mfs (SD)
9 year-old cohort								
9F	1.47	5.45	6.92	1.04	1.06	3.58	7.55	9.02
(n=313)	(1.81)	(7.90)	(7.93)	(3.17)	(1.70)	(4.07)	(8.79)	(8.80)
9 NF	1.32	11.00	12.32	3.32	1.00	5.63	15.32	16.63
(n=247)	(1.51)	(10.55)	(10.46)	(6.28)	(1.48)	(6.16)	(12.55)	(12.34)
p value[a]	0.776	**<0.001**	**<0.001**	**<0.001**	0.916	**<0.001**	**<0.001**	**<0.001**
12 year-old cohort								
12F	0.78	1.67	2.46	0	0.24	1.02	1.91	2.69
(n=294)	(1.44)	(2.99)	(3.21)		(0.55)	(1.67)	(3.15)	(3.41)
12NF	0.58	2.96	3.54	0	0.25	0.83	3.21	3.79
(n=301)	(1.12)	(4.49)	(4.52)		(0.70)	(1.28)	(4.52)	(4.54)
p value[a]	0.859	**0.007**	**0.019**	-	0.831	0.837	**0.006**	**0.013**

[a]Mann Whitney test, association between mean caries among children in fluoridated and non-fluoridated area.

$d_{1-3=}$enamel caries; $d_{4-6=}$dentine caries; $d_{1-6=}$ caries at all levels.

Similar to caries experience at tooth level, the ratio of enamel to dentine caries was lower in the non-fluroidated than the fluoridated area for both age groups [9 year-olds: 0.27 (F) vs 0.12 (NF) and 12 year-olds:0.47 (F) and 0.20 (NF)]. Results are presented in Table 5.32.

Table 5.32 Ratio of enamel (D_{1-3}) to dentine (D_{4-6}) in primary dentition at surface level by age group and area of residence

Age/Area	Mean d_{1-3}/ Mean d_{4-6}	Ratio enamel to dentine caries
9 year-old cohort		
9F	1.47/5.45	0.27
9NF	1.32/11.00	0.12
12 year-old cohort		
12F	0.78/1.67	0.47
12NF	0.58/2.96	0.20

5.4.2.1 Percentage caries prevalence in study participants

In terms of percentage caries prevalence, dentine caries ($D_{4\text{-}6}$MFT>0, $d_{4\text{-}6}$mft>0) and caries at all levels ($D_{1\text{-}6}$MFT>0, $d_{4\text{-}6}$mft>0) were used as the outcome measures and dichotomised into the absence and presence of the disease. Caries prevalence at dentine level or at all levels was significantly higher among children in the non-fluoridated area than those in the fluoridated area (Table 5.33).

Table 5.33 Caries prevalence among 9 and 12 years-old Malaysian children in fluoridated and non-fluoridated areas

	Permanent		**Primary**	
Age/ Area	**$D_{1\text{-}6}$MFT>0 %**	**$D_{4\text{-}6}$MFT>0 %**	**$d_{1\text{-}6}$mft>0 %**	**$d_{4\text{-}6}$mft>0 %**
9 year-old cohort				
9 F (n=313)	54.0	24.6	89.2	79.1
9 NF (n=247)	62.3	40.2	98.3	96.2
p value[a]	**0.047**	**<0.001**	**<0.001**	**<0.001**
12 year-old cohort				
12 F (n=294)	68.7	25.5	66.3	54.5
12 NF (n=301)	82.4	53.5	86.5	70.8
p value[a]	**<0.001**	**<0.001**	**0.001**	**0.018**

[a] Chi Square test, association between caries prevalence by age group and residency area.

F: fluoridated area, NF: non-fluoridated area.

5.4.2.2 Severity of caries lesions among study participants

Table 5.34 shows the prevalence and mean scores of severity of caries lesions in permanent teeth. In general, the number of lesions at dentine level (D_4 to D_6) was low in comparison to lesions at enamel level (D_1 to D_3) for both areas and age groups. For enamel lesions, decay at D_1 was highest followed by decay recorded at the D_2 and D_3 levels. For dentine lesions, the highest number of lesions was at the D_6 level for the 9 year-old cohort in both areas. The pattern was slightly different with the 12 year-old cohort, which the highest number of dentine lesions was at D_4 level for children in the non-fluoridated area, however no clear pattern was observed among those in the fluoridated area.

Table 5.34 Activity of caries lesions of permanent dentition by age groups in fluoridated and non-fluoridated areas at tooth level

Age	D_1 Mean (SD) Prevalence	D_2 Mean (SD) Prevalence	D_3 Mean (SD) Prevalence	D_4 Mean (SD) Prevalence	D_5 Mean (SD) Prevalence	D_6 Mean (SD) Prevalence
9 year-old cohort						
9F	0.46 (0.79)	0.19 (0.53)	0.10 (0.32)	0.03 (0.19)	0.05 (0.30)	0.14 (0.57)
(n=313)	31.3%	14.4%	9.3%	2.6%	3.5%	10.9%
9NF	0.45 (0.83)	0.18 (0.54)	0.08 (0.32)	0.06 (0.43)	0.04 (0.24)	0.14 (0.38)
(n=247)	29.1%	13.0%	6.9%	2.0%	3.6%	13.0%
12 year-old cohort						
12F	0.94 (1.31)	0.43 (0.87)	0.17 (0.56)	0.04 (0.24)	0.05 (0.25)	0.04 (0.22)
(n=294)	51.0%	26.2%	12.6%	3.1%	4.4%	3.7%
12 NF	1.02 (1.26)	0.29 (0.58)	0.20 (0.54)	0.17 (0.58)	0.05 (0.21)	0.04 (0.20)
(n=341)	56.1%	23.3%	15.3%	10.3%	4.7%	4.0%

5.4.2.3 Prevalence of fissure sealants in study participants

In addition to the water fluoridation programme, fissure sealants are one of the caries preventive strategies implemented in Malaysia. Therefore it is important to have an overview of prevalence of fissure sealants in the study population, in particular when discussing potential confounders associated with the results of this study (Section 6.3.3).

Table 5.35 shows the prevalence of fissure sealants in study participants. The frequency of sealants was analysed for sound sealant (ICDAS codes: 10, 20) and combination of sound sealant and sealant with enamel caries (ICDAS codes: 10, 11, 12, 13, 20, 21, 22, 23). There was significantly higher sealant placement among children in the non-fluoridated area than those in the fluoridated area. Similar results were observed for both age groups. The proportion of partial sealants with enamel caries was higher than proportion of complete sealant with enamel caries for both age groups.

Table 5.35 Mean score and percentage of sealed permanent teeth for 9 and 12 years old Malaysian children in fluoridated and non-fluoridate areas

	Sound Sealant (10,20)				Sound sealant & sealant with enamel caries (10, 11, 12, 13, 20, 21, 22, 23)			
	Complete Mean (SD)	%	Partial Mean (SD)	%	Complete Mean (SD)	%	Partial Mean (SD)	%
9 year-old cohort								
9 (F)	0.05 (0.24)	4.2	0.08 (0.33)	7.0	0.05 (0.24)	4.2	0.09 (0.34)	8.0
9 (NF)	0.13 (0.39)	10.9	0.41 (0.69)	32.0	0.13 (0.39)	10.9	0.49 (0.72)	37.2
p value[a]	**0.002**		**<0.001**		**<0.001**		**0.002**	
12 year-old cohort								
12 (F)	0.03 (0.18)	2.4	0.06 (0.27)	5.8	0.03 (0.19)	2.7	0.09 (0.33)	7.5
12 (NF)	0.11 (0.38)	9.3	0.28 (0.58)	22.3	0.12 (0.39)	9.6	0.37 (0.68)	27.6
p value[a]	**<0.001**		**<0.001**		**<0.001**		**<0.001**	

[a] Mann Whitney test, association between mean sealant score by age group and residency area.

5.4.3 The relationship between risk factors and dental caries

Bivariate analysis was conducted to assess risk factors (as described in earlier Sections 4.7.2.2) and dental caries. The relationship between a change in fluoride concentration in the water and caries is described first, followed by the association between other risk factors and caries. When reporting results from bivariate analysis on caries outcome, the other risk factors were divided into four sub-groups (demographic characteristics, infant feeding practices, oral hygiene habits and exposure to fluoride varnish/gel). Independent variables were di or trichotomised prior to bivariate analysis as described in Section 4.7.2.1. Significant variables from the bivariate analysis were subsequently used in the multivariate analysis as described in Section 4.7.2.2. The prevalence of caries at dentine level ($D_{4\text{-}6}$MFT>0) and caries at all levels ($D_{1\text{-}6}$MFT>0) were used for bivariate and binary logistic regression analyses.

When testing for an association between independent variables and caries, data were combined for both age groups in both areas. Separate bivariate analysis between independent variables and caries was also performed for each area, however limited difference was observed. These data are presented in Appendix 34 and are not reported in the main results section.

5.4.3.1 Association between changes of fluoride level in the water supply and dentine caries prevalence

Results in this section aim to answer the primary research question of this work, whether the reduction of fluoride level has maintained the preventive effect on dental caries. Data were analysed using bivariate analysis and multivariate analyses (Section 4.7.2.2).

Table 5.36 shows a bivariate analysis between the prevalence of caries and fluoride exposure from the water supply in the area where the study participants lived. For both caries outcome measures, children in the fluoridated area had a significantly lower caries prevalence than those living in the non-fluoridated area.

Table 5.36 Bivariate analysis between fluoride exposure from water and prevalence of caries at dentine level (D_{4-6}MFT>0) and caries at all levels (D_{1-6}MFT>0) in study participants

Variable	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Exposure to fluoride in the water	D_{4-6}MFT>0 N (%)		Odds ratio 95% CI	p value	D_{1-6}MFT>0 N (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Non-fluoridated	260 (47.4)	288 (52.6)	Ref		402 (73.4)	146 (26.6)	Ref	
Fluoridated	152 (25.0)	455 (75.0)	0.37 (0.29-0.38)	**<0.001**	371 (61.1)	236 (38.9)	0.37 (0.29-0.38)	**<0.001**

Ref: reference group

Multivariate model between change of fluoride level in the water and dental caries

In contrast to fluorosis analysis, direct comparison across birth cohorts was not possible for caries prevalence because of the different dentition present in different age groups. Therefore, two types of multivariate analyses (namely zero-inflated negative binomial and generalised linear model) were performed to explore the relationship between a change in fluoride level of the public water supply and dental caries at dentine level (Section 4.7.2.2). In both models, data were presented by age, fluoridation status and when interaction between age and fluoridation were controlled in the analysis.

Table 5.37 shows the zero-inflated negative binomial for mean caries experience (at dentine level) and generalised linear model regression for percentage caries prevalence (at dentine level) with different fluoridation status and age groups. Model 1 shows that although mean D_{4-6}MFT is lower in the fluoridated than non-fluoridated area, no significant association found between the fluoridated and non-fluoridated area when both age were considered together in the analysis. Similarly, no significant association observed between the two age groups when both areas were considered together in the analysis. After allowing for interaction between age group and fluoridation status, the results show that children who were exposed to fluoride at 0.5 ppm remained significantly associated with lower caries experience than those who did not have any exposure.

Model 2 shows that caries prevalence (D_{4-6}MFT>0) is lower in the fluoridated than non-fluoridated area. Results remained statistically significant between fluoridated and non-fluoridated area when both age group were considered together in the analysis. In terms of age, caries prevalence was significantly lower in the 9 year-old children when both areas were considered together in the analysis. Similar to model 1, after allowing for interaction between age group and fluoridation status, the results show that children who were exposed to the fluoride level (0.5 ppmF throughout life) remained significantly associated with lower caries experience than those who did not have any exposure.

Results from both models indicate that after controlling the interaction between age-fluoridation status, the difference of the differences of caries experience between fluoridated and non-fluoridated areas were statistically significant. This suggest that caries preventive effect is still maintained following the reduction of fluoride level in the water.

Table 5.37 Multivariate regression models for mean caries experience and caries prevalence following reduction of fluoride level in the water

Age group	Outcome measure	Fluoridation status		p value (95% Wald CI)
		Fluoridated^{+}	**Non-fluoridated**	
Zero-inflated negative binomial (Model 1)				
9	$D_{4\text{-}6}$MFT Mean (SD) [Median]	0.40 (0.96) [0.00]	0.73 (1.17) [0.00]	p(area) = 0.339† p(age) = 0.348† p(age×area)=<0.001†
12		0.47 (0.97) [0.00]	1.31 (1.81) [1.00]	
Generalised linear model (Model 2)				
9	$D_{4\text{-}6}$MFT>0 % caries prevalence	24.6%	40.2%	p(area)=<0.001‡ p(age)=0.021‡ p(age×area)=0.054‡
12		25.5%	53.5%	

$^{+}$9 year-old children in fluoridated area were exposed to 0.5 ppmF throughout life, and 12 year-old children in fluoridated area were exposed to 0.7 ppmF in the first two years of life and 0.5 ppmF thereafter.
†Zero-inflated negative binomial
‡ Generalised linear model
p(area): results differ by area (fluoridated and non-fluoridated) when both ages are considered together.
p(age): results differ by age groups when both areas are considered together.
p(age×area): interaction between age and fluoridation status were included together in the analysis.

To decide the best model for this analysis, results from a generalised linear modelling framework for $D_{4\text{-}6}$MFT were compared to results of a zero-inflated negative binomial model. Further inspection using Vuong test (Appendix 33) showed that zero-inflated negative binomial model (Model 1) provided a significantly ($p<0.001$) better model than the standard negative binomial model using generalised liner modelling analysis (Model 2). Although some small amount of over-dispersion in the non-zero $D_{4\text{-}6}$MFT data was also seen, the zero-inflated negative binomial model provided the best model for this data. Marginal medians were predicted correctly for each group by age and area using the zero-inflated negative binomial model.

5.4.3.2 Relationship between the prevalence of caries and demographic characteristics of study participants

Table 5.38 shows the relationship between demographic characteristics and the prevalence of caries at dentine level ($D_{4\text{-}6}$MFT>0) and caries at all levels ($D_{1\text{-}6}$MFT>0) in study participants. Girls had a higher dentine caries score compared to boys and the difference was statistically significant with caries severity at all levels. Children whose parents had only primary school education had a higher caries prevalence than those whose parents had college/university education. Children whose parents had low (<MYR 1000) monthly income also had a higher caries prevalence than those whose parents had high (≥MYR 4000) monthly income. However, the differences were not statistically significant. When looking at individual parent income, children whose father had a moderate monthly income (MYR 1000-3999) had a significantly higher caries prevalence than those whose fathers had a high monthly income. The use of two different caries severity levels (caries into dentine and combined enamel and dentine caries) did not results in major differences in these conclusions.

Table 5.38 Bivariate analysis between demographic characteristics and prevalence of caries at dentine level ($D_{4-6}MFT>0$) and caries at all levels ($D_{1-6}MFT>0$) in study participants

Variables	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Demographic characteristics	$D_{4-6}MFT>0$ N (%)		Odds ratio 95% CI	p value	$D_{1-6}MFT>0$ N (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Gender								
Boys	169 (34.1)	327 (65.9)	Ref		315 (63.5)	181 (36.5)	Ref	
Girls	243 (36.9)	416 (63.1)	1.13 (0.89-1.44)	0.325	458 (69.5)	201 (30.5)	1.31 (1.02-1.68)	**0.032**
Father Education								
College/University	85 (33.5)	169 (66.5)	Ref		173 (68.1)	81 (23.5)	Ref	
High school	248 (35.2)	457 (64.8)	1.08 (0.80-1.46)	0.623	468 (66.4)	237 (33.6)	0.93 (0.68-1.26)	0.616
≤Primary school	44 (43.1)	58 (56.9)	1.51 (0.94-2.42)	0.087	75 (68.8)	27 (33.2)	1.30 (0.78-2.17)	0.315
Mother Education								
College/University	93 (31.2)	205 (68.8)	Ref		198 (66.4)	100 (33.6)	Ref	
High school	254 (37.1)	431 (62.9)	1.30 (0.97-1.74)	0.077	463 (67.6)	222 (32.4)	1.05 (0.79-1.41)	0.724
≤Primary school	45 (41.3)	64 (58.7)	1.55 (0.99-2.44)	0.058	75 (73.5)	34 (35.5)	1.11 (0.70-1.79)	0.653
Father monthly income								
≥ MYR 4000	102 (31.2)	225 (68.8)	Ref		206 (63.0)	121 (37.0)	Ref	
MYR1000-3999	264 (37.3)	443 (62.7)	1.32 (0.99-1.74)	**0.055**	490 (69.3)	217 (30.7)	1.33 (1.01-1.75)	**0.045**
<MYR 1000	10 (37.0)	17 (63.0)	1.30 (0.57-2.93)	0.531	18 (66.7)	9 (33.3)	1.18 (0.51-2.70)	0.704
Mother monthly income								
≥ MYR 4000	76 (35.5)	138 (64.5)	Ref		144 (67.3)	70 (32.7)	Ref	
MYR 1000-3999	85 (30.9)	190 (69.1)	0.81 (0.56-1.19)	0.283	172 (62.5)	103 (37.5)	0.81 (0.56-1.18)	0.505
<MYR 1000	236 (38.5)	377 (61.5)	1.14 (0.82-1.57)	0.438	426 (69.5)	187 (30.5)	1.11 (0.79-1.55)	0.549

Ref: reference group

5.4.3.3 Relationship between the prevalence of caries and infant feeding practices

Table 5.39 shows the relationship between the prevalence of dental caries and infant feeding practices. Children who were reported as being fed with infant formula had a significantly lower dentine caries prevalence than non-formula users (p<0.001). Type of water used to reconstitute infant formula was significantly associated with dentine caries prevalence. Formula users with tap water and filtered tap water were 2.71 and 2.31 times more likely to have dentine caries than formula users with bottled water. No significant relationship was found between infant feeding practices with caries prevalence at all levels.

Table 5.39 Bivariate analysis between infant feeding practices and prevalence of caries at dentine level ($D_{4\text{-}6}MFT>0$) and caries at all levels ($D_{1\text{-}6}MFT>0$) in study participants

Variables	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Infant feeding practices	$D_{4\text{-}6}MFT>0$ n (%)		Odds ratio 95% CI	p value	$D_{1\text{-}6}MFT>0$ n (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Use of infant formula								
No	144 (46.2)	167 (53.8)	Ref		220 (70.5)	92 (29.5)	Ref	
Yes	266 (31.7)	574 (68.3)	0.54 (0.41-0.71)	**<0.001**	550 (65.5)	290 (34.5)	0.79 (0.60-1.05)	0.107
Breast feeding								
No	9 (33.3)	18 (66.7)	Ref		21 (77.8)	6 (22.2)	Ref	
Yes	403 (35.7)	725 (64.3)	1.11 (0.50-2.50)	0.798	752 (66.7)	376 (33.3)	0.57 (0.23-1.43)	0.231
Age finished breast feeding								
>12 months	251 (36.7)	433 (63.3)	Ref		457 (66.8)	227 (33.2)		
≤12 months	152 (34.0)	295 (66.0)	0.89 (0.69-1.14)	0.356	298 (66.7)	149 (33.3)	0.99 (0.77-1.28)	0.959
Age started formula								
>12 months	110 (32.3)	231 (67.1)	Ref		228 (66.9)	113 (33.1)	Ref	
≤12 months	163 (32.0)	346 (68.0)	0.99 (0.74-1.33)	0.943	329 (64.6)	180 (35.4)	0.91 (0.68-1.21)	0.503
Age finished formula								
>48 months	140 (32.3)	293 (67.7)	Ref		295 (68.1)	138 (31.9)	Ref	
≤48 months	131 (31.8)	281 (68.2)	0.98 (0.73-1.30)	0.867	260 (63.1)	152 (36.9)	0.80 (0.60-1.06)	0.125

Ref: reference group

(Table 5.39 continued)

Variables	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Infant feeding practices	D_{4-6}MFT>0 n (%)		Odds ratio 95% CI	p value	D_{1-6}MFT>0 n (%)		Odds ratio 95% CI	p value
Type of water used to prepare formula	Yes	No			Yes	No		
Bottled water	5 (15.6)	27 (84.4)	Ref		21 (65.6)	11 (34.4)	Ref	
Tap water	213 (33.4)	424 (66.6)	2.71 (1.03-7.14)	**0.043**	423 (66.4)	214 (33.6)	1.04 (0.49-2.19)	0.927
Filtered tap water	51 (30.0)	119 (70.0)	2.31 (0.84-6.35)	0.103	105 (61.8)	65 (28.2)	0.85 (0.38-1.87)	0.679
Duration of formula								
>48 months	79 (30.4)	181 (69.6)	Ref		171 (65.8)	89 (34.2)	Ref	
≤48 months	188 (32.6)	388 (67.4)	1.11 (0.81-1.52)	0.518	373 (64.8)	203 (35.2)	0.89 (0.52-1.52)	0.661
Feeding method								
Formula only	9 (33.3)	18 (66.7)	Ref		21 (77.8)	6 (22.2)	Ref	
Combine breast & formula	257 (31.6)	556 (68.4)	0.92 (0.41-2.09)	0.850	529 (65.1)	284 (34.9)	0.53 (0.21-1.33)	0.178
Breast only	144 (46.2)	168 (53.8)	1.71 (0.75-3.93)	0.203	220 (70.5)	92 (29.5)	0.68 (0.27-1.75)	0.427

Ref: reference group

5.4.3.4 Relationship between the prevalence of caries and oral hygiene habits at the time of study (2015) in study participants

In terms of oral hygiene habits, bivariate analysis were carried out for both, oral hygiene practices reported as having been practiced at age less than six years and oral hygiene practices at the time of study (in 2015). The rationale of performing this analysis is that exposure to fluoride at different stages in life may be associated with caries prevention.

Table 5.40 shows a bivariate analysis between the prevalence of caries and current (2015) oral hygiene practices. There was some variation in terms of current oral hygiene practices with caries prevalence at dentine level or at all levels, however the differences were not significant.

Table 5.40 Bivariate analysis between oral hygiene habits at the time of study (2015) and prevalence of caries at dentine level ($D_{4-6}MFT>0$) and caries at all levels ($D_{1-6}MFT>0$) in study participants

Variables	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
(Oral hygiene habits, in 2015)	$D_{4-6}MFT>0$ n (%)		Odds ratio 95% CI	p value	$D_{1-6}MFT>0$ n (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Frequency of toothbrushing								
Twice/day or more	342 (36.7)	590 (63.3)	Ref		632 (67.8)	300 (32.2)	Ref	
Once /day or less	70 (32.3)	147 (67.7)	0.82 (0.60-1.13)	0.220	139 (64.1)	78 (35.9)	0.85 (0.62-1.15)	0.289
Supervise toothbrushing								
Never	39 (37.5)	65 (62.5)	Ref		75 (72.1)	29 (27.9)	Ref	
Yes	352 (35.3)	646 (64.7)	0.91 (0.60-1.38)	0.651	662 (66.3)	336 (33.7)	0.76 (0.49-1.19)	0.234
Habits after brushing								
Spat	398 (35.4)	726 (64.6)	Ref		750 (66.7)	374 (33.3)	Ref	
Swallowed	6 (46.2)	7 (53.8)	1.56 (0.52-4.68)	0.421	10 (76.9)	3 (23.1)	1.66 (0.46-6.08)	0.442
Eating/ licking toothpaste								
Never	353 (35.6)	638 (64.4)	Ref		663 (66.9)	328 (33.1)	Ref	
Yes	56 (36.1)	99 (63.9)	1.02 (0.72-1.46)	0.902	105 (67.7)	50 (32.3)	1.04 (0.72-1.49)	0.836
Amount of toothpaste used								
Medium to large	381 (35.9)	680 (64.1)	Ref		714 (67.3)	347 (32.7)	Ref	
Small	30 (36.1)	53 (63.9)	1.01 (0.64-1.61)	0.966	55 (66.3)	28 (33.7)	0.96 (0.60-1.53)	0.847
Type of toothpaste								
Non-fluoridated	19 (40.4)	28 (59.6)	Ref		32 (68.1)	15 (31.9)	Ref	
Fluoridated	386 (35.6)	698 (64.4)	0.82 (0.45-1.48)	0.501	729 (67.3)	355 (32.7)	0.96 (0.52-1.80)	0.905

Ref: reference group

5.4.3.5 Relationship between the prevalence of caries and oral hygiene habits (at age less than six years) among study participants

Table 5.41 shows the relationship between the prevalence of dental caries and oral hygiene habits at age less than six years among the study participants. The age at which children were reported as starting to toothbrush with toothpaste was significantly associated with the prevalence of caries at dentine level (D_{4-6}MFT) and caries at both enamel and dentine levels (D_{1-6}MFT). Children who started toothbrushing with toothpaste after two years had a significantly higher caries prevalence than those started toothbrushing with toothpaste at younger age. Other variables from early exposure to fluoride from oral hygiene practices were found to be not significantly associated with caries prevalence at dentine level or at all levels.

Table 5.41 Bivariate analysis between oral hygiene habits (at age less than six years) and prevalence of caries at dentine level ($D_{4-6}MFT>0$) and caries at all levels ($D_{1-6}MFT>0$) in study participants

Variables	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Oral hygiene habits at age less than 6 years	$D_{4-6}MFT>0$ n (%)		Odds ratio 95% CI	p value	$D_{1-6}MFT>0$ n (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Frequency of toothbrushing								
Twice/day or more	226 (34.3)	432 (65.7)	Ref		446 (67.8)	212 (32.2)	Ref	
Once /day or less	185 (37.7)	306 (62.3)	1.16 (0.91-1.47)	0.244	326 (66.4)	165 (33.6)	0.94 (0.73-1.20)	0.621
Supervised toothbrushing								
Never	6 (30.0)	14 (70.0)	Ref		14 (70.0)	6 (30.0)	Ref	
Yes	393 (36.2)	694 (63.8)	1.32 (0.50-3.47)	0.571	734 (67.5)	353 (32.5)	0.89 (0.34-2.34)	0.815
Habits after brushing								
Spat	378 (35.3)	692 (64.7)	Ref		717 (67.0)	353 (33.0)	Ref	
Swallowed	29 (42.6)	39 (57.4)	1.36 (0.83-2.24)	0.224	49 (72.1)	19 (27.9)	1.27 (0.74-2.19)	0.390
Eating/ licking toothpaste								
Never	176 (36.3)	309 (63.7)	Ref		329 (67.8)	156 (32.2)	Ref	
Yes	233 (35.2)	428 (64.8)	0.96 (0.75-1.22)	0.717	441 (66.7)	220 (33.3)	0.95 (0.74-1.22)	0.690

(Table 5.41 continued)

Variables	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Oral hygiene habits at age less than 6 years	D_{4-6}MFT>0 n (%)		Odds ratio 95% CI	p value	D_{1-6}MFT>0 n (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Amount of toothpaste used								
Medium to large	216 (34.0)	419 (66.0)	Ref		422 (66.5)	213 (33.5)	Ref	
Small	194 (38.0)	317 (62.0)	1.19 (0.93-1.51)	0.166	349 (68.3)	162 (31.7)	1.09 (0.85-1.39)	0.509
Type of toothpaste								
Non-fluoridated	48 (34.0)	93 (66.0)	Ref		94 (66.7)	47 (33.3)	Ref	
Fluoridated	355 (36.1)	628 (63.9)	1.10 (0.76-1.59)	0.632	665 (67.7)	318 (32.3)	1.05 (0.72-1.52)	0.816
Age started toothbrushing								
After 2 years	292 (37.1)	495 (62.9)	Ref		524 (66.6)	263 (33.4)	Ref	
Before 2 years	120 (32.7)	247 (67.3)	0.82 (0.63-1.07)	0.146	249 (67.8)	118 (32.2)	1.06 (0.81-1.38)	0.670
Age started toothbrushing with toothpaste								
After 2 years	315 (37.7)	520 (62.3)	Ref		575 (68.9)	260 (31.1)	Ref	
Before 2 years	94 (30.3)	216 (69.7)	0.72 (0.54-0.95)	**0.020**	193 (62.3)	117 (37.7)	0.75 (0.57-0.98)	**0.035**

Ref: reference group

5.4.3.6 Relationship between the prevalence of caries and exposure to fluoride varnish/gel in study participants

For caries prevalence at dentine level, children who did not receive fluoride varnish/gel had a marginally higher dentine caries prevalence than who received fluoride varnish/gel (Table 5.42). However the difference was not significant. In contrast, for caries prevalence at all levels, children who received fluoride varnish/gel had marginally higher caries prevalence, however the difference was not significant

Table 5.42 Bivariate analysis between exposure to fluoride varnish/gel and prevalence of caries at dentine level (D_{4-6}MFT>0) and caries at all levels (D_{1-6}MFT>0) in study participants

Variables	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
	D_{4-6}MFT>0 N (%)		Odds ratio 95% CI	p value	D_{1-6}MFT>0 N (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Exposure to Fluoride varnish/gel								
No	222 (33.6)	438 (66.4)	Ref		422 (63.9)	238 (36.1)	Ref	
Yes	50 (32.5)	104 (67.5)	0.95 (0.65-1.38)	0.782	101 (65.6)	53 (34.4)	1.08 (0.74-1.55)	0.701

Ref: reference group

5.4.4 Logistic regression models for having dental caries

Binary logistic regression model using the Enter method was generated for the prevalence of caries defined by $D_{4\text{-}6}$MFT>0 and $D_{1\text{-}6}$MFT>0 separately. Significant variables ($p<0.05$) and approaching significant variables ($p<0.10$) from the bivariate analysis were entered into the model as a block. Adjusted odds ratios and 95% confidence intervals are reported in Table 5.43. This shows the multivariate logistic regression model for the prevalence of dentine caries defined by $D_{4\text{-}6}$MFT>0. Father's and mother's education level, father's monthly income, exposure to fluoride from water, use of infant formula, type of water used to reconstitute formula and age started toothbrushing with toothpaste were contributing factors to the model.

After controlling other factors in the model, exposure to fluoride from water and type of water use to reconstitute formula remained significantly associated with having higher dentine caries prevalence. Children who had been exposed to fluoridated water had a significantly lower dentine caries prevalence than those who did not have any exposure. Infant formula reconstituted with tap water or filtered tap water had a significantly higher dentine caries prevalence compared to those who used bottled water.

Table 5.43 Multivariate logistic regression model for caries prevalence at dentine level ($D_{4-6}MFT>0$)

Explanatory variable	Adjusted Odds ratio (95% CI)	p value
Father education		
College/University	Ref	
High school	0.85 (0.54-1.34)	0.473
≤Primary school	0.57 (0.25-1.29)	0.175
Mother education		
College/University	Ref	
High school	1.10 (0.73-1.66)	0.663
≤Primary school	1.59 (0.73-3.45)	0.246
Father income		
≥ MYR 4000	Ref	
MYR 1000-3999	1.15 (0.75-1.77)	0.528
<MYR 1000	1.08 (0.32-3.60)	0.906
Fluoride level from the water		
Non-fluoridated	Ref	
Fluoridated	0.43 (0.31-0.60)	**<0.001**
Infant formula		
No	Ref	
Yes	0.61 (0.14-2.61)	0.503
Type of water used to prepare formula		
Bottled water	Ref	
Tap water	4.32 (1.25-14.99)	**0.021**
Filtered tap water	4.40 (1.21-16.01)	**0.024**
Age started toothbrushing with toothpaste		
After 2 years	Ref	
Before 2 years	0.72 (0.49-1.04)	0.076

Ref: reference group

Table 5.44 shows the multivariate logistic regression model for the prevalence of caries at all levels defined by D1-6MFT>0. Gender, father's monthly income, exposure to fluoride from water and age started toothbrushing with toothpaste were contributing factors to the model.

After controlling for other factors in the model, gender and exposure to fluoride from water remained significantly associated with having lower caries prevalence at all levels. Girls had a significantly higher caries prevalence than boys. Children who had been exposed to fluoridated water had a significantly lower caries prevalence than those who did not have any exposure.

Table 5.44 Multivariate logistic regression model for caries prevalence at all levels ($D_{1\text{-}6}$MFT)

Explanatory variables	Adjusted Odds ratio (95% CI)	p value
Gender		
Boys	Ref	
Girls	1.31 (1.01-1.71)	**0.042**
Father income		
≥ RM 4000	Ref	
RM1000-3999	1.17 (0.88-1.56)	0.281
<RM 1000	1.12 (0.48-2.60)	0.802
Fluoride level		
Non-fluoridated	Ref	
Fluoridated	0.58 (0.44-0.76)	**<0.001**
Age started toothbrushing with toothpaste		
After 2 years	Ref	
Before 2 years	0.82 (0.61-1.10)	0.181

5.5 Summary of key findings

Fluorosis

- Regardless of which outcome measure was used (Deans>0 or Deans≥2), fluorosis prevalence was significantly higher among children in the fluoridated area than the non-fluoridated area.
- Reducing fluoride level in the water has resulted in narrowing of fluorosis prevalence between fluoridated and non-fluoridated areas. This implies that the decrease in fluorosis prevalence corresponds with the reduction (0.2 ppm) of fluoride in the drinking water during the time of central incisor development.
- From the bivariate analysis, the prevalence of fluorosis (Deans≥2) was significantly associated with parents' education level, parents' monthly income, fluoride exposure from the water, use of infant formula, the age at which breast feeding finished, age started formula, age finished formula, type of water used to reconstitute the formula, duration of formula use and type of feeding method.
- After controlling for other factors in the model, exposure to fluoride level from water and type of water use to reconstitute the formula remained significantly associated with the prevalence of fluorosis.

Caries

- For both age groups, the mean caries experience and percentage of caries prevalence was significantly lower among children in the fluoridated area than the non-fluoridated area. Similar results were observed for mean caries experience in permanent and primary dentitions at tooth and surface levels.

- Findings suggest that a statistically significant caries preventive effect remained following the reduction of the fluoride level in the public water supply.
- In the permanent dentition, the enamel caries prevalence was higher than the dentine caries prevalence. In contrast, dentine caries prevalence was higher than enamel caries in the primary dentition. The trends were similar when data were analysed at tooth or surface levels.
- From the bivariate analysis, the prevalence of caries at dentine level ($D_{4-6}MFT>0$) was significantly associated with parents' education level, fathers monthly income, fluoride level in water, use of infant formula, type of water used to reconstitute formula milk and age at which the participants were reported as starting toothbrushing with toothpaste.
- After controlling for other factors in the model, the fluoride level in the water supply and type of water used to reconstitute formula remained significantly associated with the prevalence of dentine caries.
- From the bivariate analysis, the prevalence of caries at all levels ($D_{1-6}MFT>0$) was significantly associated with gender, father's monthly income, exposure to fluoride level from water and age started toothbrushing with toothpaste.
- After controlling for other factors in the model, gender and exposure to fluoride level in the water supply remained significantly related to the prevalence of dental caries at all levels of severity.

6 Discussion

This chapter presents a discussion of the key findings of the thesis (Section 6.1). The findings are then discussed and compared with other published work (Sections 6.3.1 to 6.3.5). Methodological considerations, study strengths and limitations are also considered in Section 6.4.

6.1 Key findings

6.2 Systematic review

The systematic review completed in Chapter 2 reviewed the impact of removing or reducing the level of fluoride in the public water supply on dental caries and fluorosis. The findings highlighted the gap in knowledge with respect to the impact of stopping or reducing the level of fluoride in water supply on dental caries and fluorosis. There were more studies assessing the impact of cessation as opposed to reduction of fluoride level in the water supply. The available evidence on stopping water fluoridation has focused on dental caries as the primary outcome and data indicated mixed results. Studies published before the 1990s reported increased caries experience post cessation, while studies published from 1990 onwards reported a decrease in caries experience in the absence of fluoride. The limited numbers of studies that have reported on reducing fluoride level in the water supply have mainly focused on dental fluorosis as their primary outcome and indicate a decrease in fluorosis prevalence. Therefore, further investigations of these gaps in the evidence were indicated. Findings from the systematic review also highlighted issues surrounding the methods used in water fluoridation studies such as lack of examiner blinding and control of confounding factors in the analysis. These issues should be addressed to increase the quality of the studies in this area of research.

6.3 Main study

The main research undertaken in this thesis was conducted to address deficiencies in the evidence highlighted in the systematic review chapter. The opportunity arose following changes in Malaysian water fluoridation policy in 2005, when the optimum concentration of fluoride in the public water supply was reduced from 0.7 to 0.5 ppm. Therefore, this study aimed to evaluate the outcomes of a 0.2 ppm downward adjustment of fluoride concentration in the drinking water on dental fluorosis and caries. The following sections discuss the key findings of the main study compared with other published work and how some of the key methodological issues highlighted in systematic reviews have been addressed.

6.3.1 The prevalence and severity of dental fluorosis following reduction of fluoride level in the public water supply

As described in the literature review (Chapter 1), comparison across studies with regards to fluorosis prevalence is complicated by different methods and varying outcome measures used in previous studies (Section 1.2.2.4).

In the present study, fluorosis prevalence was significantly higher in fluoridated (35.7% to 42.6%) than non-fluoridated (5.5% to 9.7%) areas. This held true regardless of which threshold of fluorosis definition was used. The results confirm findings from various studies that fluorosis prevalence is strongly associated with fluoridated water (Clark, 1994, Adair et al., 1999, Maupome et al., 2003, Khan et al., 2005). Furthermore, some authorities have reported that it may not be possible to achieve effective fluoride-based caries prevention without some degree of enamel fluorosis (O' Mullane et al., 2016).

Results in the present study indicated a lower fluorosis prevalence (Deans ≥ 2= 35.7%) than the previous national survey that reported 62.3% of fluorosis prevalence in fluoridated areas (Oral Health Division Ministry of Health Malaysia, 2001). When comparing with the national and local data, fluorosis at (Deans ≥ 2) is used as case definition. A similar result was reported by another local study with 58.7% fluorosis prevalence carried out amongst a representative sample in the fluoridated state of Selangor (Tan et al., 2005). These studies were conducted among children that were exposed to 0.7 ppm fluoride in the water throughout life before the change in the fluoridation policy took place. However, with regards to those in non-fluoridated areas, there was a small increase in fluorosis prevalence observed in the present study (5.5%) over that reported in the previous national survey (3%). A similar finding of an increased prevalence of fluorosis in sub-optimal or non-fluoridated areas were also reported by another local study (31.6%) but their sample size was rather small (Shaharuddin et al., 2010). The tendency for an increase in the prevalence of fluorosis in non-fluoridated areas has also been documented by international studies in the USA (Beltrán-aguilar et al., 2002), Ireland (Whelton et al., 2004a, Whelton and O'Mullane, 2012) and Canada (McLaren, 2011). This phenomenon has several possible explanations. Firstly, due to the increased availability of fluoride from other sources such as fluoride toothpaste and other dental products such as fluoride mouthwash, and varnish/gels. Secondly by the 'diffusion effect' whereby the residents in a non-fluoridated area can be exposed to fluoride in foods or beverages that are produced in a fluoridated area and transported to the non-fluoridated area (Griffin et al., 2001).

In terms of severity, most of the fluorosis observed was in the very mild and mild category. The previous Malaysian national survey reported similar findings (Oral Health Division Ministry of Health Malaysia, 2001). This is in agreement with international studies conducted in Australia (Do and Spencer, 2007) and Canada (Maupome et al., 2003). The work carried out in the conduct of this thesis found that of those living in fluoridated areas the prevalence of moderate fluorosis was higher among children exposed to 0.7 ppm (10.9%) than 0.5 ppm (6.7%) in the first 2 years of life. The dose response in relation to the prevalence of moderate fluorosis is further illustrated by a study in 12 year-olds conducted in Quette, Pakistan, where the concentration of fluoride in the drinking water was 0.91 ppm (Sami et al., 2015). There the overall prevalence of fluorosis was reported as 63.6%. The majority (32.1%) was recorded as moderate while 27.5% was categorised as mild (Sami et al., 2015).

Little is known about the effect of reducing fluoride level to a fluoride concentration as low as 0.5 ppm. This limits the direct comparison of the present data with other studies. Findings from this study can only be compared with a series of Hong Kong studies that examined fluorosis prevalence on maxillary central incisors after downward adjustment of fluoride in Hong Kong water supply. The earlier Hong Kong studies by Evans and Stamm reported that fluorosis prevalence with Dean’s Index declined from 64% to 47% after the reduction in fluoridation level from 1.0 ppm to 0.7 ppm (Evans and Stamm, 1991b). The recent data from Hong Kong reported four cross-sectional surveys on fluorosis prevalence. The fluorosis was blind scored using photographs of maxillary incisors with DDE index. A similar trend was reported following reduction of fluoride level in the water from 1.0 (1967) to 0.7 (1978) to 0.5 (1988) (Wong et al., 2014). Fluorosis decreased from 89.3% in 1983 to 48.5% in 1991 and 32.4 % in 2001 surveys. However the follow-up survey in 2010 reported fluorosis prevalence has increased to 42.1% while the fluoride level remained the same at 0.5ppm as in 2001. The authors suggested the increase in prevalence of fluorosis might be contributed to by other sources of fluoride such as fluoridated toothpaste, infant formula and fluoride content in food (Wong et al., 2014). In 2013, the authors conducted another follow-up study and re-examined the same participants that had participated in 2010 survey. The follow-up drop-out rate was 35% (Wong et al., 2016). Results indicated a significant decrease in fluorosis prevalence from 2010 to 2013. The authors concluded that the fluorosis diminished over time. Possible explanations given were the possibility of tooth wear and the effect of remineralisation. Constant exposure to saliva, which is supersaturated with calcium and phosphate, results in continued enamel mineralization that in turn can lead to reduced opacity in affected areas (Wong et al., 2016). However results should be treated with caution because the main aim of the later study was to look at overall enamel defects not just fluorosis. Significant results were only observed for 'diffuse opacities' but not on other enamel defects such demarcated and hypoplastic enamel. Although the DDE index classifies enamel defects in a descriptive way and does not assume aetiology, one of its main types, diffuse opacities has been used synonymously as dental fluorosis.

The present study shows a decreased in fluorosis prevalence corresponds with the reduction (0.2 ppm) of fluoride in the drinking water during the time of maxillary central incisors development (Chapter 4). The results provide support for the decision to reduce the fluoride level in the public water supply in Malaysia. Findings further support previous results that the prevalence of fluorosis is sensitive to even minor changes in fluoride exposure from drinking water. This is not a novel concept and the fluorosis outcome has been addressed in a several earlier studies conducted in Hong Kong (Evans, 1989, Evans and Stamm, 1991b, Wong et al., 2014). However, the present study provides evidence that the change in fluoride level from 0.7 to 0.5 ppm not only resulted in changes on fluorosis prevalence but also has a significant impact on caries prevalence at different thresholds of severity. At 0.5 ppm fluoride in the water fluorosis prevalence is further reduced without compromising the caries preventive benefit. The impact of such reduction on caries is further discussed in Section 6.3.3. These new findings add value to the gap in literature with regards to recent movement towards lower levels of fluoride in the water.

6.3.2 Risk factors associated with fluorosis

A number of factors have been identified as associated with the prevalence of dental fluorosis in this study population (Section 5.3.2).

Socio-economic status

High fluorosis prevalence was found to be significantly associated with higher parental income and education level in bivariate analysis. The link between socio-economic status and fluorosis has also been reported by other studies in Brazil (Benazzi et al., 2012) Mexico (Pontigo-Loyola et al., 2014) and Pakistan (Sami et al., 2015). Unlike dental caries, the relationship between fluorosis and socio-economic status has not been fully established in the literature. Results across studies were mixed and the socio-economic status factors were not significant in multivariate regression model in the present study and other studies reviewed (Benazzi et al., 2012, Pontigo-Loyola et al., 2014, Sami et al., 2015). Several authors have postulated that a high fluorosis prevalence among affluent families might be due to the ability to purchase fluoride toothpaste (Benazzi et al., 2012, Pontigo-Loyola et al., 2014). However, fluorosis prevalence in the present study was strongly associated with exposure to fluoride in the water rather than to fluoride toothpaste and oral hygiene practices. Therefore, a potential reason for the bivariate association could be due to more children with parents of higher socio-economic status were exposed to fluoridated water than those in lower socio-economic status. On the other hand, a study in Pakistan reported children with better family income were less likely to have fluorosis. There, authors reported that the affluent family linked to better parental awareness about fluorosis and they were more likely to be concerned with exposure to fluoride in their children than those from low income families (Sami et al., 2015).

Infant feeding practices

Another factor associated with fluorosis prevalence was the use of infant formula. However there was lack of evidence to verify the actual fluoride content of infant formula in Malaysia. Only one publication ever reported fluoride levels in Malaysian infant formula. Authors reported the content of fluoride in infant formula when reconstituted with deionized water (0 ppmF) was low with a mean value of 0.087 ppmF ± 0.04 (Latifah and Razak, 1989). However, this study was conducted in 1980s and no data on specific fluoride content in infant formula before dilution with deionized water have been reported. No other historical data or recent publications from Malaysia are available for comparison. Effort was made to obtain fluoride content from some of the infant formula packaging, but no information was available. Therefore, further research is needed to confirm such association. Based on the international literature, infant formula generally had a low fluoride content after the 1970s (Mascarenhas, 2000). The low concentration of fluoride level in infant formula varied from (0.28 µg/F g of milk powder) in United Kingdom (Zohoori et al. 2012) to (0.41 µg/F g) in Japan (Nohno et al., 2011) and (0.49 µg/F g) in Australia (Clifford et al., 2009). Evidence in the literature suggests that fluorosis has a weak association with infant formula because of low fluoride level in infant formula (Koparal et al., 2000, Hujoel et al., 2009, Siew et al., 2009). However it shows a strong correlation with type of water used to reconstitute the formula (Nohno et al., 2011). Findings from the present study are in agreement with previous studies that reported infant formula reconstituted with fluoridated tap water increases the risk for dental fluorosis (Ekstrand et al., 1984, Ekstrand, 1989, Pagliari et al., 2006, Siew et al., 2009, Cressey, 2010). This factor remained statistically significant in the multivariate model. Duration of infant formula practice also played a role in this study. For instance, children who had a longer duration of formula use (more than 48 months), were significantly more likely to be associated with a higher fluorosis score. However this factor was no longer significant in multivariate model.

The results also indicate that children that were breast-fed during infancy were significantly less likely to have fluorosis than those who were formula usurers. This suggests that breast-feeding practices were protective against fluorosis. The findings are supported by other studies (Van Winkle et al., 1994, Brothwell and Limeback, 2003, Wondwossen et al., 2006). It is known that, even if a mother is consuming fluoridated water, human milk maintains very low fluoride concentrations (< 0.5 µM) due to the limited transfer of fluoride from plasma to breast milk (Ekstrand et al., 1984, Şener et al., 2007). Furthermore, breast-feeding duration also played a significant protective role in the current study (Section 5.3.2.3). Children who had been breastfed for a prolonged period (>12 months) were less likely to develop fluorosis. The same findings have been reported in Canada (Brothwell and Limeback, 2003) and in Ethiopia (Wondwossen et al., 2006). In addition, the practice of breast-feeding was found to be linked with the family economic status. For example in this study, those with low income and low education levels were more likely to breastfeed their children and to do so for longer. These findings confirm results from other local studies in Malaysia that have reported a higher prevalence of breast-feeding in lower socio-economic status families, and greater use of infant formula amongst the affluent (Manan, 1995, Yee and Chin, 2007, Tan, 2009). This could be due the fact the majority of the mothers of lower socio economic status were not working outside the house being full time housewives, which makes breast-feeding easier than for those who go out to work. This pattern differs from that observed in the UK (Brown et al., 2010) and USA (Heck et al., 2006), where breastfeeding is more common in higher socioeconomic groups.

6.3.3 The prevalence and severity of dental caries following reduction of fluoride level in the water

The present study design is critical in evaluating fluorosis as change of exposure to fluoride in the water during the first 2 years of life was specifically used for the analysis. The study design does not allow direct comparison to assess whether the caries preventive effect of water fluoridation at 0.5 ppm is better than at 0.7 ppm. This is because the 12 year-old children were only exposed to 0.7 ppm for the first two years of life. In addition, it is not possible to directly compare the caries experience between 9 and 12 year-olds from cross sectional data because of the different dentition present in these different age groups. However the study provided results to answer the question whether the caries preventive effect has been maintained at 0.5 ppm when compared with the non-fluoridated similarly aged control groups.

Regardless of which threshold of diagnosis was used, the mean caries experience in the permanent and primary dentitions was significantly lower in the fluoridated than non-fluoridated areas for both age groups. A higher number of teeth, missing due to caries was observed among children in the non-fluoridated area in both dentitions. The prevalence of filled surfaces was also significantly higher in the non-fluoridated area. The findings in relation to caries prevalence into dentine are in agreement with results from the Malaysian national survey (Oral Health Division Ministry of Health Malaysia, 2010) and school dental service data (Oral Health Division Ministry of Health Malaysia, 2014). Additionally, results confirmed existing evidence of the benefit of water fluoridation in caries prevention reported in other countries (McDonagh et al., 2000, Parnell et al., 2009, Iheozor-Ejiofor et al., 2015). As highlighted in many studies, it has become difficult to investigate the impact of water fluoridation alone in the community where fluoridated toothpaste use is widespread. For example in the present study, the majority of the respondents in both areas reported using fluoridated toothpaste when brushing. Results from this study also confirm findings from the York Review that the caries preventive effect is still apparent in the fluoridated community that used fluoridated toothpaste (McDonagh et al., 2000). About 37% of the children in the non-fluoridated area had fissure sealants and this proportion was found to be significantly higher than children in the fluoridated area (8%). In addition, about 14.8% of children were reported to have received fluoride varnish/gel. Although these preventive strategies were in place, children who had no exposure to fluoride in the water still had a higher caries score than those that had exposure to fluoridation. Results from this study suggest that an optimum fluoride concentration of 0.5 ppm maintained a caries preventive effect, thus supporting the decision to reduce the fluoride level in Malaysian public water supply.

Caries prevalence in this study was examined using ICDAS II criteria. The ability of the index to enable detection of early caries lesions provides an opportunity to explore caries prevalence in fluoridated and non-fluoridated populations at low levels of caries severity. Because this is a new index, only limited epidemiological studies are available for comparison. To allow comparison with other studies that use DMF index, the ICDAS II codes were collapsed at specific cut-off points for equivalence. There is ongoing debate concerning the level of equivalence to the DMF index. There is less certainty in the literature between codes 3 and 4 to be counted as sound or caries. Some authors considered the cut-off point of caries at code 3 (Braga et al., 2009, Mendes et al., 2010, Iranzo-Cortes et al., 2013). Whereas ICDAS II itself stated the codes 4, 5 and 6 are equivalent to caries score of DMF (i.e. into dentine criteria traditionally used in dental epidemiology (Pitts, 2004, Banting et al. 2005). The present study sets the ICDAS II cut-off point for comparison with the DMF score at codes 4 to 6. Enamel caries was analysed at codes 1 to 3.

When looking at caries at different thresholds, there was more enamel caries diagnosed than dentine caries in both age groups and areas of residence. When enamel caries were included, the mean DMFT and DMFS score increased by 2 to 4 times more than when only dentine caries lesions were included. For example for mean caries experience among 12 year-olds in the fluoridated area was 0.47 at (D_{4-6}MFT) and increased to 2.01 at (D_{1-6}MFT). The inclusion of early caries lesion contributed to the higher overall caries score diagnosed using ICDAS II in comparison to the traditional DMF score. Similar trends were observed in other studies that used ICDAS II criteria when assessing caries prevalence (Cadavid et al., 2010, de Amorim et al., 2012, McGrady et al., 2012a, Almerich-Silla et al., 2014). For instance in a study conducted in Spain, the mean D_{4-6}MFT was 0.83 and 3.46 (D_{1-6}MFT) according to ICDAS II criteria (Almerich-Silla et al., 2014) among 12 year-old children. Similarly in a national survey in Iceland, caries prevalence at age 12 years was 1.43 at D_{3-6}MFT and 3.93 at D_{1-6}MFT (Agustsdottir et al., 2010). Although the prevalence of early caries lesions is higher than dentine caries, results indicate that the difference in the prevalence between fluoridated and non-fluoridated is narrower when the caries is reported at this threshold (D_{1-6}MFT). For example, among the 12 years, D_{1-6}MFT was 2.01 in fluoridated and 2.83 in non-fluoridated area. Meanwhile caries prevalence at D_{4-6}MFT was 0.47 in fluoridated and 1.31 in non-fluoridated area. Similar findings were reported in a study carried out by Mc Grady and co-workers that assessed the impact of water fluoridation in Newcastle and Manchester (Mc Grady et al., 2012a). This raises another important question, whether water fluoridation prevents or merely delays the progressions of early caries. This could be answered by a longitudinal study and should be considered in future research.

Many water fluoridation studies used the DMF index and reported caries data at "dentine level". Data from this study report caries prevalence in fluoridated and non-fluoridated communities using ICDAS II index and this has allowed comparison of the relative contribution of enamel and dentine caries to overall caries experience. Therefore, the findings make an important contribution to the water fluoridation literature.

No dietary habits (i.e. sugar consumption, soft drinks) were measured in this study. National data suggest that Malaysian children consumed high added sugar in their diet and have a high frequency of snacking (Poh et al., 2013). The adult population in Kelantan was reported to have a higher sugar intake than the national average and this pattern can be speculated to be similar among the child population (Amarra et al., 2016). This factor should be considered as an important factor contributes to the high caries prevalence in Kelantan in addition to no exposure to fluoridated water.

6.3.4 Risk factors associated with dental caries

The data collected in this study allow analysis for both, pre and post-eruptive exposure to fluoride on dental caries prevention.

Oral hygiene habits at the time of study in 2015

To examine any post-eruptive effect of fluoride in caries prevention, caries data at different thresholds were analysed in relation to oral hygiene habits at the time of the study in 2015. There were some variations in terms of current oral hygiene practices with caries prevalence at dentine level or at all levels, however the differences were not statistically significant (Section 5.4.3.4).

Early exposure to fluoride from other sources

To assess any pre-eruptive effect of fluoride, data were analysed using early exposure variables that influencing caries risk (i.e. oral hygiene practices at age less than 6 years, infant feeding practices, exposure to fluoride varnish/gel at age less than 6 years). The analysis, indicated that children who started brushing with toothpaste before 2 years of age had a significantly lower caries prevalence than those who started brushing with toothpaste after 2 years. This association was significant both at the caries into dentine level and at all levels. However no significant relationship was observed for fluorosis prevalence and the age at which brushing with toothpaste started. This is reassuring and suggests that the use of fluoride toothpaste at an early age is not contributing to the prevalence of fluorosis observed. A high proportion of children were reported as being supervised during toothbrushing. Parental assistance would assist in the toothbrushing procedure and control over the use and ingestion of toothpaste. Furthermore, a majority of parents reported that their children did not have eating/licking toothpaste habits after toothbrushing.

Children who were reported as being fed with infant formula had a significantly lower dentine caries prevalence than non-formula users. This factor was associated with a high fluorosis prevalence. Because fluoride level in the infant formula is generally low, this association is likely confounded by socioeconomic status as discussed in Section 6.3.2. However the relationship between the types of water used to reconstitute infant formula and dental caries was less clear. There was an unexpected finding that the use of tap water to reconstitute infant formula was associated with higher caries prevalence than the use of bottled water. This finding may however simply reflect the small number of bottled water users and so should be treated with caution.

These significant factors were no longer significant in the multivariate analysis. In addition, other variables from early exposure to fluoride from oral hygiene practices and infant feeding practices were also found to be not significantly associated with caries prevalence for permanent teeth. These findings are consistent with other studies that the pre-eruptive effect of fluoride is less important and the primary effect of fluoride is post-eruptive (Sampaio and Levy, 2011, Hellwig and Lennon, 2004).

Demographic characteristics and socio-economic status

In terms of caries association with demographic characteristics, girls had a higher dentine caries score compared to boys and the gender difference was statistically significant with caries severity at all levels in the multivariate model. The results in this gender differences are supported by other studies conducted by (Ramezani et al., 2003, Lukacs, 2011). The factors that cause girls and women to experience more dental caries are not fully understood. Possible explanations have been proposed, including earlier tooth eruption in girls and therefore increased time of exposure to the cariogenic process (Lukacs, 2011, Martinez-Mier and Zandona, 2013). Other reported explanations include a lower salivary flow rate among females, which has lesser caries protective effect than their male counterparts. This condition may be influenced by female hormonal fluctuations (Lukacs and Largaespada, 2006).

Apart from gender, many studies have documented an inverse relationship between socio-economic status and caries prevalence. Previous studies have reported higher caries prevalence was found among the children of lower social class and a lower prevalence in children of higher social class (Lalloo et al., 1999, Reisine and Psoter, 2001). This relationship was attributed to increased oral health awareness and access to dental care among those in higher socio-economic status. These trends are similar to the findings from the present study that indicated children whose parents had a low monthly income and education level had a higher caries prevalence than those whose parents had a high monthly income and education level. However, the differences were not statistically significant.

6.3.5 Other factors

The majority of respondents reported tap water as the main source of water at home and only 11% of children in the non-fluoridated area reported that they used water from wells/rivers. In some rural parts of Malaysia, wells are still used as a source of water as they have no access to piped water (Aini et al., 2007). They are not regulated by any federal drinking water standards. Thus, this factor did not have any major impact to the data of the present study.

The high proportion of water filter use reported amongst the population in this study is of interest. Results show that higher filter use was reported among those living in the fluoridated area (60%) in comparison to those in the non-fluoridated area (42.9%). These findings are consistent with other Malaysian studies that reported there was a trend of increase usage of water filters ranging from 22.9% to 85% (Aini et al., 2007, Tan and Razak, 2013). The self-reported reasons for using water filtration devices were mainly for health reasons and to improve water quality (Aini et al., 2007, Loh et al., 2011). There are many types and brands of filters available in Malaysia. It was reported that some brands of water filters in Malaysia had no significant effect on fluoride content from drinking water. For example, a study that collected water samples among households in Selangor reported that the mean fluoride concentration of unfiltered water (0.541 ± 0.167 ppm) remained unchanged after being filtered (0.534 ± 0.192 ppm) (Tan and Razak, 2013). Another study conducted in Johor reported that 59% of the studied samples used a carbon activated water filtration system. These carbon filters had no effect on fluoride levels in drinking water, which level of fluoride remained at 0.43 ppm before and after filtration (Loh et al., 2011). However, it has been documented in the international literature that filtration systems such as reverse osmosis and distillations removed substantial amounts of fluoride (Glass, 1990, Brown and Aaron, 1991, Whitford, 1994, Jobson et al., 1999). With regards to a high number of water filter users in this study, it is likely they are not of the reverse osmosis type so it can be assumed that they did not have any significant effect on fluoride removal as reported by other local studies. This likely explains why fluoridated water remained a significant factor associated with fluorosis and preventive caries effect.

6.4 Methodological considerations, study strengths and limitations

6.4.1 Methodological considerations

6.4.1.1 Study design and data analysis

This study is a single point cross sectional survey that evaluated the effect of a change of fluoride level in the water supply on dental fluorosis and caries. Fluorosis prevalence was compared between two birth cohorts that were exposed to different fluoride levels during the critical period of maxillary central incisor development.

Dental fluorosis status was directly comparable between two birth cohorts. The comparison is possible because the main effect on fluorosis development was during the pre-eruptive period. There may be some changes in the clinical appearance post-eruption but this probably has a minimal effect of the prevalence and severity of fluorosis. In any case the ageing effect was likely to be similar between cohorts with regards to fluorosis measurement. When performing the analysis, the change in fluorosis prevalence in the fluoridated community was compared to the change in the non-fluoridated community. The baseline prevalence data were extracted from the groups that were exposed to the old fluoride level (0.7 ppm) and the ‘after’ prevalence data were extracted from the group that were exposed to the new fluoride level (0.5 ppm) after reduction occurred. This approach resembles the ‘difference of differences’ approach, which is commonly used to assess the impact of water fluoridation (Listl et al., 2016, Singhal et al., 2017).

In contrast to the fluorosis analysis, the caries status of different birth cohorts was not directly comparable because of the different stages of development of the dentition in the different age groups involved. Permanent caries experience increased with age. This pattern reflects the biological change in the process of ageing, which impacts on caries prevalence, namely the number of teeth present and the accumulation of caries over time. The ageing effect was controlled using zero-inflated negative binomial and generalized linear model regressions when estimating the difference of the differences of caries experience between fluoridated and non-fluoridated areas. Interaction between age and fluoridation status were tested and adjusted in the model when performing the analyses. Comparison of mean caries experience (D_{4-6}MFT) and caries prevalence (D_{4-6}MFT>0) between cohorts exposed to different fluoride levels (after controlling for ageing effect) revealed a significant difference. This indicates that the caries preventive effect is still maintained at 0.5 ppm following the reduction of fluoride level in the water. Children in both age groups in the fluoridated area were mainly exposed to 0.5 ppm fluoride in the water throughout their life and the full fluoridation effect can be seen at this level of concentration.

There is a common problem of dental caries data in children that data are often skewed. This is due to the fact that the counts are increasingly characterized by a large number of zero-counts as oral health has improved over time (Preisser et al., 2012). To overcome excess zeros and over-dispersion, the present study used negative binomial regression models as recommended by several authors when dealing with count regression modelling such as dental caries (Lewsey and Thomson, 2004, Preisser et al., 2012). In addition, the sample size of the present study was large enough to increase the normality of the distribution of means. Hence the models are applicable to answer the research question.

The limitations of a single point cross sectional survey when assessing the effect of water fluoridation has also been acknowledged by other authors (Singhal et al., 2017). This limitation applies to the results in the current study. Ideally a two-point survey should be carried out to confirm the findings. As this is the first study that evaluates the effect of a reduction of fluoride level in the Malaysian water supply, the results from this study could be used as a baseline data and a follow-up survey will be considered in future work. Detail discussion related to recommendations for study design in water fluoridation studies has been discussed previously in the systematic review chapter (Section 2.7.3).

6.4.1.2 Time factor for outcome measurement

Time was important in examining the prevalence of fluorosis and caries in the population. The study was considered as particularly timely for this purpose for several reasons. Fluorotic enamel maybe affected by some external factors after eruption, such as wear or dental treatment, although this change would be minimal with mild fluorosis across a limited number of years. Children aged 12 years-old were chosen instead of an adolescent group because some evidence suggests that the presentation of fluorosis might be diminished over time (Wong et al., 2016, Do et al., 2016). The population were also less likely to have aesthetic dental treatment for fluorosis condition at this age. Children who were expected to be affected by the change of fluoride level in the water supply (0.5ppm) would be at age 9 years old in 2015. This age group would have upper central incisors teeth present for clinical examination of fluorosis to take place.

6.4.2 Study strengths and limitations

6.4.2.1 Response rate

The study had achieved the required sample size (Section 4.4.2.3) and received an excellent overall response rate (81.1%) for a population study. Similarly, satisfactory response rates for individual state and age groups were also achieved. The good response rates were attributed to several factors including an incentive offered to the participants, good cooperation from the schools, teachers, parents and children. As described in (Section 4.10.2) children in this study were provided with a toothbrush and toothpaste as a token of appreciation and the parents were offered an incentive of entry to a prize draw for one of twenty shopping vouchers worth MYR 100 (USD 23) each. The teachers were also particularly helpful in distributing the questionnaire and encouraged parent's consent during data collection.

6.4.2.2 Sample selection

There might be a criticism about sample selection bias in this study because of demographic dissimilarities between the fluoridated and non-fluoridated areas. The distribution of parents' education level was almost similar in both areas, except there were more fathers with college education level in the fluoridated area (15%) than those in non-fluoridated area (10%). Similar patterns were observed in terms of mothers' education level. Most fathers in the fluoridated area had a higher income in comparison to those in non-fluoridated area. However the difference was not obvious in terms of mother's income level. The majority of the mothers' in both areas earned less than MYR 1999. Eliminating variations in socioeconomic structure may not be possible due to limited geographic areas available to act as negative controls (non-fluoridated). As highlighted in many water fluoridation studies, having a comparable comparison community is often challenging. To date, about 77% of the Malaysia population receive fluoridated water, thus the negative comparison is less readily available. In Peninsular Malaysia, Kelantan is the only state that is not fluoridated. This is due to political reasons. The state is ruled by the opposition and water fluoridation was discontinued in 1995. Many attempts were made to reinstate water fluoridation in Kelantan. In 2006, reinstitution of fluoridation began in two districts in that state namely, Pasir Mas (65.2%) and Machang (65%) (Oral Health Division Ministry of Health Malaysia, 2011). In 2012, the reinstitution of fluoridation expanded to several districts, which include Tumpat (31.8%), Pasir Puteh (22.8%) and Kota Bahru (5.5%). However the coverage for the whole population is still relatively low (Dental Division Kelantan Malaysia, 2012). These districts were among the affluent areas within the state that were excluded from the sampling frame. The exclusion of some districts explained the differences in terms of socio-economic status between study populations. One state that is most comparable in terms of demographic characteristics is Terengganu. However this state has a history of temporary cessation of water fluoridation in 1999 because of the change in political leadership and was not suitable to answer our research question. Negeri Sembilan was found to be the closest state that matched the demographic profile to non-fluoridated state and geographically feasible for data collection. The authors were aware that caries levels are expected to differ by socio-demographic characteristics and these factors were controlled for in the multivariate analysis.

6.4.2.3 Concentrations of fluoride level in the water

Concentrations of fluoride level in the water rely on the state technical report from each studied area. Although no attempt was made to validate the fluoride concentration in the water supply, data from the state technical report was considered reliable as it involves a rigorous monitoring process (Dental Division Negeri Sembilan Malaysia, 2012). In addition, Negeri Sembilan is among the fluoridated states that has been reported to be very consistent in maintaining fluoride levels as recommended by the Malaysian Ministry of Health (Oral Health Division Ministry of Health Malaysia, 2011, Oral Health Division Ministry of Health Malaysia, 2014).

6.4.2.4 Study instrument

Questionnaire development

The survey instrument used in this survey was previously used by the Malaysian National Survey (Oral Health Division Ministry of Health Malaysia, 2001, Oral Health Division Ministry of Health Malaysia, 2013) and Australian Child Dental Survey (Do, 2004) that explored fluoride exposure history, oral hygiene habits and infant feeding practices among children. Prior to use of the questionnaire, it was further revised, translated to the Malay-language, face validated and piloted among a group of Malaysian children. The internal consistency of the questionnaire was acceptable with a Cronbach's alpha (α>0.80) for this study (Field, 2009). The rigorous process of questionnaire development is an added value to this study.

Self-reported behaviour

A common limitation with this type of study is that it relied on parents' self-reported behaviour. A reliance on self-report data is common in researching into many health-related behaviours, such as diet, physical activity, smoking and alcohol use. There is a potential risk that respondents tend to answer the questions towards what is socially acceptable. For example in terms of oral hygiene habits, some parents appeared to be aware of the ideal toothbrushing frequency and fluoridated toothpaste. Several steps were taken to minimize this bias by encouraging honesty and emphasized that the results would not be individually identifiable.

In addition, effort was also made to validate parents' self-reported answer on the use of fluoridated toothpaste. A question on the 'brand of toothpaste' helped in validating fluoridated and non-fluoridated toothpaste use amongst the respondents. The brands of toothpaste used as reported by the respondents, were crosschecked with the list of type of toothpastes available in the Malaysian market from the local studies (Musa and Saub, 1998, Tan, 2003). If the brand answered by the participants was not available in the list, no correction was made in the data management, and the answer (fluoridated or non-fluoridated toothpaste) was solely based on what was reported by the respondents. Similarly, if the respondents answered more than one brand that have both fluoridated and non-fluoridated toothpastes, no validation via toothpaste brand was attempted.

Recall bias

There was a possibility of recall bias in the questionnaire data. This is a common limitation in this type of approach to data collection (Holloway and Ellwood, 1997). In addition, the ability of parents to recall the nature of oral hygiene habits and infant feeding practices were likely to become less accurate with the passage of time. It is possible that the parents of the younger children probably provided more accurate data because of the shorter time interval between the practicing of the habit and the answering of the questionnaire. Nevertheless, retrospective methods of data collection are commonly reported in the literature because of its practicality, time saving and cost-effectiveness. The possible recall bias is not expected to systematically affect the associations explored in this study.

The questionnaire in this study was designed to minimize this bias. For instance with regards to questions on oral hygiene habits (i.e. age started toothbrushing with toothpaste), a point in time reference was used to aid responses and subsequent broad categories were used in the analysis. The data management process also assists in identifying and correcting some of the recall biases. One example relates to the question on infant feeding practices. Respondents with missing or conflicting information were excluded from further analysis. An example of conflicting information is when a respondent reported ‘never fed with infant formula’ (Question 9) but answering the following question on infant formula feeding time period (Question 10 and Question 11) (Appendix 14).

6.4.2.5 Outcome assessment

Examiner reliability

Intra and inter-examiner reliability for caries and fluorosis assessment were substantial to excellent according to the classification by Landis and Koch (1977). This adds credit to the study and could be attributed to intensive training of the clinical and photographic examiners and frequent refresher sessions. Additionally, a single trained examiner carried out the clinical examination of caries and fluorosis. This approach improved the reliability of the collected data since there was no inter-examiner variation.

Blinding of examiner

It could be argued that the examiner was not blind on the children’s residential status during clinical examination. Ideally, the examination should be done at a neutral site. For example, a study in Scotland relocated the study participants for clinical examination to enable blinding assessment of oral health status between those from fluoridated and non-fluoridated areas (Stephen et al., 2002). However, in the present study, the location of the research sites made it logistically impractical and impossible to do so. However, this bias was minimized by having only one clinical examiner and blinded data entry. The caries data were recorded on clinical examination forms and entered into ICDAS software interface by research assistants, who were blinded towards children's residential status and unaware of the value of each code.

On the other hand, this consideration did not affect the quality of fluorosis data, because it was scored blind using photographs. However, concerns might be raised in terms of examiner bias of knowing the age of the children from the photographs. Although, the examiners were blinded towards residential status, those from the younger age groups may be identifiable based on the stage of dental development, apparent on the photographs. The only way to overcome this issue is by examining cropped photographs of two upper central incisors without showing other teeth. However, this approach could be technically burdensome and extremely time consuming. Furthermore there are potential issues with distortion and poor image quality with a 'cropped photographs technique' (Do, 2004). Furthermore, this may defeat other benefits of using photographs such as future use of the image for data comparison and examiner training.

Fluorosis assessment

In this study, maxillary central incisors were chosen as the sole site for the measurement of fluorosis. Using only index teeth to measure fluorosis may cause underestimation of the true fluorosis prevalence in the study population. However, restricting the analysis to maxillary central incisors help in controlling of other potential confounders when examining all erupted permanent teeth. This includes minimising variation in dental fluorosis due to tooth eruption status and variation between tooth types exposed to different fluoride levels during dentition development (Evans and Stamm, 1991b). Using the central incisors as the index teeth also has the advantage of using the teeth that are likely to be of greatest aesthetic concern.

In terms of photographic assessment of fluorosis, only 12 photographs out of 1155 photographs were discarded due to poor quality. The low proportion of discarded photographs could be attributed to the used of digital photography. This method allows the examiner to evaluate the quality of the image captured during the clinical examination immediately post exposure. The main problem faced in taking intra-oral photographs of anterior teeth is specular reflection. Two alternative methods have been recommended to overcome this problem. Firstly by using polarizing filters (Robertson and Toumba, 1999) and secondly by taking photographs at an angle to ensure that the flash is not reflected back into the lens (Cochran et al., 2004a, Pretty et al., 2012). The second method was employed in this study as it was deemed appropriate with digital camera as the image can be repeated if the quality of the image is not acceptable. This technique may be subject to variability in the angle at which the camera is held and may lead to bias in operator standardization. However this bias was likely to be of limited effect in the present study as only one examiner operated the camera.

It may be argued that teeth with dental fluorosis may be confused with other developmental defects of enamel such as molar incisor hypomineralisation (MIH). In terms of clinical appearance, enamel opacities due to fluorosis are diffuse and bilaterally distributed in contrast to the well-demarcated borders of hypomineralisation in MIH.

The similarity of appearance is more obvious in moderate to severe forms, which present as brownish and pitted enamel. It is acknowledged that the possibility of misclassification may have occurred, however the chance of misclassification is low. This is supported by data relating to the prevalence of moderate fluorosis in the non-fluoridated area (0.4%) which can act as a reference group. If there were a misclassification of MIH as fluorosis this would have also reflected on moderate fluorosis prevalence in the non-fluoridated area. In addition, looking at the prevalence of MIH in Malaysia, the available data reported prevalence of MIH was 16.9% with the majority of the condition in mild form, and affecting mainly the first permanent molars rather than the incisors (Hussein et al., 2015). Unlike fluorosis, to diagnose MIH, at least one first permanent molar has to be affected and usually the condition is more sensitive to thermal stimuli (Lygidakis et al., 2010, Alaluusua, 2010). This requirement can be assessed during clinical examination as opposed to photographic examination. Thus, further research is needed to improve assessment of MIH using photographs and how it can be done alongside other developmental defects of enamel such as fluorosis.

In addition, there could have been some misclassification bias due to the difficulty in distinguishing between moderate fluorosis with brownish pitting appearance and early caries lesions. However this bias should be minimal due to the use of single clinical examiner, adequate training and calibration exercise and substantial examiner reliability scores for caries and fluorosis diagnosis.

Caries assessment

The 9 year-old children in this study are in the mixed dentition stage of dental development. There is always some problem in determining if missing primary teeth in the mixed dentition is due to extraction due to dental caries or if the teeth have been lost by natural exfoliation. In this study, the missing teeth were coded as unerupted based on the chronological age of eruption of the primary teeth. If the primary teeth were missing earlier than the chronological age, respondents were asked reasons for missing teeth. If the respondents did not recall reasons for missing teeth (i.e. due to caries) they were considered as missing due to natural exfoliation and the successor permanent tooth recorded as unerupted. As this was an epidemiology study, radiographs were not taken to identify missing teeth due to other reasons such as congenitally missing teeth or failure of eruption.

The primary requirement for applying the ICDAS II system is the examination of clean and dry teeth. This method of examination without doubt requires more instruments that incur cost and prolong the examination period. The difference between D1 and D2 is only based on whether the detection is viewed while wet (D2) or dry using compressed air (D1). However, air drying of teeth using compressed air was not part of the diagnostic process as this was considered impractical in the community setting as used in this study (de Amorim et al., 2012). Drying teeth using gauze may not be an ideal condition to reflect early caries lesion, D1. This may cause an underestimation of the true population caries estimate for D1. However, the difference in methodology was not expected to have a major impact on comparison with other studies that use the tradition DMF index, where the threshold for a diagnosis of “decay” is into dentine.

ICDAS II consists of a two digit-code system and a new programme is required for analysis. Challenges occur in analyzing ICDAS data in particular when several combination codes exist on the same surface. For example when restoration at tooth level were observed on the same surface in the presence of one or more enamel or dentine carious lesions. Whether the condition will be counted as a filling or as a carious lesion. This problem was solved by creating cut off points for filling and caries at D1-3 (restoration supersedes caries, D4-6 caries supersedes restoration) as described in methodology chapter (Section 4.7.1.2). Guidelines on how to analyse the combination codes are not available, therefore a decision was made based on data reported from previous studies (Agustsdottir et al., 2010, Cadavid et al., 2010, de Amorim et al., 2012, Iranzo-Cortes et al., 2013).

6.4.2.6 External validity

Sample from this study is representative for the state of Kelantan and Negeri Sembilan and suitable to infer the findings for population in Peninsular Malaysia. Results may not be suitable for generalizing to the Borneo region of Malaysia due to differences in dietary patterns, ethnic and cultural background.

6.4.2.7 Causal inference from cross sectional study

The cross-sectional nature of the data did not allow for confirmation of a causal relationship. This limitation is particularly an issue for evaluation of risk factors associated with caries and fluorosis (Beck, 1998). However, this limitation may not affect the evaluation of a population-based preventive approach such as water fluoridation because the intervention is at population-level and the majority (96.5%) of the participants lived in a fluoridated community have access to fluoridated water supply.

6.5 Chapter summary

To the best of authors' knowledge, this is the first study that has investigated the impact of downward adjustment of fluoride level from 0.7 to 0.5 ppm in the public water supply on both fluorosis and dental caries as the outcome. The strengths of the study were its excellent response rate, good examiner reliability, sound sampling technique and representative sample size. The strength in outcome measurement includes blind-scoring of photographs and caries scoring at different thresholds of severity using ICDAS II criteria. The strength of the analysis is that a range of confounding factors were controlled for in the multivariate model. There exist some limitations, which include recall bias, self-reported behaviour of oral health and infant feeding practices and the cross-sectional nature of the data. The present study provides evidence that the change in fluoride level from 0.7 to 0.5 ppm has resulted in a change in fluorosis prevalence and also has significant impact on caries prevalence at different thresholds of severity. Fluorosis prevalence was associated with fluoride in the water and some factors in relation to infant-feeding practices. Caries prevalence was associated with gender, fluoride in the water and age started toothbrushing with toothpaste. The implications of the findings on practice and future research are discussed in the next chapter.

7 Conclusions and study implications

This chapter outlines the overall thesis conclusions in Section 7.1. This is followed by a discussion of the implications of the study for policy, practice and future work in Section 7.2.

7.1 Conclusions

This thesis presents the results of two projects, collectively aimed at understanding the impact on oral health of a downward adjustment of the concentration of fluoride in the public water supply from 0.7 to 0.5 ppm. In doing so, a systematic review was conducted to critically appraise the literature on stopping fluoridation or reducing the level of fluoride level in the public water supply on dental caries and fluorosis. Findings highlighted the gaps in knowledge and several methodological issues in this area of research, such as lack of examiner blinding and control of confounders. The main study aimed to evaluate the impact of a reduction in the fluoride level in the Malaysian water supply on dental fluorosis and caries and explore risk factors associated with such conditions. Effort was made to address some of the key issues with regards to methodological issues and potential confounders highlighted in the systematic review chapter.

The main study conclusions are presented to answer the research questions set out in Chapter 3 as follows:

Question 1. What is the prevalence and severity of dental fluorosis following a 0.2 ppm reduction of fluoride level in the public water supply?

The change in water fluoridation policy that reduced the concentration of fluoride in the Malaysian water supply from 0.7 to 0.5 ppm, has resulted in a decrease in the prevalence of fluorosis. The results confirm that the prevalence of dental fluorosis is sensitive to even minor changes in fluoride exposure from drinking water. The decline in the prevalence of fluorosis was observed across two birth cohorts who were at different stages of tooth development when the policy initiative was introduced. Children who were born after the introduction of the policy initiative had a lower prevalence of fluorosis compared with those who were born before the introduction of the policy initiative and whose first two years of life were not affected by the reduced fluoride level in the water supply. However the difference in fluorosis prevalence between cohorts in the fluoridated areas was not statistically significant.

Overall, fluorosis prevalence was significantly higher in the fluoridated area compared to the non-fluoridated area. In terms of severity, most of the condition was categorised as very mild and mild fluorosis.

Question 2. What is the prevalence and severity of dental caries following a 0.2 ppm reduction of fluoride level in the public water supply?

Following the change in fluoride level, results show that children who were exposed to 0.5 ppm fluoride in the water remained significantly associated with lower caries experience ($D_{4\text{-}6}$MFT) than those who did not have any exposure. Analysis was conducted between caries experience in fluoridated and non-fluoridated areas because direct comparison across birth cohorts was not possible due to different stages of dental development and tooth eruption present in the 9 and 12 year-old age groups.

Examining caries prevalence using ICDAS II criteria enabled detection of caries at different thresholds. Results showed that there was more enamel caries diagnosed than dentine caries in both age groups and areas of residence. The inclusion of early caries lesions contributed to higher overall caries score diagnosed using ICDAS II in comparison to DMF score. Although the prevalence of early caries lesion is higher than dentine caries, results indicate that the difference in the prevalence between fluoridated and non-fluoridated is narrower when the caries is reported at this threshold (D_{1-6}MFT).

Question 3. Has the policy measure to reduce the fluoride level in the water supply maintained the preventive effect of dental caries and reduced the prevalence of fluorosis?

Findings suggest that the caries preventive effect at 0.5 ppm between the fluoridated and non-fluoridated areas remained statistically significant following reduction of fluoride level in the water. However, it is important to highlight that the optimal fluoride concentration of 0.5 ppm is effective in this study population that has widespread use of fluoride toothpaste. In terms of fluorosis, the change in water fluoridation policy to 0.5 ppm has resulted in a decrease in fluorosis prevalence.

Question 4. Are there any other risk factors (in particular exposure to difference sources of fluoride) associated with dental fluorosis?

Several factors were identified as risk factors for fluorosis in this study population. These include the age at which finished breastfeeding finished, age when infant formula was started and finished, the duration of formula use, exposure to fluoride in the water, parents' education level, parents' monthly income, use of infant formula, and type of water used to reconstitute the formula. However, only two risk factors remained significantly associated with higher fluorosis prevalence in a logistic regression model, namely, exposure to fluoride from water and type of water used to reconstitute infant formula. This confirms existing evidence that fluoride in the water has important contribution to total fluoride intake and excessive exposure increases the risk of having fluorosis.

Question 5. Are there any other risk factors (in particular exposure to difference sources of fluoride) associated with dental caries?

In terms of non-modifying factors, children who were female, had parents' with low education level and low fathers' monthly income were significantly associated with high caries prevalence. After controlling for other factors in the logistic regression model, the fluoride level in the water supply and type of water used to reconstitute formula remained significantly associated with the prevalence of dentine caries (D_{4-6}MFT). Gender and exposure to fluoride level in the water supply were the significant factors related to the prevalence of dental caries at all levels of severity (D_{1-6}MFT).

Overall conclusion

This study provides evidence to further support the effectiveness of water fluoridation in caries prevention. Results provide support to the policy initiative of the reduction of fluoride level from 0.7 to 0.5 ppm in Malaysian water supply. Modification to the fluoridation policy has reduced fluorosis and maintained a caries prevention benefit. Several factors were found to be associated with fluorosis and caries prevalence. While the finding of this study contributes to the knowledge of the impact of reducing fluoride level in the water supply on dental caries and fluorosis, future research is still needed to confirm the effectiveness of such a reduction in the longer term. Future research could address some of the limitations and new research questions raised from this study.

7.2 Study implications

7.2.1 Implications for policy and practice

While results indicate a reduction in the prevalence of dental fluorosis, the population always needs close monitoring because it is an indication of the balance between the benefit and the risks of the use of fluoride in the prevention of dental caries. Findings from this study indicate that the use of fluoridated tap water to reconstitute infant formula milk was significantly associated with higher fluorosis prevalence. This poses an important question as to whether there is a need to develop a guideline with regards to infant formula preparations in Malaysia, in particular to those living in fluoridated areas. More research is needed to further examine the contribution of this factor and type of infant formula with regards to fluorosis. Looking at the international evidence, there is a variation across different countries in relation to advice regarding the use of infant formula. For example in the United States, the American Dental Association suggested that those who are concerned about their children’s exposure to fluoride should use ready-to-feed formula or should reconstituted the formula with water that has no or low levels of fluoride (Berg et al., 2011). In Canada, no specific recommendation regarding infant formula preparations was made.

This study provides evidence to further support the effectiveness of water fluoridation in the prevention of dental caries. Findings also provide support to the new Malaysian water fluoridation policy of optimum fluoride concentration at 0.5 ppm. Although results indicate that the preventive effect of water fluoridation at 0.5 ppm is still maintained alongside the use of fluoridated toothpaste, dental caries is still widespread among the Malaysian population. The high caries into dentine prevalence (40.2% at age 9 and 53.5% at age 12) in Kelantan indicates there is an urgent need to reinstate and expand the coverage of water fluoridation in Kelantan. It is acknowledged that expansion of water fluoridation programmes is politically challenging and requires lengthy and complex procedures before it can be implemented. Another issue to consider is targeting advice on fluoride use in relation to fluoridation status. At the present time in Malaysia similar advice is given regardless of exposure to fluoridated or non-fluoridated water.

In addition, advice on the information of water fluoridation status, the use of fluoride toothpaste and infant formula must be disseminated to parents and caregivers before or as soon as possible after the birth of a child. This is important since the first years of life are critical in terms of the prevention of fluorosis. While dental attendance before the age of 2 years is uncommon, contact with other health professionals (e.g. midwives) and nursery caregivers is high. Thus, collaboration with these providers is important to improve dissemination of oral health information. A system of oral health care of antenatal mothers and in early childhood care has been implemented by the Ministry of Health, Malaysia. Therefore, these programmes can be used as a platform to collaborate and emphasise on the importance of maximising caries prevention and minimising fluorosis.

Although there was a decline in terms of moderate fluorosis (from 10.9% to 6.7%) following reduction of fluoride concentration in the water supply, continuing efforts should be made to monitor fluorosis at this level of severity. This warrants another research question whether 0.5 ppm is an appropriate level of fluoride concentration in Malaysian drinking water. The optimal fluoride level in the drinking water has traditionally been calculated using Galagan formula, which estimated the daily water intake under different temperatures condition in the US during the late 1950s (Galagan et al., 1957). The formula was proposed for American children, presumed that 44% of their total fluid intake was milk with negligible fluoride levels. However, it can be argued whether this formula is appropriate for determining fluoride level in other countries with different climatic conditions and fluid consumption. A study conducted in Pakistan used a modification to the original Galagan formula on the basis of different fluid consumption patterns (especially a low intake of milk) among the Pakistan population (Khan et al., .2004). Results indicated that the appropriate level of fluoride for Pakistan with an average temperature of 29 degrees was 0.39 ppm. Therefore, further research is needed to determine any further revision to water fluoridation policy in Malaysia.

7.2.2 Implications for future research

- Results from this study were based on a single point-survey that compared children in two birth cohorts who were exposed to different fluoride level in the water supply during tooth development. This study designs is most relevant to assess dental fluorosis. Whether fluoride concentration at 0.7 ppm is better than 0.5 ppm in reducing caries prevalence remains unanswered. As a randomized control trial was not an option and a longitudinal study would be expensive to conduct, a two-point survey with a comparison group should be considered. Ideally a study with a positive control (still fluoridated at a higher level) community is needed to confirm the findings.
- Future work should incorporate measurement of tap water consumption. This information would be useful to explore the relationship of water intake and outdoor temperature among children and adults. In addition, fluid consumption from non-tap water such as processed beverages and foods should also be considered.
- The present study only collected data on feeding practices during infancy with a focus on infant formula and breast-feeding practices. Other important variables that were not measured in this study are weaning and dietary patterns. This should be addressed in future work.
- Further research is necessary to determine the actual fluoride level of infant formula and infant foods in the Malaysian market that require reconstitution with liquid prior to consumption. These data would be useful to assist in formulating advice with regards to infant feeding practices.

- Future research should also consider measurement and validation of the concentration of fluoridated toothpaste available in the Malaysian market. This is particularly useful to explore the association of the combined effect of water fluoridation at 0.5 ppm and fluoride toothpaste at specific concentrations. Therefore these two programmes can be endorsed with a coherent link with each other.
- This study only focused on the child population, future work should consider evaluating the downward adjustment of fluoride level on adults' oral health status.

7.2.3 Recommendations

In general, continuation and expansion of water fluoridation at 0.5 ppm is recommended for the Malaysian population. It is important to regularly monitor and evaluate the impact of water fluoridation on caries and fluorosis. Relevant data would assist in promotion, maintenance and regulation of water fluoridation, as well as guidance on the use of other forms of fluoride.

The World Health Organization has recommended a range of 0.5 to 1.0 ppm for artificial fluoridation (World Health Organization, 2004). It was further emphasised in the guideline that the value is not ‘fixed’ but is intended to be adapted to take account of local conditions of specific countries. The present study was conducted in a tropical country with average temperatures of 27 to 30 degrees Celsius (Malaysian Metrological Department, 2017). Findings indicate that fluoridated water at 0.5 ppm concentration further reduce fluorosis prevalence without compromising caries preventive effect. The optimal level of 0.5 ppm is appropriate in warmer climates when combined with exposure to fluoride containing toothpaste. The findings could be relevant to other tropical and subtropical countries in setting up optimal fluoride concentration in the water.

References

ADAIR, S. M., HANES, C. M., M., R. C. & WHITFORD, G. M. 1999. Dental caries and flurosis among children in a rural Georgia area. *Pediatric Dentistry,* 21**,** 81-85.

AGUSTSDOTTIR, H., GUDMUNDSDOTTIR, H., EGGERTSSON, H., JONSSON, S. H., GUDLAUGSSON, J. O., SAEMUNDSSON, S. R., ELIASSON, S. T., ARNADOTTIR, I. B. & HOLBROOK, W. P. 2010. Caries prevalence of permanent teeth: a national survey of children in Iceland using ICDAS. *Community Dentistry and Oral Epidemiology,* 38**,** 299-309.

AINI, M., FAKHRUL-RAZI, A., MUMTAZAH, O. & CHEN, J. M. 2007. Malaysian households' drinking water practices: A case study. *The International Journal of Sustainable Development and World Ecology,* 14**,** 503-510.

AL-ALOUSI, W., JACKSON, D., CROMPTON, G. & JENKINS, O. C. 1975. Enamel mottling in a fluoride and in a non-fluoride community. A study. *British Denalt Journal,* 138**,** 9-15.

ALALUUSUA, S. 2010. Aetiology of Molar-Incisor Hypomineralisation: A systematic review. *European Archives of Paediatric Dentistry,* 11, 53-58.

ALMERICH-SILLA, J. M., BORONAT-FERRER, T., M., M.-C. J. & IRANZO-CORTES, J. E. 2014. Caries prevalence in children from Valencia (Spain) using ICDAS II criteria, 2010. *Medicina Oral Patologia Oral Cirugia Bucal,* 19**,** e574-580.

AMARRA, M. S., KHOR, G. L. & CHAN, P. 2016. Intake of added sugar in Malaysia: a review. *Asia Pacific Journal of Clinical Nutrition,* 25**,** 227-240.

ANGELILLO, I., TORRE, I., NOBILE, C. & VILLARI, P. 1999. Caries and fluorosis prevalence in communities with different concentrations of fluoride in the water. *Caries Research,* 33**,** 114-122.

ATTWOOD, D. & BLINKHORN, A. 1988. Trends in dental health of ten-year-old schoolchildren in South-West Scotland after cessation of water fluoridation. *The Lancet,* 332**,** 266-268.

ATTWOOD, D. & BLINKHORN, A.S. 1989. A reassessment of the dental health of urban Scottish schoolchildren following the cessation of water fluoridation. *Community Dental Health,* 6, 207-214.

AUSTRALIAN INSTITUTE OF HEALTH AND WELFARE, CHRISOPOULOS, S., HARFORD, J. E. & ELLERSHAW, A. 2016. *Oral health status and dental care in Australia: key facts and figure 2015.* Canberra: AIHW.

AUSTRALIAN NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL 2007. *A systematic review of the efficacy and safety of fluoridation.* Canberra: National Health and Medical Research Council, Australia.

BAELUM, V., MANJI, F. & FEJERSKOV, O. 1986. Posteruptive tooth age and severity of dental fluorosis in Kenya. *European Journal of Oral Sciences,* 94, 405-410.

BANTING, D. W. 1999. International fluoride supplement recommendations. *Community Dentistry and Oral Epidemiology,* 27**,** 57-61.

BANTING, D., EGGERTSSON, H., EKSTRAND, K.R., ZANDONÁ, A.F., ISMAIL, A.I., LONGBOTTOM, C., PITTS, N.B., REICH, E., RICKETTS, D., SELWITZ, R. & SOHN, W. 2005. Rationale and evidence for the international caries detection and assessment system (ICDAS II). *Ann Arbor, 1001,* 48109-1078.

BARDSEN, A. 1999. "Risk periods" associated with the development of dental fluorosis in maxillary permanent central incisors: a meta-analysis. *Acta Odontologica Scandinavica,* 57**,** 247-256.

BARDSEN, A. & BJORVATN, K. 1998. Risk periods in the development of dental fluorosis. *Clinical Oral Investigations,* 2**,** 155-160.

BECK, J. D. 1998. Risk revisited. *Community Dentistry and Oral Epidemiology,* 26**,** 220-225.

BELTRÁN-AGUILAR, E. D., BARKER, L. & DYE, B. A. 2010. *Prevalence and severity of dental fluorosis in the United States, 1999-2004.* NCHS Data Brief, 53, 1-8. Centers for Disease Control and Prevention.

BELTRÁN-AGUILAR, E. D., GRIFFIN, S. O. & LOCKWOOD, S. A. 2002. Prevalence and trends in enamel fluorosis in the United States from the 1930s to the 1980s. *The Journal of the American Dental Association,* 133**,** 157-165.

BENAZZI, A. S., DA SILVA, R. P., DE MENEGHIM, M., AMBROSANO, G. M. & PEREIRA, A. C. 2012. Dental caries and fluorosis prevalence and their relationship with socioeconomic and behavioural variables among 12-year-old schoolchildren. *Oral Health and Preventive Dentistry,* 10**,** 65-73.

BERG, J., GERWECK, C., HUJOEL, P. P., KING, R., KROL, D. M., KUMAR, J., LEVY, S., POLLICK, H., WHITFORD, G. M., STROCK, S., ARAVAMUDHAN, K., FRANTSVE-HAWLEY, J., MEYER, D. M. 2011. Evidence-based clinical recommendations regarding fluoride intake from reconstituted infant formula and enamel fluorosis: a report of the American Dental Association Council on Scientific Affairs. *The Journal of the American Dental Association,* 142**,** 79-87.

BERKOWITZ, B., HOLLAND, G. & MOXHAM, B. 1992. *Oral Anatomy*. Woulf Publications.

BRAGA, M. M., OLIVEIRA, L. B., BONINI, G. A., BONECKER, M. & MENDES, F. M. 2009. Feasibility of the International Caries Detection and Assessment System (ICDAS-II) in epidemiological surveys and comparability with standard World Health Organization criteria. *Caries Research,* 43**,** 245-249.

BRATTHALL, D. 2000. Introducing the Significant Caries Index together with a proposal for a new global oral health goal for 12-year-olds. *International Dental Journal,* 50**,** 378-384.

BRATTHALL, D., HÄNSEL-PETERSSON, G. & SUNDBERG, H. 1996. Reasons for the caries decline: what do the experts believe? *European Journal of Oral Sciences,* 104**,** 416-422.

BROADBENT, J. & THOMSON, W. 2005. For debate: problems with the DMF index pertinent to dental caries data analysis. *Community Dentistry and Oral Epidemiology,* 33**,** 400-409.

BRONCKERS, A.L., LYARUU, D.M. & DEN BESTEN, P.K. 2009. The impact of fluoride on ameloblasts and the mechanisms of enamel fluorosis. *Journal of Dental Research*, 88, 877-893.

BROTHWELL, D. & LIMEBACK, H. 2003. Breastfeeding is protective against dental fluorosis in a nonfluoridated rural area of Ontario, Canada. *Journal of Human Lactation,* 19**,** 386-390.

BROWN, A. E., RAYNOR, P., BENTON, D. & LEE, M. D. 2010. Indices of multiple deprivation predict breastfeeding duration in England and Wales. *European Journal of Public Health,* 20**,** 231-235.

BROWN, M. D. & AARON, G. 1991. The effect of point-of-use water conditioning systems on community fluoridated water. *Pediatric Dentistry,* 13**,** 35-38.

BROWNE, D., WHELTON, H. & O'MULLANE, D. 2005. Fluoride metabolism and fluorosis. *Journal of Dentistry,* 33**,** 177-186.

BURT, B. A. 1999. The case for eliminating the use of dietary fluoride supplements for young children. *Journal of Public Health Dentistry,* 59**,** 269-274.

BURT, B. A. 2004. Pre-and posteruptive fluoride: do both actions control caries? *Journal of Public Health Dentistry,* 64**,** 47-49.

BURT, B. A., KEELS, M. A. & HELLER, K. E. 2000. The effects of a break in water fluoridation on the development of dental caries and fluorosis. *Journal of Dental Research,* 79**,** 761-769.

BURT, B. A., KEELS, M. A. & HELLER, K. E. 2003. Fluorosis development in seven age cohorts after an 11-month break in water fluoridation. *Journal of Dental Research,* 82**,** 64-68.

BUZALAF, M. A., DE ALMEIDA, B. S., OLYMPIO, K. P., DA, S. C. V. E. & DE, C. S. P. S. H. 2004. Enamel fluorosis prevalence after a 7-year interruption in water fluoridation in Jau, Sao Paulo, Brazil. *Journal of Public Health Dentistry,* 64**,** 205-208.

BUZALAF, M. A. & LEVY, S. M. 2011. Fluoride intake of children: considerations for dental caries and dental fluorosis. *Monographs in Oral Science,* 22**,** 1-19.

BUZALAF, M.A. & WHITFORD, G.M. 2011. Fluoride metabolism. *Monographs in Oral Science*, 22, 20-36.

CADAVID, A. S., LINCE, C. M. & JARAMILLO, M. C. 2010. Dental caries in the primary dentition of a Colombian population according to the ICDAS criteria. *Brazilian Oral Research,* 24**,** 211-216.

CANADIAN DENTAL ASSOCIATION. 2007. Fluoride and baby formula. *Journal of Canadian Dental Association,* 73**,** 111.

CHANKANKA, O., LEVY, S. M., WARREN, J. J. & CHALMERS, J. M. 2010. A literature review of aesthetic perceptions of dental fluorosis and relationships with psychosocial aspects/oral health-related quality of life. *Community Dentistry and Oral Epidemiology,* 38**,** 97-109.

CHEN, C. J. A., LING, K. S., ESA, R., CHIA, J. C., EDDY, A. & YAW, S. L. 2010. A school-based fluoride mouth rinsing programme in Sarawak: a 3-year field study. *Community Dentistry and Oral Epidemiology,* 38, 310-314.

CHO, H. J., JIN, B. H., PARK, D. Y., JUNG, S. H., LEE, H. S., PAIK, D. I. & BAE, K. H. 2014. Systemic effect of water fluoridation on dental caries prevalence. *Community Dentistry and Oral Epidemiology,* 42**,** 341-348.

CHUN, K.J., CHOI, H.H. & LEE, J.Y. 2014. Comparison of mechanical property and role between enamel and dentin in the human teeth. *Journal of Dental Biomechanics,* 5.

CLARK, D. C. 1994. Trends in prevalence of dental fluorosis in North America. *Community Dentistry and Oral Epidemiology,* 22**,** 148-152.

CLARK, D. C., SHULMAN, J. D., MAUPOME, G. & LEVY, S. M. 2006. Changes in dental fluorosis following the cessation of water fluoridation. *Community Dentistry and Oral Epidemiology,* 34**,** 197-204.

CLARKSON, J. 1989. Review of terminology, classifications, and indices of developmental defects of enamel. *Advances in Dental Research,* 3**,** 104-109.

CLIFFORD, H., OLSZOWY, H., YOUNG, M., HEGARTY, J. & CROSS, M. 2009. Fluoride content of powdered infant formula meets Australian Food Safety Standards. *Australian and New Zealand Journal of Public Health,* 33**,** 573-576.

COCHRAN, J. A., KETLEY, C. E., SANCHES, L., MAMAI-HOMATA, E., OILA, A. M., ARNADOTTIR, I. B., VAN LOVEREN, C., WHELTON, H. P. & O'MULLANE, D. M. 2004a. A standardized photographic method for evaluating enamel opacities including fluorosis. *Community Dentistry and Oral Epidemiology,* 32**,** 19-27.

COCHRAN, J. A., KETLEY, C. E., ARNADOTTIR, I. B., FERNANDES, B., KOLETSI-KOUNARI, H., OILA, A. M., VAN LOVEREN, C., WHELTON, H. P. & O'MULLANE, D. M. 2004b. A comparison of the prevalence of fluorosis in 8-year-old children from seven European study sites using a standardized methodology. *Community Dentistry and Oral Epidemiology,* 32, 28-33.

CRESSEY, P. 2010. Dietary fluoride intake for fully formula-fed infants in New Zealand: impact of formula and water fluoride. *Journal of Public Health Dentistry,* 70**,** 285-291.

CRUZ-ORCUTT, N., WARREN, J. J., BROFFITT, B., LEVY, S. M. & WEBER-GASPARONI, K. 2012. Examiner reliability of fluorosis scoring: a comparison of photographic and clinical examination findings. *Journal of Public Health Dentistry,* 72**,** 172-175.

DABEKA, R. W., MCKENZIE, A. D., CONACHER, H. B. & KIRKPATRICK, D. C. 1982. Determination of fluoride in Canadian infant foods and calculation of fluoride intakes by infants. *Canadian Journal of Public Health,* 73**,** 188-191.

DE AMORIM, R. G., FIGUEIREDO, M. J., LEAL, S. C., MULDER, J. & FRENCKEN, J. E. 2012. Caries experience in a child population in a deprived area of Brazil, using ICDAS II. *Clinical Oral Investigations,* 16**,** 513-520.

DEAN, H. T. 1934. Classification of mottled enamel diagnosis. *The Journal of the American Dental Association,* 21**,** 1421-1426.

DEAN, H. T. 1938. *Endemic fluorosis and its relation to dental caries,* US Government Printing Office.

DEAN, H.T. 1946. Epidemiological studies in the United States. *Dental caries and fluorine*, 5-31.

DEAN, H. T., ARNOLD, F. A. J. & ELVOVE, E. 1942. Domestic water and dental caries. V. Additional studies of relation of fluoride domestic waters to dental caries experience in 4,425 white children, aged 12-14 years, of 13 cities in 4 states. *Public Health Reports,* 57**,** 1155-1179.

DEN BESTEN, P. K. 1999. Mechanism and timing of fluoride effects on developing enamel. *Journal of Public Health Dentistry,* 59**,** 247-251.

DEN BESTEN, P. & LI, W. 2011. Chronic fluoride toxicity: dental fluorosis. *In Fluoride and the Oral Environment. Monographs in Oral Science*, 22, 81–96.

DENTAL DIVISION JOHOR MALAYSIA 1986. *Epidemiological survey of developmental defects of dental enamel in Johor.* Ministry of Health, Malaysia.

DENTAL DIVISION KELANTAN MALAYSIA. 2012. State Technical Report. *[Laporan Paras Fluorida di Loji Rawatan Air dan Retikulasi, in Malay language]*. Ministry of Health, Malaysia.

DENTAL DIVISION NEGERI SEMBILAN MALAYSIA. 2012. State Technical Report. *[Laporan Paras Fluorida di Loji Rawatan Air dan Retikulasi, in Malay language]*. Ministry of Health, Malaysia.

DEPARTMENT OF STATISTICS MALAYSIA. 2016. *Current population estimates, Malaysia 2014-2016.* Available: www.dosm.gov.my [Accessed: 20/03/2014].

DEPARTMENT OF SURVEY AND MAPPING MALAYSIA. 2013. *Malaysia Report Map*. The Department of Survey and Mapping Malaysia.

DHAR, V., & BHATNAGAR, M. 2009. Physiology and toxicity of fluoride. *Indian Journal of Dental Research,* 20, 350-355.

DHSS 1969. *The fluoridation studies in the United Kingdom and results achieved after 11 years*. A report of the Committee on Research into Fluoridation. London: Her Majesty's Stationary Office.

DO, L. C. 2004. *Fluoride exposure, dental fluorosis and caries among South Australian children.* PhD thesis, University of Adelaide, Australia.

DO, L. G., HA, D. H. & SPENCER, A.J. 2015. Factors attributable for the prevalence of dental caries in Queensland children. *Community Dentistry and Oral Epidemiology,* 43, 397-405.

DO, L. G., HA, D. H. & SPENCER, A. J. 2016. Natural history and long-term impact of dental fluorosis: a prospective cohort study. *Medical Journal of Australia,* 204**,** 25.

DO, L. G., LEVY, S. M. & SPENCER, A. J. 2012. Association between infant formula feeding and dental fluorosis and caries in Australian children. *Journal of Public Health Dentistry,* 72**,** 112-121.

DO, L. G. & SPENCER, A. J. 2007. Decline in the prevalence of dental fluorosis among South Australian children. *Community Dentistry and Oral Epidemiology,* 35**,** 282-291.

DRISCOLL, W. S., HEIFETZ, S. B. & KORTS, D. C. 1974. Effect of acidulated phosphate-fluoride chewabie tablets on dental caries in schoolchildren: results after 30 months. *The Journal of the American Dental Association*, 89**,** 115-120.

DYE, B. A., TAN, S., SMITH, V., LEWIS, B. G., BARKER, L. K., THORNTON-EVANS, G., EKE, P., BELTRÁN-AGUILAR, E., HOROWITZ, A. & LI, C. 2007. *Trends in oral health status: United States, 1988-1994 and 1999-2004.* Vital and health statistics. Series 11, Data from the national health survey**,** 1-92.

EKSTRAND, J. 1989. Fluoride intake in early infancy. *Journal of Nutrition,* 119**,** 1856-1860.

EKSTRAND, J., HARDELL, L. I. & SPAK, C. J. 1984. Fluoride balance studies on infants in a 1-ppm-water-fluoride area. *Caries Research,* 18**,** 87-92.

EKSTRAND, J., ZIEGLER, E.E., NELSON, S.E. & FOMON, S.J. 1994. Absorption and retention of dietary and supplemental fluoride by infants. *Advances in Dental Research,* 8, 175-180.

ELLWOOD, R., O'MULLANE, D., CLARKSON, J. & DRISCOLL, W. 1994. A comparison of information recorded using the Thylstrup Fejerskov index, Tooth Surface Index of Fluorosis and Developmental Defects of Enamel index. *International Dental Journal,* 44**,** 628-636.

ESA, R. & RAZAK, I. 2001. Dental fluorosis and caries status among 12-13 year-old school children in Klang district, Malaysia. *Annals of Dentistry,* 8**,** 20-24.

EVANS, R. W. 1989. Changes in dental fluorosis following an adjustment to the fluoride concentration of Hong Kong's water supply. *Advances in Dental Research,* 3**,** 154-160.

EVANS, D. J. 1991. A study of developmental defects in enamel in 10-year-old high social class children residing in a non-fluoridated area. *Community Dental Health,* 8**,** 31-38.

EVANS, R. W. & STAMM, J. W. 1991a. An epidemiologic estimate of the critical period during which human maxillary central incisors are most susceptible to fluorosis. *Journal of Public Health Dentistry,* 51**,** 251-259.

EVANS, R. W. & STAMM, J. W. 1991b. Dental fluorosis following downward adjustment of fluoride in drinking water. *Journal of Public Health Dentistry,* 51**,** 91-98.

EVANS, R. W. & DARVELL, B. W. 1995. Refining the estimate of the critical period for susceptibility to enamel fluorosis in human maxillary central incisors. *Journal of Public Health Dentistry,* 55**,** 238-249.

FANNING, E. A., GOTJAMANOS, T. & VOWLES, N. J. 1968. The use of fluoride dentifrices in the control of dental caries: methodology and results of a clinical trial. *Australian Dental Journal,* 13**,** 201-206.

FDI (WORLD HEALTH FEDERATION). 1992. A review of the developmental defects of enamel index (DDE Index). Commission on Oral Health, Research &

Epidemiology. Report of an FDI Working Group. *International Dental Journal,* 42**,** 411-426.

FEATHERSTONE, J. D. 1999. Prevention and reversal of dental caries: role of low level fluoride. *Community Dentistry and Oral Epidemiology,* 27**,** 31-40.

FEATHERSTONE, J. D. 2000. The science and practice of caries prevention. *The Journal of the American Dental Association,* 131**,** 887-899.

FEATHERSTONE, J. 2004a. The continuum of dental caries: evidence for a dynamic disease process. *Journal of Dental Research,* 83**,** C39-C42.

FEATHERSTONE, J. D. 2004b. Topical effects of fluoride in the reversal and prevention of dental decay. *Journal of Public Health Dentistry,* 64**,** 32-34.

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**,** 1-14.

FEJERSKOV, O., THYLSTRUP, A. & LARSEN, M. J. 1977. Clinical and structural features and possible pathogenic mechanisms of dental fluorosis. *European Journal of Oral Sciences*, 85, 510-534.

FEJERSKOV, O., MANJI, F. & BAELUM, V. 1988. *Dental Fluorosis: a Handbook for Health Workers.* Munksgaard, Copenhagen.

FEJERSKOV, O., MANJI, F. & BAELUM, V. 1990. The nature and mechanisms of dental fluorosis in man. *Journal of Dental Research*, 69, 692-700.

FEJERSKOV, O., LARSEN, M. J., RICHARDS, A. & BAELUM, V. 1994. Dental tissue effects of fluoride. *Advances in Dental Research,* 8**,** 15-31.

FEJERSKOV, O. 1997. Concepts of dental caries and their consequences for understanding the disease. *Community Dentistry and Oral Epidemiology,* 25, 5-12.

FIELD, A. 2009. *Discovering Statistics using SPSS,* London, Saga.

FISHER, J., GLICK, M. & COMMITTEE, F. W. D. F. S. 2012. A new model for caries classification and management: the FDI World Dental Federation caries matrix. *The Journal of the American Dental Association*, 143, 546-551.

FRENCKEN, J. E., DE AMORIM, R. G., FABER, J. & LEAL, S. C. 2011. The Caries Assessment Spectrum and Treatment (CAST) index: rational and development. *International Dental Journal,* 61**,** 117-123.

GALAGAN, D. J., VERMILLION, J. R., NEVITT, G. A., STADT, Z. M. & DART, R. E. 1957. Climate and fluid intake. *Public Health Reports,* 72**,** 484-490.

GLASS, R. 1990. Water purification systems and recommendations for fluoride supplementation. *ASDC Journal of Dentistry for Children,* 58**,** 405-408.

GOLKARI, A., SABOKSEIR, A., PAKSHIR, H. R., DEAN, M. C., SHEIHAM, A. & WATT, R. G. 2011. A comparison of photographic, replication and direct clinical examination methods for detecting developmental defects of enamel. *BMC Oral Health,* 11**,** 16.

GRIFFIN, S. Q., GOOCH, B. F., LOCKWOOD, S. A. & L., T. S. 2001. Quantifying the diffused benefit from water fluoridation in the United States. *Community Dentistry and Oral Epidemiology,* 29**,** 120-129.

GU, X. S. & SHEN, Y. M. 1989. [Effects of stopping water fluoridation on prevalence of dental caries in children, in Chinese language]. *Zhonghua Yu Fang Yi Xue Za Zhi,* 23**,** 346-348.

HAUGEJORDEN, O. & BIRKELAND, M.J. 2005. Analysis of the ups and downs of caries experience among Norwegian children aged five years between 1997 and 2003. *Acta Odontologica Scandinavica,* 63**,** 115-122.

HAUGEJORDEN, O. & BIRKELAND, M.J. 2006. Ecological time-trend analysis of caries experience at 12 years of age and caries incidence from age 12 to 18 years: Norway 1985–2004. *Acta Odontologica Scandinavica,* 64**,** 368-375.

HE, L.H. & SWAIN, M.V. 2008. Understanding the mechanical behaviour of human enamel from its structural and compositional characteristics. *Journal of the Mechanical Behavior of Biomedical Materials,* 1, 18-29.

HECK, K. E., BRAVEMAN, P., CUBBIN, C., CHAVEZ, G. F. & KIELY, J. L. 2006. Socioeconomic status and breastfeeding inititation among California mothers. *Public Health Reports,* 121**,** 51-59.

HELLWIG, E. & LENNON, A. 2004. Systemic versus topical fluoride. *Caries Research,* 38**,** 258-262.

HOLLENDER, L. & KOCH, G. 1969. Influence of topical application of fluoride on rate of progress of carious lesions in children. A long-term roentgenographic follow-up. *Odontologisk Revy,* 20**,** 37-41.

HOLLOWAY, P. J. & ELLWOOD, R. P. 1997. The prevalence, causes and cosmetic importance of dental fluorosis in the UK. A review. *Community Dental Health,* 14**,** 148-155.

HOLM, A. K. & ANDERSSON, R. 1982. Enamel mineralization disturbances in 12-year-old children with known early exposure to fluorides. *Community Dentistry and Oral Epidemiology,* 10**,** 335-339.

HOLT, R. D., MORRIS, C. E., WINTER, G. B. & DOWNER, M. C. 1994. Enamel opacities and dental caries in children who used a low fluoride toothpaste between 2 and 5 years of age. *International Dental Journal,* 44**,** 331-341.

HONG, L., LEVY, S. M., BROFFITT, B., WARREN, J. J., KANELLIS, M. J., WEFEL, J. S. & DAWSON, D. V. 2006a. Timing of fluoride intake in relation to development of fluorosis on maxillary central incisors. *Community Dentistry and Oral Epidemiology,* 34**,** 299-309.

HONG, L., LEVY, S. M., WARREN, J. J., BROFFITT, B. & CAVANAUGH, J. 2006b. Fluoride intake levels in relation to fluorosis development in permanent maxillary central incisors and first molars. *Caries Research,* 40**,** 494-500.

HOROWITZ, H. S., MAIER, F. J. & THOMPSON, M. B. 1964. The Effect of Partial Defluoridation of a Water Supply on Dental Fluorosis-Results after 11 Years. *American Journal of Public Health and the Nations Health,* 54**,** 1895-1904.

HOROWITZ, H. S. & HEIFETZ, S. B. 1972. The effect of partial defluoridation of a water supply on dental fluorosis--final results in Bartlett, Texas, after 17 years. *American Journal of Public Health,* 62**,** 767-769.

HOROWITZ, H. S., HEIFETZ, S. B. & DRISCOLL, W. S. 1972. Partial defluoridation of a community water supply and dental fluorosis. *Health Services Reports,* 87**,** 451.

HOROWITZ, H. S., DRISCOLL, W. S., MEYERS, R. J., HEIFETZ, S. B. & KINGMAN, A. 1984. A new method for assessing the prevalence of dental fluorosis--the Tooth Surface Index of Fluorosis. *The Journal of the American Dental Association,* 109**,** 37-41.

HOROWITZ, H. S. 1986. Indexes for measuring dental fluorosis. *Journal of Public Health Dentistry,* 46**,** 179-183.

HUJOEL, P. P., ZINA, L. G., MOIMAZ, S. A. & CUNHA-CRUZ, J. 2009. Infant formula and enamel fluorosis: a systematic review. *The Journal of the American Dental Association,* 140**,** 841-854.

HUSSEIN, A.,S, FAISAL, M., HARON, M., GHANIM, A.M. & ABU-HASSAN, M.I. 2015. Distribution of Molar Incisor Hypomineralization in Malaysian Children Attending University Dental Clinic. *Journal of Clinical Pediatric Dentistry,*39, 219-223.

IHEOZOR-EJIOFOR, Z., WORTHINGTON, H. V., WALSH, T., O'MALLEY, L., CLARKSON, J. E., MACEY, R., ALAM, R., TUGWELL, P., WELCH, V. & GLENNY, A. M. 2015. Water fluoridation for the prevention of dental caries. *Cochrane Database Systematic Reviews,* 6**,** CD010856.

INSTITUTE OF MEDICINE 1997. *Fluoride, In: Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride.,* Washington, DC, National Academy Press.

INTERNATIONAL CARIES DETECTION AND ASSESSMENT SYSTEM (ICDAS). *E-learning programme.* Available: https://www.icdas.org [Accessed 10/02/2014].

INTERNATIONAL CARIES DETECTION AND ASSESSMENT SYSTEM (ICDAS) COORDINATING COMMITTEE. 2009. *Criteria Manual International Caries Detection and Assessment System (ICDAS II).* Available: https://www.icdas.org/uploads/ICDAS%20Criteria%20Manual%20Revised%202009_2.pdf [Accessed: 30/01/2014].

IRANZO-CORTES, J. E., M., M.-C. J. & ALMERICH-SILLA, J. M. 2013. Caries diagnosis: agreement between WHO and ICDAS II criteria in epidemiological surveys. *Community Dental Health,* 30**,** 108-111.

ISHII, T. & SUCKLING, G. 1986. The appearance of tooth enamel in children ingesting water with a high fluoride content for a limited period during early tooth development. *Journal of Dental Research,* 65**,** 974-977.

ISMAIL, A. I. 1994. Fluoride supplements: current effectiveness, side effects, and recommendations. *Community Dentistry and Oral Epidemiology,* 22**,** 164-172.

ISMAIL, A. I. & BANDEKAR, R. R. 1999. Fluoride supplements and fluorosis: a meta-analysis. *Community Dentistry and Oral Epidemiology,* 27**,** 48-56.

ISMAIL, A. I. & HASSON, H. 2008. Fluoride supplements, dental caries and fluorosis: a systematic review. *The Journal of the American Dental Association,* 139**,** 1457-1468.

ISMAIL, A. I. & MESSER, J. G. 1996. The risk of fluorosis in students exposed to a higher than optimal concentration of fluoride in well water. *Journal of Public Health Dentistry,* 56**,** 22-27.

ISMAIL, A., SOHN, W., TELLEZ, M., AMAYA, A., SEN, A., HASSON, H. & PITTS, N. 2007. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community Dentistry and Oral Epidemiology,* 35**,** 170-178.

JOBSON, M. D., GRIMM 3RD, S., BANKS, K. & HENLEY, G. 1999. The effects of water filtration systems on fluoride: Washington, DC metropolitan area. *ASDC Journal of Dentistry for Children,* 67**,** 350-354.

JORDAN, W. 1962. The Austin School Health Study. *American Journal of Public Health,* 52**,** 301.

KALSBEEK, H., KWANT, G. W., GROENEVELD, A., DIRKS, O. B., VAN ECK, A. A. & THEUNS, H. M. 1993. Caries experience of 15-year-old children in The Netherlands after discontinuation of water fluoridation. *Caries Research,* 27**,** 201-205.

KHAN, A., MOOLA, M. H. & CLEATON-JONES, P. 2005. Global trends in dental fluorosis from 1980 to 2000: a systematic review. *The South African Dental Journal,* 60**,** 418-421.

KHAN, A.A., WHELTON, H. & O'MULLANE, D. 2004. Determining the optimal concentration of fluoride in drinking water in Pakistan. *Community Dentistry and Oral Epidemiology,* 32,166-172.

KIDD, E.A. & FEJERSKOV, O. 2004. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. *Journal of Dental Research,* 83, C35-C38.

KING, N. M. & WEI, S. H. 1986. Developmental defects of enamel: a study of 12-year-olds in Hong Kong. The *Journal of the American Dental Association,* 112**,** 835-839.

KING, N. M. 1989. Developmental defects of enamel in Chinese girls and boys in Hong Kong. *Advances in Dental Research,* 3**,** 120-125.

KING, N. M., LING, J. Y., NG, B. V. & WEI, S. H. 1986. The dental caries status and dental treatment patterns of 12-year-old children in Hong Kong. *Journal of Dental Research,* 65**,** 1371-1374.

KLEIN, H., PALMER, C. E. & KNUTSON, J. W. 1938. Studies on dental caries: I. Dental status and dental needs of elementary school children. *Public Health Reports (1896-1970)***,** 751-765.

KOBAYASHI, S., KAWASAKI, K., TAKAGI, O., NAKAMURA, M., FUJII, N., SHINZATO, M., MAKI, Y. & TAKAESU, Y. 1992. Caries experience in subjects18–22 years of age after 13 years' discontinued water fluoridation in Okinawa. *Community Dentistry and Oral Epidemiology,* 20**,** 81-83.

KOPARAL, E., ERTUGRUL, F. & OZTEKIN, K. 2000. Fluoride levels in breast milk and infant foods. *Journal of Clinical Pediatric Dentistry,* 24**,** 299-302.

KUNZEL, W. 1980. Effect of an interruption in water fluoridation on the caries prevalence of the primary and secondary dentition. *Caries Research,* 14**,** 304-310.

KUNZEL, W. & FISCHER, T. 1997. Rise and fall of caries prevalence in German towns with different F concentrations in drinking water. *Caries Research,* 31**,** 166-173.

KUNZEL, W. & FISCHER, T. 2000. Caries prevalence after cessation of water fluoridation in La Salud, Cuba. *Caries Research,* 34**,** 20-25.

KUNZEL, W., FISCHER, T., LORENZ, R. & BRUHMANN, S. 2000. Decline of caries prevalence after the cessation of water fluoridation in the former East Germany. *Community Dentistry and Oral Epidemiology,* 28**,** 382-389.

LALLOO, R., MYBURGH, N. G. & HOBDELL, M. H. 1999. Dental caries, socio-economic development and national oral health policies. *International Dental Journal,* 49**,** 196-202.

LALUMANDIER, J. A. & ROZIER, R. G. 1995. The prevalence and risk factors of fluorosis among patients in a pediatric dental practice. *Pediatric Dentistry,* 17**,** 19-25.

LANDIS, J. R. & KOCH, G. G. 1977. The measurement of observer agreement for categorical data. *Biometrics,* 33**,** 159-174.

LATIFAH, R. & RAZAK, I. A. 1989. Fluoride levels in infant formulas. *The Journal of Pedodontics,* 13**,** 323-327.

LEGEROS, R. Z. & TUNG, M. S. 1983. Chemical stability of carbonate and fluoridecontaining apatites. *Caries Research,* 17**,** 419-429.

LEKESOVA, I. 1998. [Fluorine in the prevention of dental caries, in Czech language]. *Cas Lek Cesk,* 137**,** 201-206.

LEMKE, C. W., DOHERTY, J. M. & ARRA, M. C. 1970. Controlled fluoridation: the dental effects of discontinuation in Antigo, Wisconsin. *The Journal of the American Dental Association,* 80**,** 782-786.

LEWSEY, J. & THOMSON, W. 2004. The utility of the zero-inflated Poisson and zero-inflated negative binomial models: a case study of cross-sectional and longitudinal DMF data examining the effect of socio-economic status. *Community Dentistry and Oral Epidemiology,* 32,183-189.

LIANG, C. 1998. [Evaluation on the effects of water defluoridation measures in China. Research Group Evaluation on the Effects of Water Defluoridation Measures in China, in Chinese language]. *Wei Sheng Yan Jiu,* 27**,** 16-28.

LIBERATI, A., ALTMAN, D. G., TETZLAFF, J., MULROW, C., GØTZSCHE, P. C., IOANNIDIS, J. P., CLARKE, M., DEVEREAUX, P. J., KLEIJNEN, J. & MOHER, D. 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLOS Medicine,* 6**,** e1000100.

LISTL, S., JURGES, H. & WATT, R. G. 2016. Causal inference from observational data. *Community Dentistry and Oral Epidemiology,* 44**,** 409-415.

LO, G. L. & BAGRAMIAN, R. A. 1996. Prevalence of dental fluorosis in children in Singapore. *Community Dentistry and Oral Epidemiology,* 24**,** 25-27.

LOH, K. H., YAACOB, H., ADNAN, H., OMAR, S. & JAMALUDIN, M. 2011. A study of the effect of home water filtration systems on fluoride content of drinking water in Johor. *Malaysian Dental Journal,* 33**,** 8-13.

LUKACS, J. R. 2011. Sex differences in dental caries experience: clinical evidence, complex etiology.*Clinical Oral Investigations,* 15**,** 649-656.

LUKACS, J. R. & LARGAESPADA, L. L. 2006. Explaining sex differences in dental caries prevalence: saliva, hormones and "life-history" etiologies. *American Journal of Human Biology,* 18**,** 540-555.

LUSSI, A., HELLWIG, E. & KLIMEK, J. 2012. Fluorides—mode of action and recommendations for use. *Schweizer Monatsschrift fur Zahnmedizin,* 122**,** 1030.

LYGIDAKIS, N.A, WONG, F., JALEVIK, B., VIERROU, A.M., ALALUUSUA, S. & ESPELID, I. 2010. Best Clinical Practice Guidance for clinicians dealing with children presenting with Molar-Incisor-Hypomineralisation (MIH). *European Archives of Paediatric Dentistry,* 11, 75-81.

MAGUIRE, A. & ZOHOORI, F.V. 2013. Fluoride balance in infants and young children in the U.K. and its clinical relevance for the dental team. *British Dental Journal,* 214, 587-593.

MAJID, Z., HUSSEIN, N. & BAGRAMIAN, R. 1995. The prevalence of caries and developmental defects in an adult population in Malaysia. *Dental Journal of Malaysia,* 16**,** 33-39.

MAJID, Z., HUSSEIN, N. & BAGRAMIAN, R. 1996. The prevalence of caries and enamel defects in 229 Malaysian children 16 years after water fluoridation (a pilot study). *Singapore Dental Journal,* 21**,** 11-15.

MALAYSIAN DENTAL COUNCIL. 2009. *Position document use of fluorides in Malaysia.* Putrajaya. Available http://mdc.moh.gov.my/uploads/fluorides.pdf [Accessed 04/11/2016].

MALAYSIAN METROLOGICAL DEPARTMENT. 2017. *Monthly weather bulletin.* Available: http://www.met.gov.my. [Accessed 10/01/17].

MANAN, W. A. 1995. Breast-feeding and infant feeding practice in selected rural and semi-urban communities in Kemaman, Terengganu. *Malaysian Journal of Nutrition,* 1**,** 51-61.

MANJI, F., FEJERSKOV, O., NAGELKERKE, N.J. & BAELUM, V. 1991. A random effects model for some epidemiological features of dental caries. *Community Dentistry and Oral Epidemiology,* 19, 324-328.

MARSHALL, G.W., MARSHALL, S.J., KINNEY, J.H. & BALOOCH, M.1997. The dentin substrate: structure and properties related to bonding. *Journal of Dentistry,* 25, 441-458.

MARTHALER, T. M. 1974. Caries-inhibition by an amine fluoride dentifrice results after 6 years in children with low caries activity. *Helvetica Odontologica Acta,* 18**,** 35-44.

MARTINEZ-MIER, E. A. & ZANDONA, A. F. 2013. The impact of gender on caries prevalence and risk assessment. *Dental Clinics of North America,* 57**,** 301-315.

MARTINS, C. C., CHALUB, L., LIMA-ARSATI, Y. B., PORDEUS, I. A. & PAIVA, S. M. 2009. Agreement in the diagnosis of dental fluorosis in central incisors performed by a standardized photographic method and clinical examination. *Cadernos de Saúde Pública,* 25**,** 1017-1024.

MASCARENHAS, A. K. & BURT, B. A. 1998. Fluorosis risk from early exposure to fluoride toothpaste. *Community Dentistry and Oral Epidemiology,* 26**,** 241-248.

MASCARENHAS, A. K. 2000. Risk factors for dental fluorosis: a review of the recent literature. *Pediatric Dentistry,* 22**,** 269-277.

MAUPOME, G., CLARK, D. C., LEVY, S. M. & BERKOWITZ, J. 2001a. Patterns of dental caries following the cessation of water fluoridation. *Community Dentistry and Oral Epidemiology,* 29**,** 37-47.

MAUPOME, G., SHULMAN, J. D., CLARK, D. C., LEVY, S. M. & BERKOWITZ, J. 2001b. Tooth-surface progression and reversal changes in fluoridated and no-longer-fluoridated communities over a 3-year period. *Caries Research,* 35**,** 95-105.

MAUPOME, G., SHULMAN, J. D., CLARK, D. C. & LEVY, S. M. 2003. Socio-demographic features and fluoride technologies contributing to higher fluorosis scores in permanent teeth of Canadian children. *Caries Research,* 37**,** 327-334.

MCDONAGH M, WHITING P, BRADLEY M, COOPE, J, SUTTON A, CHESTNUTT, I, MISSO, K, WILSON, P, TREASURE E, KLEIJNEN J. 2000. *A systematic review of public water fluoridation*. University of York.

MCGRADY M.G., ELLWOOD R.P. & PRETTY I.A.2010. Why fluoride? *Dental Update,* 37, 595-598.

MCGRADY, M. G., ELLWOOD, R. P., MAGUIRE, A., GOODWIN, M., BOOTHMAN, N. & PRETTY, I. A. 2012a. The association between social deprivation and the prevalence and severity of dental caries and fluorosis in populations with and without water fluoridation. *BMC Public Health,* 12**,** 1122.

MCGRADY, M. G., ELLWOOD, R. P., SRISILAPANAN, P., KORWANICH, N., WORTHINGTON, H. V. & PRETTY, I. A. 2012b. Dental fluorosis in populations from Chiang Mai, Thailand with different fluoride exposures - Paper 1: assessing fluorosis risk, predictors of fluorosis and the potential role of food preparation. *BMC Oral Health,* 12, 16.

MCKAY, F. S. 1928. The Relation of Mottled Enamel to Caries. *The Journal of the American Dental Association, 1922,* 15**,** 1429-1437.

MCLAREN, L. 2011. *Drinking water fluoridation in Canada: Review and synthesis of published literature.* Alberta Health Services, Canada.

MCLAREN, L. & SINGHAL, S. 2016. Does cessation of community water fluoridation lead to an increase in tooth decay? A systematic review of published studies. *Journal of Epidemiology and Community Health,* 70, 934-940.

MCLAREN, L., PATTERSON, S., THAWER, S., FARIS, P., MCNEIL, D., POTESTIO, M. & SHWART, L. 2016. Measuring the short-term impact of fluoridation cessation on dental caries in Grade 2 children using tooth surface indices. *Community Dentistry and Oral Epidemiology,* 44**,** 274-282.

MEJIA, G.C. & HA., D.H. 2011. Dental caries trends in Australian school children. *Australian Dental Journal,* 56**,** 227-230.

MENDES, F. M., BRAGA, M. M., OLIVEIRA, L. B., ANTUNES, J. L., ARDENGHI, T. M. & BONECKER, M. 2010. Discriminant validity of the International Caries Detection and Assessment System (ICDAS) and comparability with World Health Organization criteria in a cross-sectional study. *Community Dentistry and Oral Epidemiology,* 38**,** 398-407.

MESSER, H.H. & OPHAUG, R.H. 1993. Influence of gastric acidity on fluoride absorption in rats. *Journal of Dental Research*, 72, 619-622.

MOHAMED, A. R., THOMSON, W. M. & MACKAY, T. D. 2010. An epidemiological comparison of Dean's index and the Developmental Defects of Enamel (DDE) index. *Journal of Public Health Dentistry,* 70**,** 344-347.

MOHAPATRA, M., ANAND, S., MISHRA, B. K., GILES, D. E. & SINGH, P. 2009. Review of fluoride removal from drinking water. *Journal of Environmental Management,* 91**,** 67-77.

MOHD, M. N., SHEIHAM, A. & TSAKOS, G. 2008. Physchological impacts of dental fluorosis among Malaysian school children. *Malaysian Dental Journal,* 29**,** 20-24.

MØLLER, I. 1982. Fluorides and dental fluorosis. *International Dental Journal,* 32**,** 135-147.

MONSE, B., HEINRICH-WELTZIEN, R., BENZIAN, H., HOLMGREN, C. & VAN PALENSTEIN HELDERMAN, W. 2010. PUFA-an index of clinical consequences of untreated dental caries. *Community Dentistry and Oral Epidemiology,* 38**,** 77-82.

MURRAY, J. J. 1986. World Health Organization. *Appropriate use of fluorides for human health.* Geneva: World Health Organization.

MUSA, S. & SAUB, R. 1998. Toothpastes available in the Malaysian market, *Annals of Dentistry University of Malaya*, 5, 45-48.

MYMALAYSIABOOKS. *Travel guide on Malaysia and Singapore.* Available: http://www.mymalaysiabooks.com/ [Accessed 07/08/ 2016].

NANCI, A. 2007. *Ten Cate's Oral Histology-Pageburst on VitalSource: Development, Structure, and Function.* Elsevier Health Sciences.

NHS CENTRE FOR REVIEWS AND DISSEMINATION 1996. *Undertaking systematic reviews of research on effectiveness: CRD guidelines for those carrying out or commissioning reviews. Report Number 4,* Centre for Reviews and Dissemination.

NOHNO, K., ZOHOORI, F. V. & MAGUIRE, A. 2011. Fluoride intake of Japanese infants from infant milk formula. *Caries Research,* 45**,** 486-493.

NUNN, J. H., EKANAYAKE, L., RUGG-GUNN, A. J. & SAPARAMADU, K. D. 1993. Assessment of enamel opacities in children in Sri Lanka and England using a photographic method. *Community Dental Health,* 10**,** 175-188.

O' MULLANE, D. M., BAEZ, R. J., JONES, S., LENNON, M. A., PETERSEN, P. E., RUGG-GUNN, A. J., H., W. & WHITFORD, G. M. 2016. Fluoride and oral health. *Community Dental Health,* 33**,** 69-99.

OGANESSIAN, E., LENCOVA, E. & BROUKAL, Z. 2007. Is systemic fluoride supplementation for dental caries prevention in children still justifiable? *Prague Medical Report,* 108**,** 306-14.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA. 1998. *National oral health survey of school children 1997 (NOHSS 97).* Kuala Lumpur: OHD, Ministry of Health Malaysia.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA. 2001. *National survey of fluoride enamel opacities in 16-year-old children*. Putrajaya: OHD, Ministry of Health Malaysia.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA. 2003a. *Guidelines on oral health care for pre-school children*. Putrajaya: OHD, Ministry of Health Malaysia.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA. 2003b. *Guidelines: a school based fissure sealant programme.* Putrajaya: OHD, Ministry of Health Malaysia.Available:http://ohd.moh.gov.my/uploads/a_school_based_fissure_sealant_prog.pdf [Accessed 21/06/206].

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA 2006. *Implementation of water fluoridation in Malaysia.* Putrajaya: OHD, Ministry of Health Malaysia.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA. 2007. [*Tidak terlalu awal memulakan penjagaan kesihatan pergigian, in Malay language*]. Putrajaya: OHD, Ministry of Health Malaysia.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA 2009. *National Oral Health Survey of School Children (6 year-olds) 2007*. Putrajaya: OHD, Ministry of Health Malaysia.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA. 2010. *National Oral Health Survey of School Children (12 year-olds) 2007*. Putrajaya: OHD, Ministry of Health Malaysia.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA. 2011. *Annual report 2010.* Putrajaya: OHD, Ministry of Health Malaysia.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA. 2013. *Protocol fluoride enamel opacities in 16 year-old school children, 2013.*Putrajaya: OHD, Ministry of Health Malaysia.

ORAL HEALTH DIVISION MINISTRY OF HEALTH MALAYSIA. 2014. *Annual Report 2013*. Putrajaya: OHD, Ministry of Health Malaysia. Available: http://ohd.moh.gov.my/v3/images/pdf/ann_rpt14.pdf [Accessed 10/10/2016].

OSUJI, O. O., LEAKE, J. L., CHIPMAN, M. L., NIKIFORUK, G., LOCKER, D. & LEVINE, N. 1988. Risk factors for dental fluorosis in a fluoridated community. *Journal of Dental Research,* 67**,** 1488-92.

PAGLIARI, A. V., MOIMAZ, S. A. S., SALIBA, O., DELBEM, A. C. B. & SASSAKI, K. T. 2006. Analysis of fluoride concentration in mother's milk substitutes. *Brazilian Oral Research,* 20**,** 269-274.

PARNELL, C., WHELTON, H. & O'MULLANE, D. 2009. Water fluoridation. *European Archives Paediatric Dentistry,* 10**,** 141-148.

PENDRYS, D. G. 2000. Risk of enamel fluorosis in nonfluoridated and optimally fluoridated populations: considerations for the dental professional. *The Journal of the American Dental Association,* 131**,** 746-755.

PENDRYS, D. G. & KATZ, R. V. 1989. Risk of enamel fluorosis associated with fluoride supplementation, infant formula, and fluoride dentifrice use. *American Journal of Epidemiology,* 130**,** 1199-1208.

PENDRYS, D.G. 1990. The fluorosis risk index: a method for investigating risk factors. *Journal of Public Health Dentistry*, 50, 291-298.

PENDRYS, D. G., KATZ, R. V. & MORSE, D. E. 1994. Risk factors for enamel fluorosis in a fluoridated population. *American Journal of Epidemiology,* 140**,** 461-71.

PEREIRA, A. C., DA CUNHA, F. L., MENEGHIM MDE, C. & WERNER, C. W. 2000. Dental caries and fluorosis prevalence study in a nonfluoridated Brazilian community: trend analysis and toothpaste association. *ASDC Journal of Dentistry for Children,* 67**,** 132-5, 83.

PETERSEN, P. E., BAEZ, R. J. & LENNON, M. A. 2012. Community-oriented administration of fluoride for the prevention of dental caries: a summary of the current situation in Asia. *Advances in Dental Research,* 24**,** 5-10.

PINE, C. M., PITTS, N. B. & NUGENT, Z. J. 1997. British Association for the Study of Community Dentistry (BASCD) guidance on sampling for surveys of child dental health. A BASCD coordinated dental epidemiology programme quality standard. *Community Dental Health,* 14,10-17.

PITTS, N. 2004. "ICDAS" an international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. *Community Dental Health,* 21**,** 193-198.

PITTS, N., CHADWICK, B. & ANDERSON, T. 2015. *Children's Dental Health Survey 2013 Report 2: Dental Disease and Damage in Children England, Wales and Northern Ireland.* London: Health and Social Care Information Centre.

POH, B., NG, B., SITI HASLINDA, M., NIK SHANITA, S., WONG, J., BUDIN, S. N., RUZITA, A.T, NG, L.O., KHOUW, I. & NORIMAH A.K. 2013. Nutritional status and dietary intakes of children aged 6 months to 12 years: findings of the Nutrition Survey of Malaysian Children (SEANUTS Malaysis). *British Journal of Nutrition,* 110**,** S21-S35.

PONTIGO-LOYOLA, A. P., MEDINA-SOLIS, C. E., LARA-CARRILLO, E., PATINO-MARIN, N., ESCOFFIE-RAMIREZ, M., MENDOZA-RODRIGUEZ, M., DE LA ROSA-SANTILLANA, R. & MAUPOME, G. 2014. Impact of socio-demographic, socioeconomic, and water variables on dental fluorosis in adolescents growing up during the implementation of a fluoridated domestic salt program. *Odontology,* 102**,** 105-115.

PREISSER, J.S., STAMM, J.W., LONG, D.L. AND KINCADE, M.E. 2012. Review and recommendations for zero-inflated count regression modeling of dental caries indices in epidemiological studies. *Caries Research*, 46, 413-423.

PRETTY, I. A., MCGRADY, M., ZAKIAN, C., ELLWOOD, R. P., TAYLOR, A., SHARIF, M. O., IAFOLLA, T., MARTINEZ-MIER, E. A., SRISILAPANAN, P., KORWANICH, N., GOODWIN, M. & DYE, B. A. 2012. Quantitative light fluorescence (QLF) and polarized white light (PWL) assessments of dental fluorosis in an epidemiological setting. *BMC Public Health,* 12**,** 366.

RAMEZANI, G. H., NOROZI, A. & VALAEL, N. 2003. The prevalence of nursing caries in 18 to 60 months old children in Qazvin. *Journal of Indian Society of Pedodontics and Preventive Dentistry,* 21**,** 19-26.

RAZAK. I. & NIK, H. N. 1986. Enamel defects in 11-12 year-old subjects in a fluoridated area. *Dental Journal of Malaysia,* 9**,** 23-28.

REISINE, S. T. & PSOTER, W. 2001. Socioeconomic status and selected behavioural determinants as risk factors for dental caries. *Journal of Dental Education,* 65**,** 1009-1016.

RIORDAN, P. J. 1993a. Dental fluorosis, dental caries and fluoride exposure among 7-year-olds. *Caries Research,* 27**,** 71-77.

RIORDAN, P. J. 1993b. Fluoride supplements in caries prevention: a literature review and proposal for a new dosage schedule. *Journal of Public Health Dentistry,* 53**,** 174-189.

RIORDAN, P. J. 1996. The place of fluoride supplements in caries prevention today. *Australian Dental Journal,* 41**,** 335-342.

RIORDAN, P. J. 1999. Fluoride supplements for young children: an analysis of the literature focusing on benefits and risks. *Community Dentistry and Oral Epidemiology,* 27**,** 72-83.

ROBERTSON, A. J. & TOUMBA, K. J. 1999. Cross-polarized photography in the study of enamel defects in dental paediatrics. *The Journal of Audiovisual Media in Medicine,,* 22**,** 63-70.

ROZIER, R. G. 1994. Epidemiologic indices for measuring the clinical manifestations of dental fluorosis: overview and critique. *Advances in Dental Research,* 8**,** 39-55.

ROZIER, R.G., ADAIR, S., GRAHAM, F., IAFOLLA, T., KINGMAN, A., KOHN, W., KROL, D., LEVY, S., POLLICK, H., WHITFORD, G. AND STROCK, S. 2010. Evidence-based clinical recommendations on the prescription of dietary fluoride supplements for caries prevention: a report of the American Dental Association Council on Scientific Affairs. *The Journal of the American Dental Association,* 2010; 141, 1480-1489.

RUGG-GUNN, A. J., SPENCER, A. J., WHELTON, H. P., JONES, C., BEAL, J. F., CASTLE, P., COONEY, P. V., JOHNSON, J., KELLY, M. P., LENNON, M. A., MCGINLEY, J., O'MULLANE, D., SGAN-COHEN, H. D., SHARMA, P. P., THOMSON, W. M., WOODWARD, S. M. & ZUSMAN, S. P. 2016. Critique of the review of 'Water fluoridation for the prevention of dental caries' published by the Cochrane Collaboration in 2015. *British Dental Journal,* 220**,** 335-340.

SABIEHA, A. M. & ROCK, W. P. 1998. A comparison of clinical and photographic scoring using the TF and modified DDE indices. *Community Dental Health,* 15**,** 82-87.

SAMI, E., VICHAYANRAT, T. & SATITVIPAWEE, P. 2015. Dental Fluorosis and Its Relation to Socioeconomic Status, Parents' Knowledge and Awareness among 12-Year-Old School Children in Quetta, Pakistan. *Southeast Asian Journal of Tropical Medicine and Public Health,* 46**,** 360-368.

SAMPAIO, F. C. & LEVY, S. M. 2011. Systemic fluoride. *Monographs in Oral Science,* 22**,** 133-45.

SCHLUTER, P. J. & LEE, M. 2016. Water fluoridation and ethnic inequities in dental caries profiles of New Zealand children aged 5 and 12–13 years: analysis of national cross-sectional registry databases for the decade 2004–2013. *BMC Oral Health,* 16**,** 21.

SEAMAN, S., THOMAS, F. D. & WALKER, W. A. 1989. Differences between caries levels in 5-year-old children from fluoridated Anglesey and non-fluoridated mainland Gwynedd in 1987. *Community Dental Health,* 6**,** 215-221.

ŞENER, Y., TOSUN, G., KAHVECIOGLU, F., GÖKALP, A. & KOÇ, H. 2007. Fluoride levels of human plasma and breast milk.*European Journal of Dentistry,,* 1**,** 21-24.

SEPPA, L., HAUSEN, H., KARKKAINEN, S. & LARMAS, M. 2002. Caries occurrence in a fluoridated and a nonfluoridated town in Finland: a retrospective study using longitudinal data from public dental records. *Caries Research,* 36**,** 308-314.

SEPPA, L., KARKKAINEN, S. & HAUSEN, H. 1998. Caries frequency in permanent teeth before and after discontinuation of water fluoridation in Kuopio, Finland. *Community Dentistry and Oral Epidemiology,* 26**,** 256-262.

SEPPA, L., KARKKAINEN, S. & HAUSEN, H. 2000a. Caries in the primary dentition, after discontinuation of water fluoridation, among children receiving comprehensive dental care. *Community Dentistry and Oral Epidemiology,* 28**,** 281-288.

SEPPA, L., KARKKAINEN, S. & HAUSEN, H. 2000b. Caries Trends 1992–1998 in Two Low–Fluoride Finnish Towns Formerly with and without Fluoridation. *Caries Research,* 34**,** 462-468.

SHAHARUDDIN, M., KIDAHUS, M. N., SUMARLAN, S., KAMIL, Y. M., ISMAIL, Y. M., FIRUZ, R. M., YUNUS, A. M., AIZAT, I. S. & DASRILSYAH, S. 2010. Dental fluorosis (DF) and its relationship with fluoride levels in drinking water in three states in Malaysia. *Research Journal of Medical Sciences,* 4**,** 20-24.

SIEW, C., STROCK, S., RISTIC, H., KANG, P., CHOU, H.-N., CHEN, J.-W., FRANTSVE-HAWLEY, J. & MEYER, D. M. 2009. Assessing a potential risk factor for enamel fluorosis: a preliminary evaluation of fluoride content in infant formulas. *The Journal of the American Dental Association,* 140**,** 1228-1236.

SILVA, M. & REYNOLDS, E. C. 1996. Fluoride content of infant formulae in Australia. *Australian Dental Journal,* 41**,** 37-42.

SINGER, L. & OPHAUG, R. 1979. Total fluoride intake of infants. *Pediatrics,* 63**,** 460-6.

SINGHAL, S., FARMER, J. & MCLAREN, L. 2017. Methodological considerations for designing a community water fluoridation cessation study. *Community Dentistry and Oral Epidemiology,* 45,193-200.

SMITH, F., & EKSTRAND J. 1988. *Fluoride in the environment and intake in man. In: Ekstrand, J. et al. eds. Fluoride in dentistry.* 1st edition ed. Munksgaard, Copenhagen, 13-24.

SMITH, F. A., & EKSTRAND, J. 1996. *The occurance and chemistry of fluoride. In: Fejerskov, O. et al. eds. Fluoride in Dentistry*. 2nd ed. Munksgaard, Copenhagen, 17-26.

SOHN, W., ISMAIL, A. I. & TAICHMAN, L. S. 2007. Caries risk-based fluoride supplementation for children. *Pediatric Dentistry,* 29**,** 23-31.

SOTO-ROJAS, A. E., MARTINEZ-MIER, E. A., URENA-CIRETT, J., JACKSON, R. D. & STOOKEY, G. K. 2008. Development of a standardisation device for photographic assessment of dental fluorosis in field studies. *Oral Health and Preventive Dentistry,* 6**,** 29-36.

SPENCER, A. J. & DO, L. G. 2016. Caution needed in altering the 'optimum' fluoride concentration in drinking water. *Community Dentistry and Oral Epidemiology,* 44**,** 101-108.

STEPHEN, K. W., MACPHERSON, L. M. D., GILMOUR, W. H., STUART, R. A. M. & MERRETT, M. C. W. 2002. A blind caries and fluorosis prevalence study of school-children in naturally fluoridated and nonfluoridated townships of Morayshire, Scotland. *Community Dentistry and Oral Epidemiology,* 30**,** 70-79.

STEPHEN, K., MCCALL, D. & TULLIS, J. 1987. Caries prevalence in northern Scotland before, and 5 years after, water defluoridation. *British Dental Journal,* 163**,** 324.

SUJAK, S. L., KADIR, R. A. & DOM, T. N. M. 2004. Esthetic perception and psychosocial impact of developmental enamel defects among Malaysian adolescents. *Journal of Oral Science,* 46**,** 221-226.

TAN, B.S. 2003. *Fluorosis and fluoride exposure among Malaysian schoolchildren.* PhD thesis, University of Malaya, Malaysia.

TAN, B. & RAZAK, I. 2013. Impact of Water Filters and Consumption of Bottled Water on Fluoride Intake. *Sains Malaysiana,* 42**,** 115-121.

TAN, B. S., RAZAK, I. A. & FOO, L. C. 2005. Fluorosis prevalence among schoolchildren in a fluoridated community in Malaysia. *Community Dental Health,* 22**,** 35-39.

TAN, K. 2009. Knowledge, attitude and practice on breastfeeding in Klang, Malaysia. *The International Medical Journal of Malaysia,* 8, 17-21.

TAVENER, J. A., DAVIES, G. M., DAVIES, R. M. & ELLWOOD, R. P. 2006. The prevalence and severity of fluorosis in children who received toothpaste containing either 440 or 1,450 ppm F from the age of 12 months in deprived and less deprived communities. *Caries Research,* 40**,** 66-72.

TAVENER, J., DAVIES, R. M. & ELLWOOD, R. P. 2007. Agreement amongst examiners assessing dental fluorosis from digital photographs using the TF index. *Community Dental Health,* 24**,** 21-25.

TEN CATE, J. & FEATHERSTONE, J. 1991. Mechanistic aspects of the interactions between fluoride and dental enamel. *Critical Reviews in Oral Biology and Medicine,* 2**,** 283-296.

TEN CATE, J. M. 1999. Current concepts on the theories of the mechanism of action of fluoride. *Acta Odontologica Scandinavica,* 57**,** 325-329.

TEN CATE, J.M. 2013. Contemporary perspective on the use of fluoride products in caries prevention. *British Dental Journal,* 214, 161-167.

THE BIRTISH FLUORIDATION SOCIETY. 2012. *One in a Million: the facts about water fluoridation* [Online]. Available: https://www.bfsweb.org/one-in-a-million.

THOMAS, F., KASSAB, J. & JONES, B. 1995. Fluoridation in Anglesey 1993: a clinical study of dental caries in 5-year-old children who had experienced sub-optimal fluoridation. *British Dental Journal,* 178**,** 55-59.

THYLSTRUP, A. & FEJERSKOV, O. 1978. Clinical appearance of dental fluorosis in permanent teeth in relation to histologic changes. *Community Dentistry and Oral Epidemiology,* 6**,** 315-328.

TRAUTNER, K. & EINWAG, J.1989. Influence of milk and food on fluoride bioavailability from NaF and Na2FPO3 in man. *Journal of Dental Research,* 68, 72-77.

TREASURE, E. T. & DEVER, J. G. 1992. The prevalence of caries in 5-year-old children living in fluoridated and non-fluoridated communities in New Zealand. *New Zealand Dental Journal,* 88**,** 9-13.

TREASURE, E. T. & DEVER, J. G. 1994. Relationship of caries with socioeconomic status in 14-year-old children from communities with different fluoride histories. *Community Dentistry and Oral Epidemiology,* 22**,** 226-230.

TUBERT-JEANNIN, S., AUCLAIR, C., AMSALLEM, E., TRAMINI, P., GERBAUD, L., RUFFIEUX, C., SCHULTE, A. G., KOCH, M. J., REGE-WALTHER, M. & ISMAIL, A. 2011. Fluoride supplements (tablets, drops, lozenges or chewing gums) for preventing dental caries in children. *Cochrane Database Systematic Reviews,* 12, CD007592.

VAN WINKLE, S., LEVY, S., KIRITSY, M., HEILMAN, J., WEFEL, J. & MARSHALL, T. 1994. Water and formula fluoride concentrations: significance for infants fed formula. *Pediatric Dentistry,* 17**,** 305-310.

WALVEKAR, S. V. & QURESHI, B. A. 1982. Endemic fluorosis and partial defluoridation of water supply - A public health concern in Kenya. *Community Dentistry and Oral Epidemiology,* 10**,** 156-160.

WANG, H. Y., PETERSEN, P. E., BIAN, J. Y. & ZHANG, B. X. 2002. The second national survey of oral health status of children and adults in China. *International Dental Journal,* 52**,** 283-290.

WANG, N. J., GROPEN, A. M. & OGAARD, B. 1997. Risk factors associated with fluorosis in a non-fluoridated population in Norway. *Community Dentistry and Oral Epidemiology,* 25**,** 396-401.

WEI, Z.-D. & WEI, Y. 2002. Fluoridation in China: a clouded future. *Fluoride,* 35**,** 1-4.

WHELTON, H., BROWNE, D., FELICIA P. & WHELTON J. *Dean's Index, e-learning programme*. Oral Health Services Research Centre, University College Cork, Ireland. Available: www.fluorosisindex.com [Accessed 12/02/2014].

WHELTON, H., CROWLEY, E., O'MULLANE, D., DONALDSON, M., KELLEHER, V. & CRONIN, M. 2004a. Dental caries and enamel fluorosis among the fluoridated and non-fluoridated populations in the Republic of Ireland in 2002. *Community Dental Health,* 21**,** 37-44.

WHELTON, H. P., KETLEY, C. E., MCSWEENEY, F. & O'MULLANE, D. M. 2004b. A review of fluorosis in the European Union: prevalence, risk factors and aesthetic issues. *Community Dentistry and Oral Epidemiology,* 32**,** 9-18.

WHELTON, H. & O' MULLANE, D. 2012. Monitoring the effectiveness of water fluoridation in the Republic of Ireland. *Journal of Irish Dental Association,* 58; S6-S8.

WHITFORD, G. M. 1994. Intake and metabolism of fluoride. *Advances in Dental Research,* 8**,** 5-14.

WHITFORD, G. M. 1999. Fluoride metabolism and excretion in children. *Journal of Public Health Dentistry,* 59, 224-228.

WHITFORD, G.M., 1996. Overview of fluoride metabolism and intake. In the metabolism and toxicity of fluoride. *Monographs in Oral Science,* 16, 1-9.

WONDWOSSEN, F., ÅSTRØM, A., BJORVATN, K. & BÅRDSEN, A. 2006. Sociodemographic and behavioural correlates of severe dental fluorosis. *International Journal of Paediatric Dentistry,* 16**,** 95-103.

WONG, H. M., MCGRATH, C. & KING, N. M. 2014. Diffuse opacities in 12-year-old Hong Kong children - four cross-sectional surveys. *Community Dentistry and Oral Epidemiology,* 42**,** 61-69.

WONG, H. M., MCGRATH, C., LO, E. C. & KING, N. M. 2006. Association between developmental defects of enamel and different concentrations of fluoride in the public water supply. *Caries Research,* 40**,** 481-486.

WONG, H. M., WEN, Y. F., KING, N. M. & MCGRATH, C. P. 2016. Longitudinal changes in developmental defects of enamel. *Community Dentistry and Oral Epidemiology,* 44**,** 255-262.

WONG, M. C., CLARKSON, J., GLENNY, A. M., LO, E. C., MARINHO, V. C., TSANG, B. W., WALSH, T. & WORTHINGTON, H. V. 2011. Cochrane reviews on the benefits/risks of fluoride toothpastes. *Journal of Dental Research,* 90**,** 573-579.

WONG, M. C., GLENNY, A. M., TSANG, B. W., LO, E. C., WORTHINGTON, H. V. & MARINHO, V. C. 2010. Topical fluoride as a cause of dental fluorosis in children. *Cochrane Database Systematic Reviews***,** 1, CD007693.

WORLD HEALTH ORGANIZATION. 1997. *Oral Health Surveys-Basic Methods.* Geneva: World Health Organization.

WORLD HEALTH ORGANIZATION. 2004. *Guidelines for drinking-water quality (Vol. 1).* Geneva: World Health Organization.

WU, J., ZHANG, J., CHEN, Z. & DU, G. 2000. [Survey on children's dental fluorosis and fluoride content in urine after defluridation to improve drinking water, in Chinese language]. *Wei Sheng Yan Jiu*, 29**,** 218-220.

YEE, C. F. & CHIN, R. 2007. Parental perception and attitudes on infant feeding practices and baby milk formula in East Malaysia. *International Journal of Consumer Studies,* 31**,** 363-370.

YUSOFF, N., JAAFAR, N., RAZAK, I., CHEW, Y., ISMAIL, N. & BULGIBA, A. 2008. The prevalence of enamel opacities in permanent teeth of 11-12 year-old school children in Kuala Lumpur, Malaysia. *Community Dental Health*, 25**,** 55-58.

ZERO, D. T. 1999. Dental caries process. *Dental Clinics of North America*, 43**,** 635-664.

ZOHOORI, F.V., MOYNIHAN, P.J., OMID, N., ABUHALOOB, L. & MAGUIRE, A. 2012. Impact of water fluoride concentration on the fluoride content of infant foods and drinks requiring preparation with liquids before feeding. *Community Dentistry and Oral Epidemiology,* 40, 432-440.

Appendices

Appendix 1 ICDAS II Criteria

	Restoration and Sealant Codes		**Caries Code**
0=	Not sealed or restored	0=	Sound tooth surface
1=	Sealant, partial	1=	First visual change in enamel
2=	Sealant, full enamel	2=	Distinct visual change in enamel
3=	Tooth coloured restoration	3=	Enamel breakdown, no dentine visible
4=	Amalgam restoration	4=	Dentinal shadow (not cavitated into dentine)
5=	Stainless steel crown	5=	Distinct cavity with visible dentine
6=	Porcelain, gold, PFM crown or veneer	6=	Extensive distinct cavity with visible dentine
7=	Lost or broken restoration		
8=	Temporary restoration		**MISSING TEETH**
		97=	Extracted due to caries
06	RETAINED ROOT	98=	Missing for other reason
		99=	Unerupted

First digit=Restoration and sealant code.
Second digit=Caries code.

Appendix 2 Nomenclature for recording dental caries in the present study

In the present study dental caries was recorded using ICDAS II criteria. Due to the potential for confusion between the different codes and thresholds used to describe dental caries when using ICDAS and previous caries indices / scoring systems, the following Table (Table 1) describes the terms used to define thresholds and levels of dental caries experience. In this study dental caries status is described using the following principal terms; caries free, free of caries into dentine; enamel caries and dentine caries.

Table 1 The terms used to describe dental caries status

Terms used in this study (designation)	**ICDAS caries codes**	**Traditional caries scores, (e.g. BASCD, WHO)**	**Notes**
Caries free	00	Sound	Describes the condition free of either enamel or dentine caries
Free of caries into dentine	00, 01, 02 and 03	Sound, D1 and D2.	This is the status traditionally regarded as “caries-free”. This is the principal diagnostic level used for both primary and secondary outcomes in the study.
Enamel caries (d_{1-3}/D_{1-3})	01, 02, 03	D1 and D2	Caries lesions limited to enamel
Dentine caries (d_{4-6}/D_{4-6})	04, 05 and 06	D3, both cavitated and non-cavitated	Caries lesions involving dentine, also referred to as obvious dental decay

BASCD = British Association for the Study of Community Dentistry.
WHO = World Health Organisation.

Appendix 3 Search strategy: EMBASE

Details of literature search (perfomed on 11th February 2016. All databases were searched from their start date to 11th February 2016

Embase via Ovid SP searched (start year: 1947)

1 exp Fluoridation/ (6055)
2 exp Fluorides/ (29993)
3 exp Fluorine/ (10661)
4 fluorid*.ti,ab. (45003)
5 fluorin*.ti,ab. (20872)
6 flurid*.ti,ab. (144)
7 flurin*.ti,ab. (14)
8 1 or 2 or 3 or 4 or 5 or 6 or 7 (77484)
9 exp Water Supply/ (30918)
10 water.ti,ab. (687788)
11 8 or 9 (694388)
12 cessation.ti,ab. (72046)
13 break.ti,ab. (40198)
14 interruption.ti,ab. (31288)
15 discontinu*.ti,ab. (138056)
16 re-introduc*.ti,ab. (1823)
17 (adjust* adj1 down*).ti,ab. (204)
18 defluoridation.ti,ab. (291)
19 defluoridation/ (241)
20 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 (277593)
21 (oral adj1 health).ti,ab. (15154)
22 exp Tooth Disease/ (191821)
23 caries.ti,ab. (34107)
24 dental.ti,ab. (177962)
25 tooth.ti,ab. (66335)
26 teeth.ti,ab. (85767)
27 dentition.ti,ab. (12988)
28 enamel.ti,ab. (24290)
29 exp tooth/ (142556)
30 fluorosis.ti,ab. (3544)
31 flurosis.ti,ab. (15)
32 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 (415756)
33 8 and 11 and 20 and 32 (185)

Number of articles retrieved: 185

After removed duplicates: 70

Appendix 4 Search strategy: Medline

Medline via Ovid SP searched (start year: 1946)

1 exp Fluoridation/ (5417)
2 exp Fluorides/ (31105)
3 exp Fluorine/ (6905)
4 fluorid*.ti,ab. (32142)
5 fluorin*.ti,ab. (12741)
6 flurid*.ti,ab. (110)
7 flurin*.ti,ab. (5)
8 1 or 2 or 3 or 4 or 5 or 6 or 7 (60475)
9 exp Water Supply/ (28475)
10 water.ti,ab. (443155)
11 9 or 10 (449419)
12 cessation.ti,ab. (50176)
13 break.ti,ab. (27654)
14 interruption.ti,ab. (19125)
15 discontinu*.ti,ab. (83383)
16 re-introduc*.ti,ab. (1127)
17 (adjust* adj1 down*).ti,ab. (144)
18 defluoridation.ti,ab. (153)
19 12 or 13 or 14 or 15 or 16 or 17 or 18 (178224)
20 exp Oral Health/ (10671)
21 (oral adj1 health).ti,ab. (13552)
22 caries.ti,ab. (29070)
23 dental.ti,ab. (153813)
24 tooth.ti,ab. (56828)
25 teeth.ti,ab. (72464)
26 dentition.ti,ab. (10968)
27 enamel.ti,ab. (21164)
28 Dental Caries Susceptibility/ (2040)
29 exp Tooth/ (71599)
30 exp Tooth Diseases/ (144299)
31 fluorosis.ti,ab. (2308)
32 flurosis.ti,ab. (5)
33 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 (329750)
36 8 and 11 and 19 and 33 (92)

Number of articles retrieved: 92
After removed duplicates: 20

Appendix 5 Search strategy: The Cochrane central register of controlled trials

Cochrane Central Register of Controlled Trials searched (start year:1990)

Title, abstract and keywords were searched for the following terms:

(fluorid* OR fluorin* OR flurid* OR flurin*)
AND
(water)
AND
(cessation OR break OR interrupt* OR discontinu* OR re-introduc* OR (adjust* NEAR/1 down*) OR defluoridation)
AND
("oral health" OR caries OR dental OR tooth OR teeth OR dentition OR enamel OR fluorosis OR flurosis)

Number of articles retrieved: 36

Appendix 6 Search strategy: The web of science

Web of Science searched (Start year: 1990)

(fluorid* OR fluorin* OR flurid* OR flurin*) AND (water) AND (cessation OR break OR interrupt* OR discontinu* OR re-introduc* OR (adjust* NEAR/1 down*) OR defluoridation) in title, abstract, keywords or Keywords Plus

AND

("oral health" OR caries OR dental OR tooth OR teeth OR dentition OR enamel OR fluorosis OR flurosis) in title

Number of articles retrieved: 72

Appendix 7 Search strategy: unpublished papers

Attempt was made to contact the following authors, however failed to access the articles.

1. Hobbs D 1994. Annual report of the Director of Dental Public Health to Powys
2. Wragg K. 1992..Health Authority. Dental caries experience of 5 year olds in South Derbyshire.

Appendix 8 Data extraction form

Study tittle:
Authors and year of publication:

Country of study	
Geographic location	
Year study started	
Year study ended	
Year of change in fluoridation	
Study design	
Inclusion criteria	
Exclusion criteria	
Other sources of fluoride	
Social class	
Ethnicity	
Other confounding	
Fluoride level at baseline	
Fluoride level at the end	
No of subject (caries)	
Age groups (caries)	
Caries Index & outcome	
DMFT/DMFS/ S.D (baseline)	
DMFT/DMFS/ S.D (after)	
No of subjects (fluorosis)	
Age groups (fluorosis)	
Fluorosis Index & outcome	
Fluorosis % prevalence (baseline)	
Fluorosis % prevalence (after)	
Funding:	
Notes	

Appendix 9 Validity assessment scoring and definition of terms in the tables (adapted from NHS Center for Reviews and Dissemiantion, 1996 in York Review, 2000)

Cohort, Before-After, Ecological and Cross-Sectional Study Designs

Prospective	Was the study prospective? Was it planned and started prior to the outcome of interest occurring? Score =1 or 0
Study Design	The study design hierarchy for this review= cohort > before-after> ecological> cross-sectional. Score range between 0.25 -1, with cohort=1, cross-sectional =0.25
Fluoride measurement	Was the fluoride level reliably measured? Scores range between 0-1
Confounding factors	Were confounding factors addressed (measured)? Scores range between 0-1, with 3 or more factors measure=1
Control for confounding	Was the adjustment for the possible effect of confounding factors in the analysis? Score range between 0-1, with stratification by age and sex=0.5, other types of analysis (regression)=1
Blinding	Were those measure outcomes (e.g. fluorosis) blind to the exposure status of the person being assessed? Score=0 or 1
Baseline Survey	Was the baseline survey at the point of discontinuation of water fluoridation? Score = 0 or 1
Follow-up	Was the final survey an adequate time after the discontinuation of water fluoridation to assess effects (2 years for caries, 5 years for other effects)? Score 0 or 1
Score	Sum of the score of the above questions. Total score is out of 8 possible
Level of Evidence	A, B or C based on the levels defined in the methods section

Case-control Study Designs

Disease validated	Was the disease state of the cases reliably assessed and validated? Score =0 or 1
Cases in Series	Are the cases representative of a series (or is there a potential for selection bias)? Score =0 or 1
Controls Similar	Are the controls selected from a similar population to the cases? Score=0 or 1
Controls Disease-Free	Is there evidence that the controls are free from disease? Score=0 or 1
Confounding factors	Are cases and controls comparable with respect to confounding factors? Score range between 0-1, with 3 or more factors measured=1
Exposure Assessment Similar	Was exposure (e.g. to fluoridated water) assessed in the same way for cases and controls? Score 0 or 1
Response Rate Adequate	Was the response rate adequate (meaning numbers of people included into the study out of those possible)? Score 0 or 1
Non-response similar	Was the non-response rate in the same in cases and controls? Score 0 or 1
Statistical Analysis	Was an appropriate statistical analysis performed (e.g. use of matching)? Score= 0 or 1
Score	Sum of the scores of the above questions. Total score is out of 9 possible
Level of Evidence	A, B or C based on the levels defined in the methods section

Appendix 10 Summary of the included studies that assess impact of reducing or stopping fluoride level in the water on caries and fluorosis.

CARIES STUDIES

Studies that assess impact of stopping water fluoridation on caries prevalence

Stopping water fluoridation and caries
Cross sectional survey with no control group

Country	**Reference**	**Title**	**Comments**
Antigo, Wisconsin, USA	Lemke et al. 1970	Controlled fluoridation: the dental effects of discontinuation in Antigo, Wisconsin	Study design: Cross sectional survey with no control group Index/outcome measure: DMFT, dmft, caries free Validity score: 3.5/8 Level of evidence: C Funding: Not stated Key Findings: Caries increased post-cessation
Scotland, UK	Stephen et al. 1987	Caries prevalence in Northern Scotland before and 5 years after water defluoridation	Study design: Cross sectional survey with no control group Index/outcome measure: dmft, caries free Validity score: 3.25/8 Level of evidence: C Funding: Not stated Key Findings: Caries increased post-cessation
Germany	Kunzel and Fisher 1997	Rise and fall of caries prevalence in German towns with different fluoride concentrations in drinking water	Study design: Cross sectional survey with no control group Index/outcome measure: DMFT Validity score: 4.25/8 Level of evidence: C Funding: Not stated Key Findings: Caries decreased post-cessation

La Salud, Cuba	Kunzel and Fisher 2000	Caries prevalence after cessation of water fluoridation in La Salud, Cuba	Study design: Cross sectional survey with no control group Index/outcome measure: DMFT, DMFS, caries free Validity score: 3.25/8 Level of evidence: C Funding: German Research Council & Cuban Ministry of Health Key Findings: Caries decreased post-cessation
East Germany	Kunzel, Fischer, Bruhmann, 2000.	Decline of caries prevalence after the cessation of water fluoridation in the former East Germany	Study design: Cross sectional survey with no control group Index/outcome measure: DMFT, DMFS Validity score: 3.75/8 Level of evidence: C Funding: Not stated Key Findings: Caries decreased post-cessation
Gongzhou, China	Wei and Wei 2002	Fluoridation in China, a clouded future	Study design: Cross sectional survey with no control group Index/outcome measure: DMFT Validity score: 3.25/8 Level of evidence: C Funding: Not stated Key Findings: Caries decreased post-cessation
Austin, USA	Jordan 1962	The Austin School Health Study	Study design: Cross sectional survey with no control group Index/outcome measure: DMFT Validity score: 3.25/8 Level of evidence: C Funding: Not stated Key Findings: Caries increased post-cessation

Stopping water fluoridation and caries
Cross sectional survey with a negative control group

Country	Reference	Title	Comments
Scotland, UK	Attwood and Blinkhorn 1989	A reassessment of dental health of urban Scottish schoolchildren following the cessation of water fluoridation	Study design: Cross sectional survey with a negative control group Index/outcome measure: DMFT, dmft Validity score: 3.5/8 Level of evidence: C Funding: Not stated Key Findings: Mixed results on different dentition. Caries increased in permanent teeth post-cessation, caries decreased in primary teeth post-cessation
Netherlands	Kalsbeek et al. 1993	Caries experience of 15 year-old children in the Netherlands after discontinuation of water fluoridation	Study design: Cross sectional survey with a negative control group Index/outcome measure: DMFT, DMFS Validity score: 5 Level of evidence: B Funding: Not stated Key Findings: Caries increased post-cessation
Kuopio and Jyvaskayla, Finland	Seppa et al. 1998	Caries frequency in permanent teeth before and after discontinuation of water fluoridation in Kuopio, Finland	Study design: Cross sectional survey with a negative control group Index/outcome measure: DMFS Validity score: 4.5 Level of evidence: C Funding: Academy of Finland Key Findings: Mixed results in different age group. Caries increased in 12 & 15 years, caries decreased in 6 & 9 years (permanent dentition)

Kuopio & Jyvaskayla, Finland	Seppa et. al. 2000a	Caries in the primary dentition after discontinuation of water fluoridation, among children receiving comprehensive dental care	Study design: Cross sectional survey with a negative control group Index/outcome measure: dmft Validity score: 4.5/8 Level of evidence: C Funding: Yrjo Jahnsson Foundation and the Academy Finland Key Findings: Caries decreased post-cessation
Kuopio and Jyvaskayla, Finland	Seppa et al. 2000b	Caries trends 1992-1998 in the low-fluoride finnish towns formerly with and without fluoridation	Study design: Cross sectional survey with a negative control group Index/outcome measure: Caries free Validity score: 5.5/8 Level of evidence: B Funding: Not stated Key Findings: Caries decreased post-cessation
Kilmarnock, Scotland	DHSS 1969	The fluoridation studies in the UK & results achieved after 11 years. A report of the committee in research into fluoridation.	Study design: Cross sectional survey with a negative control group Index/outcome measure: dmft, caries free Validity score: 3.5/8 Level of evidence: C Funding: Not stated Key Findings: Caries increased post-cessation

Stopping water fluoridation and caries
Cross sectional survey with a positive control group

Country	**Reference**	**Title**	**Comments**
British Columbia, Canada	Maupome et. al. 2001a	Patterns of dental caries following the cessation of water fluoridation	Study design: Cross sectional survey with a positive control group Index/outcome measure: D1D2MFS Validity score: 5.5/8 Level of evidence: B Funding: NHRDF Operating Grant 6610-2225-002 Key Findings: Caries decreased post-cessation
Calgary & Edmonton, Canada	McLaren et al. 2016	Measuring the short-term impact of fluoridation cessation on dental caries in Grade 2 children using tooth surface indices	Study design: Cross sectional survey with a positive control group Index/outcome measure: Validity score: 5.5/8 Level of evidence: B Funding: Canadian Institutes of Health Research (GIR 127083), Alberta Health and Alberta Health Services. Key Findings: Mixed results on different dentition. Caries decreased in permanent teeth post-cessation, caries increased in primary teeth post-cessation.

Studies that assess impact of reducing fluoride level in the water supply on caries prevalence

Reduction of fluoride level in the water and caries			
Country	**Reference**	**Title**	**Comments**
Germany	Kunzel 1980	Effect of an interruption in water fluoridation on caries prevalence of the primary and secondary dentition	Study design: Cross sectional survey with historical control Index: DMFT, dft Validity score: 3.75/8 Level of evidence: C Funding: Not stated Key Findings: Caries in primary and permanent dentition decreased post-reduction

FLUOROSIS STUDIES

Studies that assess impact of stopping water fluoridation on fluorosis prevalence

Stopping water fluoridation and fluorosis			
Country	**Reference**	**Title**	**Comments**
British Columbia, Canada	Clark et al. 2006	Changes in dental fluorosis following the cessation of water fluoridation	Study design: Cross sectional survey with no control group Outcome measure: Fluorosis prevalence Index: TF Index Validity score: 5.25/8 Level of evidence: B Funding: Canadian Institutes of Health Research Operating Grant (MOP-57721) and the National Health Research Development Program Operating Grant (6610-2225-002). Key Findings: Fluorosis decreased post-cessation

Studies that assess impact of reducing fluoride level in the water supply on fluorosis prevalence

Reduction of fluoride level in the water and fluorosis			
Country	**Reference**	**Title**	**Comments**
Britton, USA	Horowitz et al. 1972	Partial defluoridation of a Community water supply and dental fluorosis	Study design: Cross sectional survey with no control group Index: Dean's Index Validity score: 3.75/8 Level of evidence: C Funding: Not stated Key Findings: Fluorosis decreased post-reduction
Bartlett, Texas, USA	Horowitz and Heifetz 1972	The effect of partial defluoridation of a water supply on dental fluorosis- final results in Bartlett, Texas, after 17 years	Study design: Cross sectional survey with historical control Index: Dean's Index Validity score: 3.25/8 Level of evidence: C Funding: Not stated Key Findings: Fluorosis decreased post-reduction
Hong Kong	Evans et al. 1989	Changes in dental fluorosis following an adjustment to the fluoride concentration of Hong Kong's water supply	Study design: Multiple birth cohorts analysis Index: Dean's Index Validity score: 3.75/8 Level of evidence: C Funding: Not stated Key Findings: Fluorosis decreased post-reduction
Hong Kong	Evans and Stamm 1991b	Dental fluorosis following downward adjustment of fluoride in drinking water	Study design: Multiple birth cohorts analysis Index: Dean's Index Validity score: 3.25/8 Level of evidence: C Funding: Not stated Key Findings: Fluorosis decreased post-reduction

Hong Kong	Wong et al. 2014	Diffuse opacities in 12-year-old Hong Kong children- four cross sectional surveys	Study design: Cross sectional survey with no control group Index: DDE Validity score: 5.25/8 Level of evidence: B Funding: Research Grants Council of the Special Administration Region, China (Project No: 782811) Key Findings: Fluorosis decreased post-reduction

Appendix 11. Characteristics of included studies

Characteristic of included studies, ordered by type of study and year of publication

Stopping water fluoridation and caries, cross sectional studies with no control group

Study tittle: The Austin School Health Study

Authors and year of publication: Jordan, 1962

<table>
<tr><td rowspan="6">**Study details**</td><td>Country of study</td><td>USA</td></tr>
<tr><td>Geographic location</td><td>Austin</td></tr>
<tr><td>Year study at baseline</td><td>1955</td></tr>
<tr><td>Year study at follow up</td><td>1959</td></tr>
<tr><td>Year of change in fluoridation</td><td>1952 (begin WF) ,April 1956 (WF ceased)</td></tr>
<tr><td>Study design</td><td>Cross sectional with no control</td></tr>
<tr><td rowspan="2">**Inclusion & exclusion criteria**</td><td>Inclusion criteria</td><td>Not stated</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">**Confounding factors**</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Not stated</td></tr>
<tr><td rowspan="2">**Fluoride levels**</td><td>Fluoride level at baseline</td><td>1.2 ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>0 (ceased)</td></tr>
<tr><td rowspan="2">**Outcome measure**</td><td>Caries index</td><td>6-12 years</td></tr>
<tr><td>Teeth examined</td><td>Permanent</td></tr>
<tr><td rowspan="3">**Outcomes**</td><td>No of subjects (N)</td><td>4321 (total for 6,7 and 8 years)</td></tr>
<tr><td>Age groups</td><td>6-12 years examined. Children aged 6,7 and 8 years with complete pre and post-cessation data.</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><td>Age</td><td colspan="2">6</td><td colspan="2">7</td><td colspan="2">8</td></tr>
<tr><td>Survey</td><td>1955</td><td>1959</td><td>1956</td><td>1959</td><td>1956</td><td>1959</td></tr>
<tr><td>DMFT</td><td>0.4</td><td>0.51</td><td>1.2</td><td>1.38</td><td>2.1</td><td>2.07</td></tr>
<tr><td>N</td><td>629</td><td>721</td><td>705</td><td>821</td><td>698</td><td>747</td></tr>
</table>
</td></tr>
<tr><td>**Funding**</td><td colspan="2">Not stated</td></tr>
<tr><td>**Comment**</td><td colspan="2">~Water fluoridation began 1952 and WF ceased in April 1956.
~Serial survey in 1952, 1953, 1954, 1955, 1956*, 1957, 1958, 1959. Only data of children aged 6,7 and 8 were available for pre and post cessation.</td></tr>
</table>

Study tittle: Controlled fluoridation, the dental effects of discontinuation in Antigo, Wisconsin

Authors and year of publication: Lemke et al., 1970

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>USA</td></tr>
<tr><td>Geographic location</td><td>Antigo, Wisconsin</td></tr>
<tr><td>Year study at baseline</td><td>1960</td></tr>
<tr><td>Year study at follow up</td><td>1966</td></tr>
<tr><td>Year of change in fluoridation</td><td>1960 (WF cessation)
1965 (WF reinstate)</td></tr>
<tr><td>Study design</td><td>Serial cross sectional with historical control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Life long resident (children whose use of the local water was continuous except for periods not exceeding 90 days in any calendar year); consented;and excellent cooperation</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Not stated</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>Not stated (assume 1ppm)</td></tr>
<tr><td>Fluoride level at the end</td><td>0 (cessation)</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>DMFT, dmft and % caries free</td></tr>
<tr><td>Teeth examined</td><td>Permanent and primary teeth</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>Total for 3 surveys: 1266</td></tr>
<tr><td>Age groups</td><td>Kindergarten, 8, 10 and 12 year-old</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><td>Age</td><td colspan="3">Kindergarten</td><td colspan="3">8 year-old</td><td colspan="3">10 year-old</td></tr>
<tr><td>Year</td><td>N</td><td>dmft</td><td>% caries free</td><td>N</td><td>DMFT</td><td>% caries free</td><td>N</td><td>DMFT</td><td>% caries free</td></tr>
<tr><td>1960</td><td>125</td><td>2.5</td><td>39.0</td><td>143</td><td>0.6</td><td>71.0</td><td>137</td><td>1.7</td><td>35.0</td></tr>
<tr><td>1964</td><td>131</td><td>4.8</td><td>19.8</td><td>109</td><td>1.7</td><td>38.5</td><td>130</td><td>2.4</td><td>26.2</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Comment</td><td colspan="2">~No information on specific baseline F level (assume optimum level of WF at 1ppm).
~ Data for 12 year-old children were excluded because no information about this age group in follow up survey.
~Kindergarten children: exact age not stated. Assume average age 5/6.</td></tr>
</table>

Study tittle: Caries experience in Northern Scotland before and 5 years after water defluoridation

Authors and year of publication: Stephen et al., 1987

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Scotland</td></tr>
<tr><td>Geographic location</td><td>Wick</td></tr>
<tr><td>Year study at baseline</td><td>1979</td></tr>
<tr><td>Year study at follow up</td><td>1984</td></tr>
<tr><td>Year of change in fluoridation</td><td>1979 (WF ceased)</td></tr>
<tr><td>Study design</td><td>Serial cross sectional survey with no control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Lifelong resident</td></tr>
<tr><td>Exclusion criteria</td><td>Refusal of parental consent, use of fluoride tablet</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Children were group by different social class
(S.C I+II), (S.C III), (S.C IV & V)
(all social class)</td></tr>
<tr><td>Ethnicity</td><td>Scottish</td></tr>
<tr><td>Other confounding</td><td>Not stated</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>1 ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>0.02 ppm</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>dmft, dmfs and % caries free</td></tr>
<tr><td>Teeth examined</td><td>Primary</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>1979 (N=106), 1984 (N=126)</td></tr>
<tr><td>Age groups</td><td>5-6</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><td>Year</td><td>dmft mean (s.e)</td><td>dmfs mean (s.e)</td><td>caries free</td></tr>
<tr><td>1979 (baseline)</td><td>2.6 (±0.19)</td><td>7.80 (±1.11)</td><td>27.4%</td></tr>
<tr><td>1984 (after)</td><td>3.92 (±0.20)</td><td>13.33 (±1.45)</td><td>24.6%</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Comment</td><td colspan="2">Clinical and radiograph examination. No blinding of examiner.</td></tr>
</table>

Study tittle: Rise and fall of caries prevalence in German Towns with different fluoride concentrations in drinking water

Authors and year of publication: Kunzel & Fischer, 1997

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td colspan="2">German</td></tr>
<tr><td>Geographic location</td><td colspan="2">Chemnitz & Plauen</td></tr>
<tr><td>Year study started</td><td colspan="2">1963</td></tr>
<tr><td>Year study ended</td><td colspan="2">1995</td></tr>
<tr><td>Year of change in fluoridation</td><td colspan="2">Chemnitz= 1990, Plauen=1984;
(1971:22 months fluoride interruption in Chemnitz)</td></tr>
<tr><td>Study design</td><td colspan="2">Cross sectional with no control (historical control)</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td colspan="2">Life long resident</td></tr>
<tr><td>Exclusion criteria</td><td colspan="2">Non-continuous resident, disable children</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td colspan="2">Fluoride varnish (after 1971), fluoride toothpaste, fluoride salt (after 1992)</td></tr>
<tr><td>Social class</td><td colspan="2">Not stated</td></tr>
<tr><td>Ethnicity</td><td colspan="2">Not stated</td></tr>
<tr><td>Other confounding</td><td colspan="2">Sugar consumption, fissure sealant</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td colspan="2">1.0 ± 0.1ppm</td></tr>
<tr><td>Fluoride level at the end</td><td colspan="2">0</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td colspan="2">DMFT (Only mean DMFT provided , no S.D reported)</td></tr>
<tr><td>Teeth examined</td><td colspan="2">Permanent teeth</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td colspan="2">Overall for all age groups. Chemnitz (N=219 594), Plauen (N=66, 582)</td></tr>
<tr><td>Age groups</td><td colspan="2">6-15 years old</td></tr>
<tr><td>Caries experience (before & after)</td><td colspan="2"><table>
<tr><td>Age</td><td colspan="2">Chemnitz –DMFT & (N*)</td><td colspan="2">Plauen-DMFT & (N*)</td></tr>
<tr><td></td><td>1987
(N=29,432)
0.9ppm</td><td>1995
(N=12,229)
0.2ppm</td><td>1983
(N=7587)
0.9ppm</td><td>1995
(N=4852)
0.2ppm</td></tr>
<tr><td>8</td><td>0.75 (N=2452)</td><td>0.32 (N=1019)</td><td>0.7 (N=632)</td><td>0.58 (N=404)</td></tr>
<tr><td>12</td><td>2.55 (N=2452)</td><td>1.87 (N=1019)</td><td>3.5 (N=632)</td><td>1.98 (N=404)</td></tr>
<tr><td>15</td><td>4.87 (N=2452)</td><td>3.78 (N=1019)</td><td>6.2 (N=632)</td><td>3.47 (N=404)</td></tr>
</table></td></tr>
<tr><td>Funding</td><td colspan="3">Not stated</td></tr>
<tr><td>Comment</td><td colspan="3">*Only an overall sample size per year was provide (not broken down by age). Values (N) in the results table were determined by dividing the total N (year survey conducted) by the number of age group (12 groups).
~For Chemnitz, baseline data is refer to 1987 (0.9 ppm), follow-up data is 1995 (0.2ppm). For Plauen, baseline data is taken for 1983 (0.9 ppm), follow-up data is 1995 (0.2ppm). Water fluoridation was implemented in 1972. Authors also provide survey data in 1959 (pre-fluoridation).</td></tr>
</table>

Study tittle: Caries prevalence after cessation of water fluoridation in La Salud, Cuba

Authors and year of publication: Kunzel & Fischer, 2000

Study details	Country of study	Cuba
	Geographic location	La, Salud
	Year study at baseline	1982
	Year study at follow up	1997
	Year of change in fluoridation	1990
	Study design	Cross sectional with no control group
Inclusion & exclusion criteria	Inclusion criteria	-School children who were born in the community (lifelong residents) -Gender: girls to boys ratio balanced
	Exclusion criteria	-Those who moved into town (non-lifelong residents);those who were ill at the date of caries examination; and those who merely attended school in La Salud.
Confounding factors	Other sources of fluoride	After cessation (1990), all children received mouthrinses with a 0.2% NaF solution fortnightly (=15times/year). Children aged 2 and 5 in kindergartens received one or two applications of F varnish annually. No fluoride toothpaste available to the studied community.
	Social class	Life and nutritional conditions are similar in all rural communities in Cuba including La Salud
	Ethnicity	Not stated
	Other confounding	-Sugar-enriched drinking water and excessive oranges used by families as an additional calorie supplement for children. -Gender balance among study sample
Fluoride levels	Fluoride level at baseline	0.8 ppm ± 0.1
	Fluoride level at the end	0 ± 0.05 ppm (fluoridation cessation)
Outcome measure	Caries index	DMFT, DMFS and % caries free
	Teeth examined	Permanent
Outcomes	No of subjects (N)	414
	Age groups	6-13 years
	Caries experience (before & after)	DMFT

	1982		1997	
Age	N	DMFT (s.d)	N	DMFT (s.d)
6-13 (all)	470	0.8 (1.47)	414	0.70 (1.26)
6/7	107	0.07 (0.34)	82	0.07 (0.31)
8/9	159	0.5 (1.04)	123	0.6 (0.98)
10/11	126	1.1 (1.51)	104	0.8 (1.21)
12/13	78	2.1 (2.11)	105	1.1 (1.75)

DMFS

	1982		1997	
Age	N	DMFS (s.d)	N	DMFS (s.d)
6-13 (all)	470	1.2 (2.34)	414	0.91 (1.83)
6/7	107	0.1 (0.73)	82	0.07 (0.31)
8/9	159	0.7 (1.64)	123	0.7 (1.19)
10/11	126	1.5 (2.21)	104	1.2 (2.12)
12/13	78	3.1 (3.75)	105	1.5 (2.46)

% Caries free

Age	N	1982	N	1997
6-13 (all)	287	61.6	277	66.9
6/7	101	95.2	77	93.9
8/9	118	75.6	80	65.0
10/11	42	54.8	62	59.6
12/13	26	33.3	58	55.2

Funding	German Research Council & Cuban Ministry of Health
Comment	~Water fluoridation was introduced in 1973. Data available for survey in 1973, 1982, 1997. Survey in 1982 were used as a baseline data (survey that available few years before water fluoridation cessation). ~The same clinical examiners were used for all surveys.

Study tittle: Decline of caries prevalence after the cessation of water fluoridation in the former East Germany

Authors and year of publication: Kunzel et al., 2000

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Germany</td></tr>
<tr><td>Geographic location</td><td>Spremberg & Zittau</td></tr>
<tr><td>Year study at baseline</td><td>1993</td></tr>
<tr><td>Year study at follow up</td><td>1996</td></tr>
<tr><td>Year of change in fluoridation</td><td>1993</td></tr>
<tr><td>Study design</td><td>Serial cross sectional survey with no control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Life long resident in Spremberg & Zittau</td></tr>
<tr><td>Exclusion criteria</td><td>Non-continuous resident, disable children</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Fluoridated toothpaste, fluoride salt, oral hygiene habits</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Fissure sealant, reduce sugar consumption, pattern of food consumption (fast food) – not adjusted in analysis</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>0.9 ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>0.2 ppm (range 0.12 to 0.19ppm)</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>DMFT</td></tr>
<tr><td>Teeth examined</td><td>Permanent</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>See table</td></tr>
<tr><td>Age groups</td><td>8-12 years</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><th colspan="5">Spremberg</th><th colspan="5">Zittau</th></tr>
<tr><th>Age</th><th colspan="2">1993</th><th colspan="2">1996</th><th>Age</th><th colspan="2">1993</th><th colspan="2">1997</th></tr>
<tr><th></th><th>N</th><th>DMFT</th><th>N</th><th>DMFT</th><th></th><th>N</th><th>DMFT</th><th>N</th><th>DMFT</th></tr>
<tr><td>8</td><td>224</td><td>0.51 (0.97)</td><td>158</td><td>0.34 (0.79)</td><td>8</td><td>333</td><td>0.56 (1.02)</td><td>-</td><td>-</td></tr>
<tr><td>9</td><td>259</td><td>0.69 (1.15)</td><td>190</td><td>0.50 (1.02)</td><td>9</td><td>324</td><td>0.92 (1.38)</td><td>-</td><td>-</td></tr>
<tr><td>12</td><td>323</td><td>2.36 (2.11)</td><td>89</td><td>1.45 (1.67)</td><td>12</td><td>337</td><td>2.47 (2.06)</td><td>184</td><td>1.96 (1.96)</td></tr>
<tr><td>13</td><td>327</td><td>2.59 (2.19)</td><td>180</td><td>1.63 (2.02)</td><td>13</td><td>334</td><td>3.16 (2.54)</td><td>-</td><td>-</td></tr>
<tr><td>15</td><td>313</td><td>4.13 (3.10)</td><td>91</td><td>3.74 (3.64)</td><td>15</td><td>264</td><td>4.71 (3.33)</td><td>-</td><td>-</td></tr>
<tr><td>16</td><td>294</td><td>5.03 (3.32)</td><td>125</td><td>3.86 (3.21)</td><td>16</td><td>205</td><td>4.93 (3.82)</td><td>-</td><td>-</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Comment</td><td colspan="2">~Clinical examination only. Cross sectional survey in two communities in Germany.
~Fluoridation status not stable before 1993 (therefore only include data from 1993-1996). Only extract DMFT data, no baseline data for % caries free in 1993.
~In Zittau area: before and after intervention data only available for 12 year-old group.</td></tr>
</table>

Study tittle: Fluoridation in China, a clouded future

Authors and year of publication: Wei & Wei, 2002

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>China</td></tr>
<tr><td>Geographic location</td><td>Gongzhou</td></tr>
<tr><td>Year study at baseline</td><td>1982</td></tr>
<tr><td>Year study at follow up</td><td>1990</td></tr>
<tr><td>Year of change in fluoridation</td><td>1983 (WF ceased)</td></tr>
<tr><td>Study design</td><td>Cross sectional survey with no control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Not stated</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Not stated</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>0.7 to 0.8 ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>0.3 ppm</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>DMFT</td></tr>
<tr><td>Teeth examined</td><td>Permanent</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>137</td></tr>
<tr><td>Age groups</td><td>15</td></tr>
<tr><td>Caries experience (before & after)</td><td><table><tr><td>Year</td><td>N</td><td>DMFT</td></tr><tr><td>1982</td><td>75</td><td>0.90</td></tr><tr><td>1990</td><td>62</td><td>0.44</td></tr></table></td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Comment</td><td colspan="2">For baseline survey in 1982 (data available from 12-18 years) but for follow up survey (1990), data only available for 15 years of age.</td></tr>
</table>

Stopping water fluoridation and caries, cross sectional studies with a negative control group

Study tittle: The fluoridation studies in the UK & results achieved after 11 years. A report of the committee in research into fluoridation. London: Her Majesty's Stationary Office; Reports on Public Health Medical Subjects No. 122.

Authors and year of publication: DHSS, 1969

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Scotland, UK</td></tr>
<tr><td>Geographic location</td><td>Kilmarnock (WF ceased) & Ayr (control-NF)</td></tr>
<tr><td>Year study at baseline</td><td>1956</td></tr>
<tr><td>Year study at follow up</td><td>1968</td></tr>
<tr><td>Year of change in fluoridation</td><td>1962 (Oct 1982_WF discontinued)</td></tr>
<tr><td>Study design</td><td>Cross sectional with negative control group</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Not stated</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Not stated</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>Not clearly stated (Assume 1.0ppm)</td></tr>
<tr><td>Fluoride level at the end</td><td>0 (after cessation)</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>dmft and % caries free</td></tr>
<tr><td>Teeth examined</td><td>3 & 4 year-old (full deciduos), 5 & 7 y.old
(molar & canine deciduous)</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>Not stated</td></tr>
<tr><td>Age groups</td><td>3 to 7</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><td>Year</td><td colspan="2">Kilmanork (WF ceased)</td><td colspan="2">Ayr (control-NF)</td></tr>
<tr><td></td><td>Mean dmft</td><td>% caries free</td><td>Mean dmft</td><td>% caries free</td></tr>
<tr><td>1961</td><td>3.99</td><td>20</td><td>6.89</td><td>4</td></tr>
<tr><td>1968</td><td>5.81</td><td>7</td><td>5.98</td><td>7</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Comment</td><td colspan="2">~Data available for survey in 1956 (prior to fluoridation), 1961, 1964, 1968.
~Sample size was not reported.
~Of those examined, only 5 year-old children were having full effect of fluoridation.</td></tr>
</table>

Study tittle: A reassessment of the dental health of urban Scottish schoolchildren following the cessation of water fluoridation

Authors and year of publication: Attwood & Blinkhorn, 1989

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>UK</td></tr>
<tr><td>Geographic location</td><td>Stranrear (WF ceased) & Annan (control)</td></tr>
<tr><td>Year study started</td><td>1980</td></tr>
<tr><td>Year study ended</td><td>1986</td></tr>
<tr><td>Year of change in fluoridation</td><td>1983</td></tr>
<tr><td>Study design</td><td>Cross sectional negative control group</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Life time residents</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Similar social class for both groups</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Not stated</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>1 ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>0 (WF ceased)</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>DMFT and dmft index.</td></tr>
<tr><td>Teeth examined</td><td>Permanent and primary teeth</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>980 (total)</td></tr>
<tr><td>Age groups</td><td>5 & 10</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><td></td><td colspan="4">Stranraer (WF ceased)</td><td colspan="4">Annan (NF)-control</td></tr>
<tr><td>Year</td><td>N</td><td>5 y.o dmft (s.d)</td><td>N</td><td>10 y.o DMFT (s.d)</td><td>N</td><td>5 y.o dmft (s.d)</td><td>N</td><td>10 y.o DMFT (s.d)</td></tr>
<tr><td>1980</td><td>129</td><td>2.48 (3.16)</td><td>147</td><td>1.66 (1.63)</td><td>121</td><td>4.38 (4.31)</td><td>141</td><td>3.35 (2.30)</td></tr>
<tr><td>1986</td><td>120</td><td>1.17 (1.76)</td><td>127</td><td>1.72 (1.56)</td><td>90</td><td>3.82 (3.63)</td><td>105</td><td>2.81 (2.22)</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Comment</td><td colspan="2">Data for 15 year-old only available for 1986 survey. No baseline for this age group, therefore it was excluded from analysis.</td></tr>
</table>

Study tittle: Caries experience of 15 years old children in the Netherlands after discontinuation of water fluoridation

Authors and year of publication: Kalsbeek et al., 1993

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>The Netherlands</td></tr>
<tr><td>Geographic location</td><td>Tiel (WF ceased) & Culemborg (NF control)</td></tr>
<tr><td>Year study started</td><td>1968</td></tr>
<tr><td>Year study ended</td><td>1988</td></tr>
<tr><td>Year of change in fluoridation</td><td>1973</td></tr>
<tr><td>Study design</td><td>Repeated cross sectional survey with negative control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Life long resident, parental consent</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Professional fluoride application (gel), fluoride tablet, fluoride toothpaste, frequency of toothbrushing</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td></td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>1.1 ppm (Tiel); Culemborg (Non-fluoridated)</td></tr>
<tr><td>Fluoride level at the end</td><td>0 (for both areas)</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>DMFT and DMFS</td></tr>
<tr><td>Teeth examined</td><td>Permanent teeth</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>Total for all 6 surveys both groups, N=3545 (Tiel=2051, Colemborg= 1494)</td></tr>
<tr><td>Age groups</td><td>15</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><td></td><td colspan="3">Tiel (WF ceased)</td><td colspan="3">Colemborg (Non-fluoridated)</td></tr>
<tr><td>Year</td><td>N</td><td>DMFT (s.d)</td><td>DMFS (s.d)</td><td>N</td><td>DMFT (s.d)</td><td>DMFS (s.d)</td></tr>
<tr><td>1968/69</td><td>285</td><td>7.4 (±4.0)</td><td>10.8 (±7.7)</td><td>261</td><td>14.1 (±5.7)</td><td>27.7 (±14.6)</td></tr>
<tr><td>1987/ 88</td><td>297</td><td>5.5 (±4.7)</td><td>9.6 (±9.9)</td><td>241</td><td>4.8 (±4.2)</td><td>7.7 (±8.2)</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Comment</td><td colspan="2">~Clinical & radiographs examination.
~Data from six surveys were data available: 1968/69, 1979/80, 1981/82, 1983/84, 1985/86, 1987/88
~The survey in 1968/69 as (pre) and survey in 1987/88 was used as (post-intervention)</td></tr>
</table>

Study tittle: Caries frequency in permanent teeth before and after discontinuation of water fluoridation in Kuopio, Finland

Authors and year of publication: Seppa et al., 1998

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Finland</td></tr>
<tr><td>Geographic location</td><td>Kuopio (WF ceased)& Jyvaskyla (control-NF)</td></tr>
<tr><td>Year study at baseline</td><td>1992</td></tr>
<tr><td>Year study at follow up</td><td>1995</td></tr>
<tr><td>Year of change in fluoridation</td><td>1992</td></tr>
<tr><td>Study design</td><td>Repeated cross sectional survey with negative control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Children age 6,9,12, and 15</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Fluoride varnish, fluoride toothpaste, fluoride tablets/lozenges</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Finnish</td></tr>
<tr><td>Other confounding</td><td>Xylitol chewing gum, fluoride varnish, sealant application,</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>1 ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>0.1 ppm</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>DMFS</td></tr>
<tr><td>Teeth examined</td><td>Permanent</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>Total 1992 (n=550), 1995 (n=1198)</td></tr>
<tr><td>Age groups</td><td>6,9,12 and 15 years</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><td></td><td></td><td colspan="4">Mean DMFS (S.E)</td></tr>
<tr><td>Year/ Age</td><td>N</td><td>6</td><td>9</td><td>12</td><td>15</td></tr>
<tr><td colspan="6">Fluoridated</td></tr>
<tr><td>1992</td><td>278</td><td>0.06 (0.04)</td><td>0.88 (0.16)</td><td>1.88 (0.37)</td><td>4.00 (0.59)</td></tr>
<tr><td>1995</td><td>617</td><td>0.07 (0.04)</td><td>0.69 (0.10)</td><td>1.62 (0.19)</td><td>3.19 (0.28)</td></tr>
<tr><td colspan="6">Non-Fluoridated</td></tr>
<tr><td>1992</td><td>272</td><td>0.03 (0.03)</td><td>0.70 (0.16)</td><td>2.99 (0.47)</td><td>5.62 (0.60)</td></tr>
<tr><td>1995</td><td>581</td><td>0.11 (0.03)</td><td>0.70 (0.15)</td><td>1.63 (0.23)</td><td>3.91 (0.43)</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Academy of Finland</td></tr>
<tr><td>Comment</td><td colspan="2">Clinical examination & radiographs</td></tr>
</table>

Study tittle: Caries in primary dentition, after discontinuation of water fluoridation, among children receiving comprehensive dental care

Authors and year of publication: Seppa et al., 2000a.

Study details	Country of study	Finland
	Geographic location	Kuopio (WF cessation) & Jyväskylä (control, naturally fluoridated 0.1ppm)
	Year study at baseline	1992
	Year study at follow up	1995
	Year of change in fluoridation	1992 (WF ceased- end of the year)
	Study design	Serial cross sectional survey with control group
Inclusion & exclusion criteria	Inclusion criteria	Consented children Non-life time resident was included in the analyses (in Kuopio) Children resident in Kuopio and Jyvaskyla
	Exclusion criteria	Non consented
Confounding factors	Other sources of fluoride	-Fluoride varnish, fluoride toothpaste, fluoride tablet or lozenges (in Non-F area).
	Social class	2 towns are similar with regard to the distribution of sources of livelihood.
	Ethnicity	Finnish?? (not clearly stated)
	Other confounding	-Family income, socio economic status (controlled with logistic regression) -Fluoride varnish, fluoride toothpaste, fluoride tablet or lozenges (in Non-F area).
Fluoride levels	Fluoride level at baseline	1.0ppm
	Fluoride level at the end	0.1ppm (after cessation in Kuopio)
Outcome measure	Caries index	Invited participant= 1315 , participant rate =917 (for both towns)
	Teeth examined	3, 6, and 9 years
Outcomes	No of subjects (N)	Kuopio; 1992, n= 222 and 1995, n=453 Jyväskylä; 1992, n=199 and 1995, n=441
	Age groups	3,6,9

Seppa et al., 2000a (continued)

<table>
<tr>
<td></td>
<td>Caries experience (before & after)</td>
<td>
Number of participant
<table>
<tr><th></th><th colspan="2">WF ceased (Kuopio)</th><th colspan="2">Non-F (Jyväskylä)</th></tr>
<tr><td>Age</td><td>1992 (N %)</td><td>1995 (N %)</td><td>1992 (N %)</td><td>1995(N %)</td></tr>
<tr><td>3</td><td>74 (74%)</td><td>142 (79%)</td><td>64 (64%)</td><td>144 (80%)</td></tr>
<tr><td>6</td><td>68 (68%)</td><td>152 (84%)</td><td>66 (66%)</td><td>148 (82%)</td></tr>
<tr><td>9</td><td>80 (80%)</td><td>159 (88%)</td><td>69 (69%)</td><td>149 (83%)</td></tr>
</table>
dmfs(Mean, SD)
<table>
<tr><th></th><th></th><th colspan="3">WF ceased (Kuopio)</th><th></th><th colspan="3">Non-F (Jyväskylä)</th></tr>
<tr><td></td><td></td><td>1992</td><td></td><td>1995</td><td></td><td>1992</td><td></td><td>1995</td></tr>
<tr><td>Age</td><td>N</td><td>Mean (SD)</td><td>N</td><td>Mean (SD)</td><td>N</td><td>Mean (SD)</td><td>N</td><td>Mean (SD)</td></tr>
<tr><td>3</td><td>74</td><td>0.47 (1.87)</td><td>142</td><td>0.39 (2.25)</td><td>64</td><td>0.33 (1.57)</td><td>144</td><td>0.28 (1.66)</td></tr>
<tr><td>6</td><td>68</td><td>2.26 (2.91)</td><td>152</td><td>1.90 (3.61)</td><td>66</td><td>1.32 (2.51)</td><td>148</td><td>1.26 (2.73)</td></tr>
<tr><td>9</td><td>80</td><td>4.90 (5.13)</td><td>159</td><td>3.55 (4.39)</td><td>69</td><td>2.91 (4.30)</td><td>149</td><td>2.22 (3.55)</td></tr>
</table>
</td>
</tr>
<tr><td>**Funding**</td><td colspan="2">Yrjö Jahnsson Foundation and the Academy Finland</td></tr>
<tr><td>**Comment**</td><td colspan="2"></td></tr>
</table>

Study tittle: Caries trends 1992-1998 in Two Low-Fluoride Finish Towns Formerly with and without fluoridation

Authors and year of publication: Seppa et al., 2000b.

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Finland</td></tr>
<tr><td>Geographic location</td><td>Kuopio (WF cessation); Jyaskyla (control-NF)</td></tr>
<tr><td>Year study at baseline</td><td>1992</td></tr>
<tr><td>Year study at follow up</td><td>1998</td></tr>
<tr><td>Year of change in fluoridation</td><td>1992</td></tr>
<tr><td>Study design</td><td>Serial cross sectional surveys with negative control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Not stated</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Fluoride varnish, fluoride toothpaste</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Information on fissure sealant and fluoride varnish collected</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>1.0ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>0.1ppm(after cessation)</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>DMFT/dmft, DMFS/dmfs , % caries free</td></tr>
<tr><td>Teeth examined</td><td>Permanent and primary</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>1992=688 ,1995=824, 1998=851 (both areas) N for responded subjects</td></tr>
<tr><td>Age groups</td><td>3,6,9,12 and 15 years</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><td></td><td colspan="6">% caries free</td></tr>
<tr><td rowspan="2">Age</td><td colspan="3">Kuopio (WFceased)</td><td colspan="3">Jyvaskyla (control)-NF</td></tr>
<tr><td>1992</td><td>1995</td><td>1998</td><td>1992</td><td>1995</td><td>1998</td></tr>
<tr><td>3</td><td>85</td><td>91</td><td>98</td><td>92</td><td>93</td><td>94</td></tr>
<tr><td>6</td><td>44</td><td>57</td><td>67</td><td>68</td><td>66</td><td>69</td></tr>
<tr><td>9</td><td>21</td><td>30</td><td>35</td><td>45</td><td>48</td><td>40</td></tr>
<tr><td>12</td><td>44</td><td>38</td><td>34</td><td>29</td><td>46</td><td>48</td></tr>
<tr><td>15</td><td>27</td><td>27</td><td>25</td><td>10</td><td>22</td><td>41</td></tr>
<tr><td>N</td><td>352</td><td>421</td><td>437</td><td>336</td><td>403</td><td>414</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Comment</td><td colspan="2">~Clinical examination and radiographs.
~Mean DMFT & dmfs showed in graph, no exact figure stated except for caries free . Can’t access raw data as DMFT & dmfs in graph.</td></tr>
</table>

Stopping water fluoridation and caries, cross sectional studies and a positive control group

Study tittle: Patterns of dental caries following the cessation of water fluoridation

Authors and year of publication: Maupome et al., 2001a

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Canada</td></tr>
<tr><td>Geographic location</td><td>British Colombia (Comox/Courtney:WF ceased, Kamloops: positive control)</td></tr>
<tr><td>Year study at baseline</td><td>1993/94</td></tr>
<tr><td>Year study at follow up</td><td>1996/97</td></tr>
<tr><td>Year of change in fluoridation</td><td>1992</td></tr>
<tr><td>Study design</td><td>Repeated cross sectional and a longitudinal investigation with concurrent positive control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Consented</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Fluoride toothpaste, fluoride supplement, oral hygiene practice</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Social economic levels, snacking practices (including beverages), oral hygiene practices</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>Comox/Courtney =1ppm; Kamloops=1ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>Comox/Courtney =0; Kamloops=1ppm</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>Modified D1D2MFS index</td></tr>
<tr><td>Teeth examined</td><td>Permanent teeth</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>All subjects (N=5927); life long residents (N=2994)</td></tr>
<tr><td>Age groups</td><td>Baseline, 1994/94: Grades 2,3,8 & 9
Follow-up, 1996/96: Grades 5,6,11 & 12</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><th>Study site/Grades</th><th>Measure</th><th>93/94 survey</th><th>96/96 survey</th></tr>
<tr><td>WF ceased</td><td>Subjects -N</td><td>1468</td><td>1067</td></tr>
<tr><td>Grade 2 & 3</td><td>Mean age</td><td>8.3</td><td>8.2</td></tr>
<tr><td></td><td>D1D2MFS Mean (SD)</td><td>1.29 ± 2.10</td><td>0.63 ± 1.69</td></tr>
<tr><td>Still fluoridated</td><td>Subjects- N</td><td>1239</td><td>1111</td></tr>
<tr><td>Grade 2 & 3</td><td>Mean age</td><td>8.3</td><td>8.3</td></tr>
<tr><td></td><td>D1D2MFS Mean (SD)</td><td>0.37 ± 1.11</td><td>0.30 ± 0.94</td></tr>
<tr><td>WF ceased</td><td>Subjects -N</td><td>1716</td><td>1144</td></tr>
<tr><td>Grade 8 & 9</td><td>Mean age</td><td>14.3</td><td>14.3</td></tr>
<tr><td></td><td>D1D2MFS Mean (SD)</td><td>4.93 ± 6.43</td><td>3.86 ± 5.67</td></tr>
<tr><td>Still fluoridated</td><td>Subjects- N</td><td>1504</td><td>608</td></tr>
<tr><td>Grade 8 & 9</td><td>Mean age</td><td>14.4</td><td>14.3</td></tr>
<tr><td></td><td>D1D2MFS Mean (SD)</td><td>2.27 ± 3.88</td><td>2.41 ± 4.58</td></tr>
</table>
</td></tr>
</table>

Maupome et al., 2001a (continued)

Funding	NHRDF Operating Grant 6610-2225-002
Comment	~Data were analysed for both – all subjects & life long residents ~The same children examine in 1993/94 and in the follow up survey 1996/97.

Study tittle: Measuring the short-term impact of fluoridation cessation on dental caries in Grade 2 children using tooth surface indices

Authors and year of publication: McLaren et al., 2016

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Canada</td></tr>
<tr><td>Geographic location</td><td>Calgary (WF ceased) and Edmonton (control)</td></tr>
<tr><td>Year study at baseline</td><td>2004/2005</td></tr>
<tr><td>Year study at follow up</td><td>2013/2014</td></tr>
<tr><td>Year of change in fluoridation</td><td>2011</td></tr>
<tr><td>Study design</td><td>Cross sectional with concurrent positive control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Signed parental consent & child verbal consent, life long resident who reported usually drinking water.</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Matched population: both large urban centres with diverse demographic profiles.</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Sugar consumption (mentioned in discussion but no detail information)</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>Not stated (assume 1.0ppm)</td></tr>
<tr><td>Fluoride level at the end</td><td>0 (ceased)</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Caries index</td><td>DMFS and defs</td></tr>
<tr><td>Teeth examined</td><td>Permanent teeth:12 teeth-central incisors, lateral incisors, first molars).
Primary teeth: all primary teeth</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>Total (12,581)</td></tr>
<tr><td>Age groups</td><td>Grade 2</td></tr>
<tr><td>Caries experience (before & after)</td><td>
<table>
<tr><td></td><td colspan="4">Calgary (WF ceased)
Mean (95 CI%)</td><td colspan="4">Edmonton (WF continued)
Mean (95 CI%)</td></tr>
<tr><td>Index</td><td>N</td><td>2004/05</td><td>N</td><td>2013/14</td><td>N</td><td>2004/05</td><td>N</td><td>2013/14</td></tr>
<tr><td>defs</td><td>599</td><td>2.6 (2.2-3.0)</td><td>3230</td><td>6.4 (5.9-6.9)</td><td>6445</td><td>4.5 (4.1-4.8)</td><td>2307</td><td>6.6 (6.0-7.2)</td></tr>
<tr><td>DMFS</td><td>590</td><td>0.45 (0.37-0.52)</td><td>3182</td><td>0.15 (0.13-0.17)</td><td>6373</td><td>0.25 (0.22-0.28)</td><td>2263</td><td>0.21 (0.17-0.25)</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Canadian Institutes of Health Research (funding reference GIR 127083), Alberta Health and Alberta Health Services. Lindsay McLaren holds an Applied Public Health Chair award funded by the Canadian Institutes of Health Research (Institute of Population and Public Health, Institutes of Musculoskeletal Health and Arthritis), the Public Health Agency of Canada and Alberta Innovates- Health Solutions.</td></tr>
<tr><td>Comment</td><td colspan="2">Total sample size (N) was calculated based on children with primary teeth</td></tr>
</table>

Reduction of fluoride level in the water and caries, cross sectional survey with no control group

Study tittle: Effect of an interruption in water fluoridation on the caries prevalence of the primary and secondary dentition

Authors and year of publication: Kunzel, 1980

<table>
<tr><td rowspan="6">**Study details**</td><td>Country of study</td><td>Germany</td></tr>
<tr><td>Geographic location</td><td>Karl-Marx-Stadt</td></tr>
<tr><td>Year study at baseline</td><td>1959 (fluoridation begin December 1959)</td></tr>
<tr><td>Year study at follow up</td><td>1977</td></tr>
<tr><td>Year of change in fluoridation</td><td>1970 to 1973 (technical problem)-fluoride level reduced from optimum 1ppm</td></tr>
<tr><td>Study design</td><td>Series of cross sectional with historical control</td></tr>
<tr><td rowspan="2">**Inclusion & exclusion criteria**</td><td>Inclusion criteria</td><td>Lifelong resident
Aged 6-15 years (permanent dentition)
Aged 3-8 years (deciduous canines and molars)</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">**Confounding factors**</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>No gender difference in study sample</td></tr>
<tr><td rowspan="2">**Fluoride levels**</td><td>Fluoride level at baseline</td><td>1.0ppm (± 0.1)</td></tr>
<tr><td>Fluoride level at the end</td><td>1970 (0.5ppm), 1971 (0.2ppm), 1972 (0.4ppm)</td></tr>
<tr><td rowspan="2">**Outcome measure**</td><td>Caries index</td><td>DMFT and dft</td></tr>
<tr><td>Teeth examined</td><td>DMFT (all permanent teeth), dft (deciduous canine and molars)</td></tr>
<tr><td rowspan="3">**Outcomes**</td><td>No of subjects (N)</td><td>6-15 years (n=20,000), 3-18 years (12,000).</td></tr>
<tr><td>Age groups</td><td>3-8 years (primary), 6-15 years (permanent)</td></tr>
<tr><td colspan="2">Caries experience (before & after)
<table>
<tr><td rowspan="2">Age</td><td colspan="3">Year /ppm F</td><td></td></tr>
<tr><td>1961
1.0ppm</td><td>1970
0.5ppm</td><td>1971
0.2ppm</td><td>1972
0.4ppm</td></tr>
<tr><td></td><td colspan="3">Mean DMFT (permanent)</td><td></td></tr>
<tr><td>8</td><td>1.7</td><td>0.4</td><td>0.2</td><td>0.3</td></tr>
<tr><td>12</td><td>4.5</td><td>1.7</td><td>1.7</td><td>1.4</td></tr>
<tr><td>15</td><td>7.1</td><td>3.6</td><td>3.1</td><td>3.0</td></tr>
<tr><td></td><td colspan="3">Mean dft (primary)</td><td></td></tr>
<tr><td>5</td><td>3.9</td><td>1.3</td><td>0.9</td><td>1.1</td></tr>
<tr><td>6</td><td>4.0</td><td>1.9</td><td>1.6</td><td>1.4</td></tr>
</table></td></tr>
<tr><td>**Funding**</td><td colspan="2">Not stated</td></tr>
<tr><td>**Comment**</td><td colspan="2">~Fluoridation begin December 1959. Fluoride level was reduced from the water supply due to technical reason. Detailed of fluoride concentration reported for each year with appropriate reference.
~ For permanent teeth, only data for age 8,12 and 15 were extracted. For primary teeth, only data for age 5 were extracted.
~Only overall sample size per year provided. Number of subjects not broken down by age group. Values (N) in the results table were determined by dividing the N for each year by the number of age group.</td></tr>
</table>

FLUOROSIS STUDIES

Stopping water fluoridation and fluorosis

Study tittle: Changes in dental fluorosis following the cessation of water fluoridation

Authors and year of publication: Clark et al., 2006.

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Canada</td></tr>
<tr><td>Geographic location</td><td>Comox/Courtenay and Campbell River communities in British Columbia</td></tr>
<tr><td>Year study started</td><td>1993/94</td></tr>
<tr><td>Year study ended</td><td>2002/03</td></tr>
<tr><td>Year of change in fluoridation</td><td>1992 (water fluoridation cessation)</td></tr>
<tr><td>Study design</td><td>Serial cross sectional with no control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Permanent residency status, school children in second or third grades with parental consent</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Fluoride supplement, fluoride dentifrice, oral hygiene habits</td></tr>
<tr><td>Social class</td><td>Parental education level</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Not stated</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>1 ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>0 ppm</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Fluorosis index</td><td>Thylstrup-Fejerskov Index(TFI) . Any fluorosis TFI>0</td></tr>
<tr><td>Teeth examined</td><td>All teeth and maxillary anterior teeth</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>1137 (total)</td></tr>
<tr><td>Age groups</td><td>6.2 to 9</td></tr>
<tr><td>Fluorosis prevalence (%), before and after</td><td>
<table>
<tr><th rowspan="2">Group (N)</th><th rowspan="2">Year survey</th><th colspan="2">% Any fluorosis</th></tr>
<tr><th>All teeth</th><th>Anterior teeth</th></tr>
<tr><td>1 (437)</td><td>93/94 (baseline)</td><td>58.6</td><td>45.4</td></tr>
<tr><td>2 (261)</td><td>93/94 *[F]</td><td>57.4</td><td>47.4</td></tr>
<tr><td>3 (293)</td><td>96/97</td><td>23.0</td><td>33.4</td></tr>
<tr><td>4 (146)</td><td>2002/03 (after)</td><td>24.4</td><td>22.0</td></tr>
</table>
*[F]: with fluoride supplements</td></tr>
<tr><td>Funding</td><td colspan="2">The study was supported by Canadian Institutes of Health Research operating grant (MOP-57721) and the National Health Research Development Program operating grant (6610-2225-002)</td></tr>
<tr><td>Note</td><td colspan="2"></td></tr>
</table>

Reduction of fluoride level in the water and fluorosis

Study tittle: Partial defluoridation of a community water supply and dental fluorosis

Authors and year of publication: Horowitz et al., 1972

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>USA</td></tr>
<tr><td>Geographic location</td><td>Britton</td></tr>
<tr><td>Year study started</td><td>1948</td></tr>
<tr><td>Year study ended</td><td>1970</td></tr>
<tr><td>Year of change in fluoridation</td><td>1954 (reduction of F level)</td></tr>
<tr><td>Study design</td><td>Cross sectional with no control (historical control)</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Life long resident</td></tr>
<tr><td>Exclusion criteria</td><td>Not stated</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Not stated</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>6.7 ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>1.56 ppm</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Fluorosis index</td><td>Dean (any fluorosis Deans>0)</td></tr>
<tr><td>Teeth examined</td><td>Not stated</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>Total: 436</td></tr>
<tr><td>Age groups</td><td>8</td></tr>
<tr><td>Fluorosis prevalence (%), before and after</td><td><table>
<tr><td>Year</td><td>N</td><td>Any fluorosis % (Deans>0)</td></tr>
<tr><td>1948 (before)</td><td>71</td><td>100</td></tr>
<tr><td>1960</td><td>97</td><td>77.3</td></tr>
<tr><td>1965</td><td>114</td><td>80.7</td></tr>
<tr><td>1970 (after)</td><td>154</td><td>79.2</td></tr>
</table></td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Note</td><td colspan="2"></td></tr>
</table>

Study tittle: The effect of partial defluoridation of a water supply on dental fluorosis- final results in Bartlett, Texas

Authors and year of publication: Horowitz, 1972

Study details	Country of study	USA
	Geographic location	Bartlett,Texas
	Year study started	1954
	Year study ended	1969
	Year of change in fluoridation	1952
	Study design	Cross sectional with no control group (historical control)
Inclusion & exclusion criteria	Inclusion criteria	Life long resident (from birth to 8 with absence from the city for no more than 90 days in any one year).
	Exclusion criteria	Absent on examination day
Confounding factors	Other sources of fluoride	Not stated
	Social class	Not stated
	Ethnicity	Not stated
	Other confounding	Not stated
Fluoride levels	Fluoride level at baseline	8.0 ppm
	Fluoride level at the end	1.0 ppm (average)
Outcome measure	Fluorosis index	Dean (any fluorosis Deans>0)
	Teeth examined	Not stated
Outcomes	No of subjects (N)	Total= 289 ; survey 1954 (n=132); survey1969 (n=157)
	Age groups	8-11 years old
	Fluorosis prevalence (%), before and after	Year (N) / Any fluorosis 1954 (n=132) / 97.7% 1969 (n=157) / 51.0%
Funding	Not stated	
Note		

Study tittle: Changes in dental fluorosis following an adjustment to the fluoride concentration of the Hong Kong's water supply

Authors and year of publication: Evans,1989

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Hong Kong</td></tr>
<tr><td>Geographic location</td><td>4 districts; Aberdeen, Kowloon, Yuen Long and Peng Chau</td></tr>
<tr><td>Year study at baseline</td><td>Not stated</td></tr>
<tr><td>Year study at follow up</td><td>Not stated</td></tr>
<tr><td>Year of change in fluoridation</td><td>1978</td></tr>
<tr><td>Study design</td><td>Cross sectional survey with no control group</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>Lifelong residents, consented children.</td></tr>
<tr><td>Exclusion criteria</td><td>Non-lifelong residents</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Not stated</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Equal numbers boys and girls in total samples</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>1ppm (average). The exact F level varies across district</td></tr>
<tr><td>Fluoride level at the end</td><td>0.7 ppm</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Fluorosis index</td><td>Deans Index</td></tr>
<tr><td>Teeth examined</td><td>Upper central right incisor</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>Total=2382 (lifelong residents); Aberdeen (n=859); Yuen Long (n=684), Kowloon (n=562), Peng Chau (n=274)</td></tr>
<tr><td>Age groups</td><td>7-13 years</td></tr>
<tr><td>Fluorosis prevalence (%), before and after</td><td>
<table>
<tr><th>District</th><th>Age group</th><th>sample</th><th>Fluoride Level (ppm)</th><th>Any fluorosis %</th></tr>
<tr><td colspan="5">Birth cohorts that exposed to higher F level</td></tr>
<tr><td>Aberdeen</td><td>12-13</td><td>138</td><td>0.90</td><td>92.8</td></tr>
<tr><td>Yueng Long</td><td>13</td><td>108</td><td>0.83</td><td>78.7</td></tr>
<tr><td>Kowloon</td><td>11-12</td><td>81</td><td>0.78</td><td>90.1</td></tr>
<tr><td>Peng Chau</td><td>11-12</td><td>33</td><td>0.85</td><td>81.8</td></tr>
<tr><td colspan="5">Birth cohorts that exposed to lower F level</td></tr>
<tr><td>Aberdeen</td><td>7-8</td><td>129</td><td>0.63</td><td>82.5</td></tr>
<tr><td>Yueng Long</td><td>7-8</td><td>63</td><td>0.63</td><td>85.7</td></tr>
<tr><td>Kowloon</td><td>7-8</td><td>40</td><td>0.63</td><td>95.0</td></tr>
<tr><td>Peng Chau</td><td>7-8</td><td>23</td><td>0.61</td><td>95.7</td></tr>
</table>
</td></tr>
<tr><td>Funding</td><td colspan="2">Not stated</td></tr>
<tr><td>Comment</td><td colspan="2">This study compare different birth cohorts that exposed to different fluoride level during development of enamel</td></tr>
</table>

Study tittle: Dental fluorosis following downward adjustment of fluoride in drinking water

Authors and year of publication: Evans & Stamm 1991a

Study details	Country of study	Hong Kong
	Geographic location	Not specific (2 metropolitan & 2 rural cities)
	Year study started	1973 (not clear)
	Year study ended	1986
	Year of change in fluoridation	1978 (reduced F level)
	Study design	Cross sectional with no control
Inclusion & exclusion criteria	Inclusion criteria	Parental consent, lifelong resident, erupted upper right central incisors
	Exclusion criteria	Non-continuous resident (n=172), author provides number and reason of exclusion table
Confounding factors	Other sources of fluoride	Fluoridated toothpaste (starting 1983), formula milk reconstituted with tap water
	Social class	Not stated
	Ethnicity	Not stated
	Other confounding	Not stated
Fluoride levels	Fluoride level at baseline	1.0 ppm
	Fluoride level at the end	0.7 ppm
Outcome measure	Fluorosis index	Dean's Index & CFI
	Teeth examined	Upper right central incisors
Outcomes	No of subjects (N)	1062
	Age groups	7 to 12
	Fluorosis prevalence (%), before and after	Age / F level (ppm) / Any fluorosis % 12 / 0.8 / 88 11 / 0.8 / 85 10 / 0.8+0.6 / 86 9 / 0.8+0.6 / 79 8 / 0.8+0.6 / 76 7 / 0.6 / 77
Funding	Note stated	
Note	This study compare different birth cohorts that exposed to different fluoride level during development of enamel	

Study tittle: Diffuse opacities in 12 year-old Hong Kong children

Authors and year of publication: Wong et al., 2014

<table>
<tr><td rowspan="6">Study details</td><td>Country of study</td><td>Hong Kong</td></tr>
<tr><td>Geographic location</td><td>Not stated</td></tr>
<tr><td>Year study started</td><td>1983</td></tr>
<tr><td>Year study ended</td><td>2010</td></tr>
<tr><td>Year of change in fluoridation</td><td>1978=0.7ppm, 1988: 0.5ppm</td></tr>
<tr><td>Study design</td><td>Serial cross sectional survey with no control</td></tr>
<tr><td rowspan="2">Inclusion & exclusion criteria</td><td>Inclusion criteria</td><td>12 year-old children
Availability of intra-oral photographs of previous surveys</td></tr>
<tr><td>Exclusion criteria</td><td>Presence of fixed orthodontic appliances, overlapping tooth surfaces on the photographs, missing tooth, fracture of a tooth, plaque/stain, restoration & decay on tooth, gingival hyperplasia.</td></tr>
<tr><td rowspan="4">Confounding factors</td><td>Other sources of fluoride</td><td>Fluoridated toothapaste, toothbrushing habits</td></tr>
<tr><td>Social class</td><td>Not stated</td></tr>
<tr><td>Ethnicity</td><td>Not stated</td></tr>
<tr><td>Other confounding</td><td>Not stated</td></tr>
<tr><td rowspan="2">Fluoride levels</td><td>Fluoride level at baseline</td><td>1.0 ppm</td></tr>
<tr><td>Fluoride level at the end</td><td>0.5 ppm</td></tr>
<tr><td rowspan="2">Outcome measure</td><td>Fluorosis index</td><td>DDE (diffuse opacities =fluorosis)</td></tr>
<tr><td>Teeth examined</td><td>Photograph scores on maxillary incisors (4 teeth)</td></tr>
<tr><td rowspan="3">Outcomes</td><td>No of subjects (N)</td><td>2658 (total)</td></tr>
<tr><td>Age groups</td><td>12</td></tr>
<tr><td>Fluorosis prevalence (%)
Before and after</td><td><table>
<tr><td>Year</td><td>N</td><td>F level</td><td>Fluorosis %</td></tr>
<tr><td>1983</td><td>700</td><td>1.0</td><td>89.3</td></tr>
<tr><td>1991</td><td>670</td><td>0.7</td><td>48.5</td></tr>
<tr><td>2001</td><td>620</td><td>0.5</td><td>32.4</td></tr>
<tr><td>2010</td><td>668</td><td>0.5</td><td>42.1</td></tr>
</table></td></tr>
<tr><td>Funding</td><td colspan="2">Research Grants Council of the special Administrative Region, China (Project No: 782811)</td></tr>
<tr><td>Note</td><td colspan="2">Caries data cited from different studies was presented in graph (caries data such as mean caries cannot be extracted). Full text references retrieved (2 articles), however relevant data needed is not available.</td></tr>
</table>

Appendix 12 Validity score for each study

CARIES STUDIES

Author	**Country**	**Study design**	**Prospective**	**Fluoride Measurement**	**Confounding Factors**	**Control for Confounding**	**Blinding**	**Baseline Survey**	**Follow Up**	**Score**	**Level of Evidence**
Reduction studies and caries [historical control]											
Kunzel 1980	Germany	CS (1/4)	1	1/2	0	0	0	1	1	3.75	C
Cessation studies and caries [historical control]											
Lemke et al. 1970	Wisconsin, USA	CS (1/4)	1	0	0	0	0	1	1	3.25	C
Stephen et al. 1987	Scotland, UK	CS (1/4)	1	0	0	0	0	1	1	3.25	C
Kunzel & Fischer, 1997	Germany	CS (1/4)	1	0	1	0	0	1	1	4.25	C
Kunzel & Fischer, 2000	La Salud, CUba	CS (1/4)	1	0	0	0	0	1	1	3.25	C
Kunzel et al. 2000	Germany	CS (1/4)	1	1/2	0	0	0	1	1	3.75	C
Wei & Wei	Gongzhou, China	CS (1/4)	1	0	0	0	0	1	1	3.25	C
Jordan 1962	USA	CS (1/4)	1	0	0	0	0	1	1	3.25	C

WF cessation & caries studies with positive control											
Maupome et. al., 2001	British Columbia, Canada	CS with a positive control (2/4)	1	0	1	1	0	1	1	5.5	B
McLaren et al., 2016	Calgary & Edmonton, Canada	CS with a positive control	1	0	1	1	0	1	1	5.5	B
WF cessation & caries studies with negative control											
Atwood & Blinkhorn, 1989	Scotland, UK	CS with NF control (2/4)	1	0	0	0	0	1	1	3.5	C
Kalsbeek et al. 1993	Netherlands	CS with NF control	1	0	1	0	1/2	1	1	5	B
Seppa et al., 1998 (permanent)	Kuopio & Jyvaskayla, Finland	CS with NF control	1	0	1	0	0	1	1	4.5	C
Seppa et al., 2000a (primary)	Kuopio & Jyvaskayla, Finland	CS with NF control	1	0	1	1	0	1	1	5.5	B
Seppa et al., 2000b	Kuopio & Jyvaskayla, Finland	CS with NF control	1	0	1	0	0	1	1	4.5	C
DHSS, 1969	Kilmanork, Scotland	CS with NF control	1	0	0	0	0	1	1	3.5	C

FLUOROSIS STUDIES

Author	**Country**	**Study design**	**Prospective**	**Fluoride Measurement**	**Confounding Factors**	**Control for Confounding**	**Blinding**	**Baseline Survey**	**Follow Up**	**Score**	**Level of Evidence**
Reduction studies and fluorosis											
Wong et al., 2014	Hong Kong	CS (1/4)	1	0	1	0	1	1	1	5.25	B
Evans et al., 1989	Hong Kong	CS birth cohorts analysis (1/4)	1/2	1/2	0	0	1	1	1/2	3.75	C
Evans & Stamm, 1991	Hong Kong	CS birth cohorts analysis (1/4)	1/2	0	1	0	0	1	1/2	3.25	C
Horowitz & Heifetz, 1972	Texas, USA	CS (1/4)	1	0	0	0	0	1	1	3.25	C
Horowitz et al. 1972	Britton, USA	CS (1/4)	1	1/2	0	0	0	1	1	3.75	C
Cessation studies and fluorosis [historical control]											
Wei & Wei, 2002	Gongzhou, China	CS (1/4)	1	0	0	0	0	1	1	3.25	C
Clark et al, 2006	British Columbia, Canada	CS (1/4)	1	0	1	1	0	1	1	5.25	B

Note: CS=cross sectional

- Study with historical control (1/4)=0.25, studies with control group (2/4)=0.5
- Fluorosis studies (one survey with birth cohorts analysis)= prospective: 1/4 (= 0.5), with follow-up 1/2 (=0.5)
- Blinding (not blincing clinically but with with xray)=1/2 (=0.5)
- Fluoride measurement: measure F level as part of study (1), if mention reference of appropriate F measurement in the water (0.5

Appendix 13 Sampling of school and calculated sample size in fluoridated and non-fluoridated areas

a) Sampling of school and calculated sample size in fluoridated area

FLUORIDATED AREA (NEGERI SEMBILAN)								
Age	Districts	No of schools A	No of children B	% of population C	Large schools	Small Schools	Sample required D	Selected schools
				A/B x100			C x 400	D/50
9	Seremban	108	9120	0.54	78	30	212	4
	P.Dickson	54	2169	0.13	19	35	52	1
	Rembau	30	635	0.04	4	26	16	1*
	Kuala Pilah	51	1122	0.07	12	39	28	1**
	Jelebu	25	616	0.04	6	19	16	*
	Jempol	45	1616	0.10	20	25	40	**
	Tampin	43	1543	0.09	21	22	36	1
	TOTAL	356	16,821		160	196	400	8
12	Seremban	108	9147	0.53	78	30	210	
	P.Dickson	54	2201	0.13	19	35	50	
	Rembau	30	782	0.05	4	26	20	
	Kuala Pilah	51	1249	0.07	12	39	28	
	Jelebu	25	635	0.04	6	19	16	
	Jempol	45	1691	0.10	20	25	40	
	Tampin	43	1586	0.09	21	22	36	
	TOTAL	356	17,291		160	196	400	

*/** Due to small number needed per district, two neighbouring districts were combined (Rembau and Jelebu; Kuala Pilah and Jempol). Similar schools were used for both age groups.

b) Sampling of school and calculated sample size in non-fluoridated area

NON-FLUORIDATED AREA (KELANTAN)								
Age	Districts	No of schools A	No of children B	% of population C	Large schools	Small Schools	Sample required D	Selected schools
				A/B x100			C x 400	D/50
9	Tanah Merah	33	2185	0.24	28	5	96	2
	Jeli	17	914	0.10	13	4	40	1
	Bachok	34	2482	0.27	32	2	108	3
	Kuala Krai	39	1774	0.19	25	14	76	1
	Gua Musang	39	1868	0.20	20	19	80	1*
	TOTAL	162	9233		118	44	400	8
12	Tanah Merah	33	2488	0.24	28	5	96	
	Jeli	17	1066	0.10	13	4	40	
	Bachok	34	2810	0.27	32	2	108	
	Kuala Krai	39	1900	0.19	25	14	76	
	Gua Musang	39	1999	0.19	20	19	80	
		162	10,263		118	14	400	

*Substitute with spare school in Bachok due to flood issue in that particular district during data collection.

Participant ID:

Appendix 14 Survey instrument

a. Questionnaire (English version)

Dear parent/guardian,

Pupils in your child's class have been selected to participate in this survey. To ensure the success of this survey, we would appreciate if you could answer the following questions.
Please return the completed questionnaire to the school teacher tomorrow. Your child will receive a toothbrush and toothpaste as a token of appreciation for participation in this survey. We will offer all parents who send back a completed questionnaire, an incentive of entry to a prize draw for one of five RM 100 shopping vouchers.
Thank you very much for your assistance. If you have any questions about the questionnaire, feel free to get in touch with Dr Nor Azlida Mohd Nor (Tel:03-79674805, email: azlida@um.edu.my)

PART A: Your child's personal details and residential status

Please provide the following information about your child

1. Your child's name

……………………………………………

…………………………………………..

…………………………………………

2. Your child's date of birth (dd/mm/yyyy)

3. Your child's gender

☐ Male ☐ Female

4. Your child's ethnicity

☐ Malay ☐ Chinese ☐ Indian ☐ Others (specify)…………………………………

5. Is your child a life-long resident in the area where you currently live?

☐ NO (Go to Q6) ☐ YES (Go to Q7)

6. If NO, please provide the address(es) of all your child's previous residence. (*Refer example below*)

Previous residential		Years of residence	
District	State	From (Month/Year)	To (Month/Year)
Example: Kuala Lipis	*Pahang*	*Sept/2007*	*Oct/2009*

PART B: In this section we want you to recall your child's previous feeding practice

7. **Was your child breast-fed?**

☐ YES (go to Q8)   ☐ NO (go to Q9)

8. **At what age did your child finish breast feeding?**

……………Months

9. **Was your child ever fed with an infant formula?**

☐ Yes (go to Q10)   ☐ No (go to Q14)

10. **At what age did your child start an infant formula?** →

………….Years   ……………Months

11. **At what age did your child finish an infant formula?**

………….Years   ……………Months

12. **What brands of the infant formula was he/she fed?**
(Please write brands) →

…………………………………………....
……………………………………………
……………………………………………

13. **What type of water did you usually use to prepare the infant formula for your child?**
(Tick one box only)

☐ Tap water
☐ Filtered tap water/ Reverse osmosis
☐ Bottled water

PART C: In this section, we would like to ask you about the oral hygiene practices of your child

14. **Does your child brush their teeth?**

☐ Yes (go to Q15)   ☐ No (go to Q24)

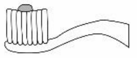
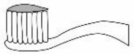
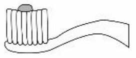
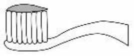
15. **At what age did you start brushing your child's teeth?**

………….Years   ……………Months

16. **At what age did your child first use toothpaste?** *(Tick one box only)*

☐ Before 2 years old   ☐ After 4 years old
☐ Between 2 to 4 years old   ☐ Does not use toothpaste

For the following questions, please tick one box only about the oral hygiene practices of your child at two different times: (i) when your child was aged less than 6 years old (ii) current practice

	When your child was aged less than 6 years old	**Now (current practice)**
17. How often did/does your child brush his/her teeth?	☐ Less than once a day ☐ Once a day ☐ Twice a day ☐ More than twice a day	☐ Less than once a day ☐ Once a day ☐ Twice a day ☐ More than twice a day
18. How often did/do you usually supervise your child's toothbrushing?	☐ Everyday ☐ Sometimes ☐ Never ☐ Not sure	☐ Everyday ☐ Sometimes ☐ Never ☐ Not sure
19. After tooth brushing did/does your child usually	☐ Just swallow ☐ Rinse and swallow ☐ Rinse and spit ☐ Just spit ☐ Don't know	☐ Just swallow ☐ Rinse and swallow ☐ Rinse and spit ☐ Just spit ☐ Don't know
20. Have you noticed your child eating or licking toothpaste	☐ Often ☐ Sometimes ☐ Never	☐ Often ☐ Sometimes ☐ Never
21. Usually, how much toothpaste did your child use when brushing? *(Tick one box only)*	 A smear on the brush ☐  A pea-sized amount ☐  Moderate amount ☐  Cover all the brush bristles ☐	 A smear on the brush ☐  A pea-sized amount ☐  Moderate amount ☐  Cover all the brush bristles ☐
22. What type of toothpaste did/does your child usually use?	☐ Fluoridated adult toothpaste ☐ Fluoridated children's toothpaste ☐ Non-fluoridated toothpaste ☐ Don't know	☐ Fluoridated adult toothpaste ☐ Fluoridated children's toothpaste ☐ Non-fluoridated toothpaste ☐ Don't know
23. What toothpaste brands did/do you most frequently used at home for your child? *(Only one answer allowed)*	………………………………….. ………………………………….. *(Please write the most common brand used)*	…………………………………… …………………………………… *(Please write the most common brand used)*

24. **Has your child ever had fluoride varnish or gel applied by their dentist/dental nurse before age 6?** *(Tick one box only)*

☐ Yes ☐ No ☐ Don’t know

25. **What is the source(s) of drinking water in your home?** *(You can tick more than one answer)*

☐ Tap water

☐ River/stream water

☐ Bottled water

☐ Others (specify)……………………

→ **26.** **If tap water is used, is the tap water filtered?**

☐ Yes ☐ No

PART D: Characteristics of the household

The following questions are about your household. These questions will help us to understand if different methods of preventing dental problems work equally well for all groups within the community and to ensure that the researchers obtain representative samples of the population.

QUESTION	Father or Guardian (A)	Mother or Guardian (B)
a) What is your occupation? *(Please write)*	……………………………………	……………………………………
b) What is the highest level of education you have? *(Tick one box only)*	☐ Never been to school ☐ Did not complete primary school ☐ Completed standard 6 ☐ Completed Form 3 ☐ Completed Form 5 ☐ Completed STPM/Certificate/Diploma ☐ Completed a degree ☐ Completed a postgraduate degree ☐ Others:…………………………	☐ Never been to school ☐ Did not complete primary school ☐ Completed standard 6 ☐ Completed Form 3 ☐ Completed Form 5 ☐ Completed STPM/Certificate/Diploma ☐ Completed a degree ☐ Completed a postgraduate degree ☐ Others:…………………………
c) Which category does your monthly income fall into? *(Tick one box only)*	☐ No monthly income ☐ Less than RM1000 ☐ RM1001-1999 ☐ RM2000-3999 ☐ RM4000-4999 ☐ More than RM5000	☐ No monthly income ☐ Less than RM1000 ☐ RM1001-1999 ☐ RM2000-3999 ☐ RM4000-4999 ☐ More than RM5000

ID Peserta:

b.Questionnaire (Malay version)

Kepada Ibu/Bapa/Penjaga,

Anak tuan/puan adalah di antara pelajar yang terpilih untuk menyertai kajiselidik ini. Untuk menjayakan kajiselidik ini, kami amat menghargai sekiranya tuan/puan dapat bekerjasama menjawab soalan-soalan berikut. **Borang yang telah lengkap di isi perlulah di kembalikan kepada pihak sekolah pada hari berikutnya**. Anak anda akan menerima berus gigi dan ubat gigi sebagai tanda penghargaan menyertai kaji selidik ini. Ibu bapa/penjaga yang mengembalikan borang kajiselidik yang lengkap berpeluang untuk memenangi cabutan bertuah *voucher* membeli belah bernilai RM100 setiap satu.

Terima kasih di atas kerjasama yang diberikan. Jika anda mempunyai sebarang pertanyaan mengenai kaji selidik ini, boleh menghubungi Dr. Nor Azlida Mohd Nor (Tel:03-79674805, email: azlida@um.edu.my)

PART A: Maklumat anak anda dan status penempatan

Sila isikan maklumat di bawah

1. Nama anak

…………………………………………

…………………………………………

…………………………………………

2. Tarikh lahir anak (dd/mm/yyyy)

3. Jantina anak

☐ Lelaki ☐ Perempuan

4. Kumpulan etnik anak

☐ Melayu ☐ Cina ☐ India ☐ Lain-lain (nyatakan)………………………………..

5. Adakah anak anda bermaustatin secara tetap sejak lahir di kawasan anda tinggal sekarang?

☐ TIDAK (jawab soalan 6) ☐ YA (jawab soalan 7)

6. Jika TIDAK, sila nyatakan alamat kediaman tempat tinggal anda terdahulu.
(*Rujuk contoh di bawah*)

Kediaman terdahulu		Tahun menetap	
Daerah	Negeri	Dari (Bulan/Tahun)	Hingga (Bulan/Tahun)
Contoh: Kuala Lipis	*Pahang*	*Sept/2007*	*Okt/2009*

PART B: Di bahagian ini anda perlu mengimbas kembali amalan pemakanan anak anda

7. Adakah anak anda pernah menyusu dengan susu ibu?

☐ YA (jawab soalan 8) ☐ TIDAK (jawab soalan 9)

8. Pada umur berapakah anak anda berhenti menyusu dengan susu ibu?

……………Bulan

9. Adakah anak anda pernah menyusu dengan susu formula?

☐ YA (jawab soalan 10) ☐ TIDAK (jawab soalan 14)

10. Pada umur berapakah anak anda mula menyusu dengan susu formula? →

…………Tahun ……………Bulan

11. Pada umur berapakah anak anda berhenti menyusu dengan susu formula?

…………Tahun ……………Bulan

12. Apakah jenama susu formula yang digunakan oleh anak anda *(Tuliskan jenama)* →

…………………………………………......

……………………………………………..

……………………………………………..

13. Jenis air yang digunakan untuk membancuh susu formula anak anda? *(Tandakan satu jawapan sahaja)*

☐ Air paip (dimasak)

☐ Air paip ditapis/*reverse* osmosis (RO)

☐ Air mineral/ air minuman di dalam botol

PART C: Di bahagian ini anda perlu mengimbas kembali amalan kesihatan pergigian anak anda

14. Adakah anak anda memberus gigi?

☐ Ya (jawab soalan 15) ☐ Tidak (jawab soalan 24)

15. Sejak umur berapakah anak anda mula memberus gigi?

…………Tahun ……………Bulan

16. Sejak umur berapakah anak anda menggunakan ubat gigi? *(Tandakan satu jawapan sahaja)*

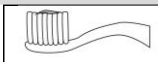
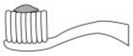
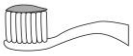
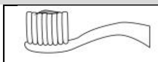
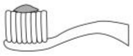
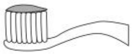
☐ Sebelum umur 2 tahun

☐ Di antara umur 2 hingga 4 tahun

☐ Selepas umur 4 tahun

☐ Tidak menggunakan ubat gigi

Bagi soalan-soalan berikutnya, sila tandakan satu jawapan berkaitan amalan pergigian anak anda bagi setiap tempoh masa yang diberikan: **(i) Sewaktu anak anda berumur 6 tahun ke bawah & (ii) pada umur sekarang**

	Amalan pergigian sewaktu anak anda berumur 6 tahun ke bawah	**Amalan pergigian pada umur sekarang**
17. Kekerapan anak anda memberus gigi?	☐ Kurang dari satu kali sehari ☐ Sekali sehari ☐ Dua kali sehari ☐ Lebih dari dua kali sehari	☐ Kurang dari satu kali sehari ☐ Sekali sehari ☐ Dua kali sehari ☐ Lebih dari dua kali sehari
18. Kekerapan anda mengawasi anak anda ketika dia memberus gigi?	☐ Setiap hari ☐ Kadang kala ☐ Tidak pernah ☐ Tidak pasti	☐ Setiap hari ☐ Kadang kala ☐ Tidak pernah ☐ Tidak pasti
19. Sebaik sahaja selesai memberus gigi, apakah yang dilakukan oleh anak anda?	☐ Telan sahaja ☐ Kumur dan telan ☐ Kumur dan ludah ☐ Ludah sahaja ☐ Tidak tahu	☐ Telan sahaja ☐ Kumur dan telan ☐ Kumur dan ludah ☐ Ludah sahaja ☐ Tidak tahu
20. Adakah anda perasan anak anda menjilat atau menelan ubat gigi?	☐ Kerap kali ☐ Kadangkala ☐ Tidak pernah	☐ Kerap kali ☐ Kadangkala ☐ Tidak pernah
21. Pada kebiasaannya berapa banyak ubat gigi digunakan oleh anak anda? *(Tandakan satu jawapan sahaja)*	 Secalit ubat gigi ☐  Saiz kacang 'pea' ☐  Saiz sederhana ☐  Sepanjang berus gigi ☐	 Secalit ubat gigi ☐  Saiz kacang 'pea' ☐  Saiz sederhana ☐  Sepanjang berus gigi ☐
22. Apakah jenis ubat gigi yang kebiasaannya digunakan oleh anak anda?	☐ Ubat gigi berfluorida (dewasa) ☐ Ubat gigi berfluorida (kanak-kanak) ☐ Ubat gigi tanpa fluorida ☐ Tidak tahu	☐ Ubat gigi berfluorida (dewasa) ☐ Ubat gigi berfluorida (kanak-kanak) ☐ Ubat gigi tanpa fluorida ☐ Tidak tahu
23. Apakah jenama ubat gigi yang biasanya digunakan anak anda di rumah? *(Satu jenama sahaja)*	…………………………………… …………………………………… *(Tuliskan jenama yang paling kerap diguna)*	…………………………………… …………………………………… *(Tuliskan jenama yang paling kerap diguna)*

24. Pernahkah anak anda menerima sapuan gel/varnish fluorida oleh doktor/jururawat pergigian sewaktu umur di bawah 6 tahun? *(Tandakan satu jawapan sahaja)*

☐ Ya ☐ Tidak ☐ Tidak Tahu

25. Apakah sumber-sumber air di rumah anda?
(Boleh tanda lebih dari satu jawapan)

→ **26. Jika air paip digunakan adakah penapis air digunakan?**

☐ Ya ☐ Tidak

☐ Air paip
☐ Air sungai
☐ Air mineral/*reverse* osmosis di dalam botol
☐ Lain-lain (nyatakan)………………..

PART D: Maklumat isi rumah

Soalan berikut adalah mengenai isi rumah. Soalan-soalan ini dapat membantu kami untuk memahami perbezaan kaedah pencegahan masalah pergigian untuk semua golongan di dalam masyarakat. Ia juga bagi memastikan penyelidik memperolehi sampel mencukupi untuk mewakili masyarakat yang pelbagai.

SOALAN	Bapa atau Penjaga (A)	Ibu atau Penjaga (B)
a) Apakah pekerjaan anda *(Sila tuliskan)*	…………………………………..	……………………………………
b) Taraf pendidikan *(Tandakan satu jawapan sahaja)*	☐ Tidak pernah bersekolah ☐ Tidak habis sekolah rendah ☐ Tamat Darjah 6 ☐ Tamat Tingkatan 3 ☐ Tamat Tingkatan 5 ☐ Tamat STPM/sijil/diploma ☐ Tamat pengajian sarjana muda ☐ Tamat pengajian pasca-ijazah ☐ Lain-lain:………………………	☐ Tidak pernah bersekolah ☐ Tidak habis sekolah rendah ☐ Tamat Darjah 6 ☐ Tamat Tingkatan 3 ☐ Tamat Tingkatan 5 ☐ Tamat STPM/sijil/diploma ☐ Tamat pengajian sarjana muda ☐ Tamat pengajian pasca-ijazah ☐ Lain-lain:………………………
c) Jumlah pendapatan sebulan *(Tandakan satu jawapan sahaja)*	☐ Tiada pendapatan bulanan ☐ Kurang dari RM1000 ☐ RM1001-1999 ☐ RM2000-3999 ☐ RM4000-4999 ☐ Lebih dari RM5000 dan ke atas	☐ Tiada pendapatan bulanan ☐ Kurang dari RM1000 ☐ RM1001-1999 ☐ RM2000-3999 ☐ RM4000-4999 ☐ Lebih dari RM5000 dan ke atas

Appendix 15 Guidelines of questionnaire distribution

(English version)

Dear teacher,

Thank you for helping us to distribute the questionnaire. There are ….. sets of survey forms which consist of a **questionnaire**, **consent form** and **patient information sheet**. This set of survey forms need to be distributed to the parents/guardians of children in Grade 3 and Grade 6 of …… classes namely ……………………………………………………………………

1) **Patient information sheet**: provides a detailed explanation to the parents/guardians about the survey. The parents/guardians can detached the sheet and keep it for their own reference.

2) **Consent form**: written approval is required from the parents/guardians for their child to participate in this study.

3) **Questionnaire**: comprises of questions relating to the survey objectives. Parents/guardians who consent to participate are required to complete the questionnaire and return to the school no later than the **27th February 2015.**

If you have any questions about the form, feel free to get in touch with me.

Dr. Nor Azlida Mohd Nor
Department of Community Oral Health and Clinical Prevention
Faculty of Dentistry
University of Malaya
50603 Kuala Lumpur
Tel:03-79674805/ 010-2716747 Email: azlida@um.edu.my

Guidelines of questionnaire distribution (Malay version)

Kepada Guru yang berkenaan,

Terima kasih diucapkan atas kerjasama dari pihak sekolah untuk mengagihkan borang kaji selidik ini. Terdapat …… set borang kaji selidik telah di hantar kepada pihak sekolah. Terdapat 3 jenis borang yang perlu di agihkan kepada setiap ibu bapa/penjaga pelajar yang telah di jemput untuk menyertai kaji selidik ini (Tahun 3 dan Tahun 6) dari kelas ……………………………………………………….. Set borang-borang ini mengandungi butiran seperti berikut.

1) **Risalah maklumat** adalah bertujuan untuk memberitahu dengan lebih lanjut tentang kaji selidik ini kepada ibu bapa/penjaga. Risalah ini boleh diceraikan dari borang yang lain untuk simpanan ibu bapa/penjaga.

2) **Borang kebenaran ibu bapa/penjaga** bertujuan mendapatkan persetujuan bertulis dari ibu bapa/penjaga untuk mereka dan anak mereka menyertai kaji selidik ini secara suka rela. Ibu bapa/penjaga yang bersetuju perlu mengisi bahagian ini dan mengembalikan kepada pihak sekolah.

3) **Borang soal selidik (questionnaire)** adalah borang yang perlu di isi secara lengkap oleh ibu bapa/ penjaga yang bersetuju untuk menyertai kajian ini dan dikembalikan kepada pihak sekolah.

Untuk makluman, kajian ini hanya melibatkan pemeriksaan gigi dan menjawab borang soal selidik. Kami memohon jasa baik guru yang terlibat untuk memastikan para pelajar mengembalikan borang kepada pihak sekolah dalam jangka waktu masa yang ditetapkan iaitu pada selewat-lewatnya pada **27 Februari 2015.** Insentif di tawarkan bagi ibu bapa yang menghantar borang kaji selidik yang lengkap iaitu berpeluang memenangi 20 *voucher* cabutan bertuah bernilai RM 100 setiap satu.

Kerjasama dan sokongan guru-guru dalam kaji selidik ini amat dihargai dan didahului dengan ucapan ribuan terima kasih.

Sebarang permasalahan boleh di rujuk kepada penyelidik seperti alamat di bawah.

Dr Nor Azlida Mohd Nor
Pensyarah kanan
Jabatan Pergigian Masyarakat & Pencegahan Klinikal
Fakulti Pergigian
Universiti Malaya
50603 Kuala Lumpur.
Tel:03-79674805/ 010-2716747 Email: azlida@um.edu.my

Appendix 16 Participant information Sheet (English Version)

PARTICIPANT INFORMATION SHEET

Title: Oral health following adjustment of fluoride levels in Malaysian public water supply

1. Introduction

You and your child are being invited to take part in a research survey. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Participation in this research is voluntary and we hope you will help us with this survey.

2. What is the purpose of the study?

The survey aims to look for cavities and white patches that may or may not be present on the tooth surface among 9 and 12-year-old school children who are continuous life-long residents in identified fluoridated and non-fluoridated areas. The information obtained from the survey will be used to monitor children's oral health status in Malaysia.

3. Why have I been chosen?

Nine and 12-year-old school children have been randomly chosen to participate in this survey. This survey will be carried out in selected Malaysian primary schools and carried out by a government dentist in school. You and your child have been chosen to participate in this survey at random from the list of names in your child's class.

4. Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

5. What will happen to me if I take part?

If you agree to take part in this survey, we will ask for two things:
Your consent for your child to have a simple dental examination in school.
In addition, the front teeth of your child will be photographed. None of the images will contain any identifying aspects of your child face. An example of the intraoral image is shown below (see Figure 1).
We will also send you a questionnaire about your child's dental health.

Figure 1: Intraoral image of front teeth

6. What about confidentiality?

All information obtained will be strictly confidential and will only be used for the purpose of this survey.

7. What do I have to do?

If you agree to take part in this survey, you will have to do the following:
Return the signed consent form to indicate your agreement for your child to participate in the survey.
We will ask you to complete a questionnaire about your child's dental health. This will take about 5-10 minutes to complete.

8. Are there any risks?

There are no risks involved in this survey. The survey will be carried out with the highest standard of hygiene and only disposable materials and instruments will be used for the oral examination.

9. Will I receive an incentive for returning the questionnaire?

Yes, we will offer all parents who send back a completed questionnaire, an incentive of entry to a prize draw for one of twenty RM100 (£20) shopping vouchers.

10. What will happen to the results of the research study?

The results of the survey will help us to understand the current state of children's dental health and the impact of oral hygiene practices in young children. The results of the survey will be published as a PhD thesis. You will not be identified in any report or publication. A copy of the published results will be available by contacting Dr Nor Azlida Mohd Nor at the address below.

11. Who is organising and funding the research?

This survey is a joint project between Cardiff University and University Malaya funded by Ministry of Education, Malaysia.

12. Contact for further Information

If you have any questions about the survey, please contact the dentist who will be examining your child during the survey as follows:

Dr. Nor Azlida Mohd Nor
Department of Community Oral Health and Clinical Prevention
Faculty of Dentistry
University of Malaya
50603 Kuala Lumpur
Tel:03-79674805/ 010-2716747 Email: azlida@um.edu.my

Appendix 17 Consent form (English version)

CONSENT FORM

Title: Oral health following adjustment of fluoride levels in Malaysian public water supply

Researcher: Dr. Nor Azlida Mohd Nor

Please tick (/)

1. I confirm that I have read and understand the information sheet dated 30th April 2014 (version 1.1) for the above survey and have the opportunity to ask questions. ☐

2. I understand that my child's participant in this survey is voluntary and that I am free to withdraw my child at any time without giving any reason. ☐

3. I am happy for my child to take part in the above survey. I consent to photograph(s) of my child's teeth being taken for dental records for this survey. I consent to the photograph(s) of my child being published in open access journal, textbook or other form of medical publication. ☐

____________________ ____________________

Child's name Relationship with the child

____________________ ____________________ __________

Parent/Guardian's name Signature Date

Appendix 18 Patient Information Sheet (Malay version)

RISALAH MAKLUMAT (untuk ibu bapa/penjaga)

Tajuk: Status kesihatan pergigian selepas penyelarasan kepekatan fluorida di dalam bekalan air di Malaysia

1. Pengenalan

Anda dan anak anda dijemput untuk mengambil bahagian di dalam kajiselidik ini. Risalah maklumat ini menerangkan dengan lebih terperinci mengenai kajian dan membantu anda untuk membuat keputusan jika anda ingin mengambil bahagian. Kajiselidik ini adalah projek bersama di antara Universiti Malaya dan Cardiff University, United Kingdom di bawah tajaan Kementerian Pendidikan Malaysia.

2. Apakah tujuan kaji selidik ini?

Kaji selidik ini bertujuan untuk mengenalpasti gigi yang berlubang, tompok keputihan yang mungkin ada atau tiada pada permukaan gigi kanak-kanak yang berumur 9 dan 12 tahun yang merupakan penduduk yang bermastautin secara berterusan di kawasan-kawasan yang telah dikenalpasti mempunyai air berfluorida atau air tidak berfluorida.

3. Kenapa saya terpilih?

Murid-murid sekolah berumur 9 dan 12 tahun telah dipilih secara rawak untuk mengambil bahagian dalam kajian ini. Kajian ini dijalankan di sekolah-sekolah terpilih di Malaysia oleh seorang doktor pergigian. Anda dan anak anda terpilih secara rawak untuk mengambil bahagian dalam kajian ini daripada senarai nama pelajar di dalam kelas anak anda.

4. Perlukah saya mengambil bahagian?

Penyertaan di dalam kajian ini adalah secara sukarela. Jika anda mengambil keputusan untuk menyertai kajian ini, anda akan di berikan risalah maklumat ini untuk di simpan dan anda di minta untuk menandatangi borang keberanan sebagai tanda persetujuan. Anda berhak menarik diri pada bila-bila masa tanpa sebarang sebab.

5. Apa yang akan berlaku sekiranya saya mengambil bahagian?

Jika anda bersetuju untuk meyertai kajiselidik ini, kami memohon melakukan perkara di bawah:

Kebenaran bertulis untuk anak anda menjalani pemeriksaan gigi di sekolah. Selain itu, gambar gigi hadapan anak anda akan di rakam. Hanya imej gigi hadapan di rekodkan dan tiada gambar wajah atau identiti diri anak anda akan di ambil (Gambar 1 sebagai rujukan)

Kami juga akan menghantar borang soal-selidik mengenai kesihatan mulut anak anda.

Gambar 1: Imej gigi hadapan

6. Bagaimana dengan kerahsiaan identiti?

Segala maklumat yang dikumpul adalah sulit dan hanya digunakan untuk tujuan kajiselidik ini sahaja.

7. Apa yang perlu saya lakukan?

Jika anda bersetuju untuk meyertai kajian ini, anda perlu melakukan perkara berikut:
Mengembalikan kebenaran bertulis untuk keizinan anak anda menyertai kajian ini
Anda di minta untuk mengisi borang kaji selidik mengenai kesihatan pergigian anak anda. Ini mengambil masa sekitar 5-10 minit.

8. Adakah terdapat sebarang risiko?

Tiada sebarang risiko untuk anak anda di dalam kajiselidik ini. Kajian ini akan dijalankan dengan tahap kebersihan dan kawalan infeksi mengikut piawaian dan hanya peralatan pakai buang akan digunakan semasa pemeriksaan mulut.

9. Adakah saya akan menerima sebarang insentif sekiranya melengkapkan dan mengembalikan borang kajiselidik?

Ya, ibu bapa/ penjaga berpeluang untuk memenangi hadiah cabutan bertuah iaitu 20 *voucher* membeli belah bernilai rm100 setiap satu sekiranya mengembalikan borang soal-selidik yang lengkap di isi.

10. Apa akan berlaku kepada hasil kajian penyelidikan?

Hasil kajian ini akan membantu kami untuk memahami kesihatan gigi dan impak penjagaan kesihatan mulut di kalangan kanak-kanak. Keputusan kaji selidik itu akan diterbitkan sebagai *thesis PhD*. Identiti anda tidak akan dikenal pasti dalam apa-apa laporan atau penerbitan. Salinan kajian yang diterbitkan boleh didapati dengan menghubungi Dr Nor Azlida Mohd Nor di alamat di bawah.

11. Siapakah yang menaja penyelidikan ini?

Kajian ini adalah projek kerjasama di antara *Cardiff University* dan Universiti Malaya yang di taja oleh Kementerian Pelajaran Malaysia.

12. Butiran lanjut

Sekiranya anda mempunyai sebarang pertanyaan lanjut, sila hubungi:

Dr. Nor Azlida Mohd Nor
Pensyarah Kanan
Jabatan Kesihatan Pergigian dan Pencegahan Klinikal
Fakulti Pergigian, Universiti Malaya
50603 Kuala Lumpur.
Pejabat : 0379674805 Mobile: 010-2716747 Email: azlida@um.edu.my

Appendix 19 Consent form (Malay Version)

BORANG KEBENARAN

Tajuk: Status kesihatan pergigian selepas penyelarasan kepekatan fluorida di dalam bekalan air di Malaysia

Nama Penyelidik: Dr. Nor Azlida Mohd Nor

Sila tandakan (/)

1. Saya telah membaca dan memahami risalah maklumat bertarikh 30 April 2014 (versi 1.1) untuk kaji selidik ini. ☐

2. Saya memahami bahawa penyertaan anak saya adalah secara sukarela dan boleh menarik diri pada bila-bila masa, tanpa sebarang sebab. ☐

3. Saya bersetuju anak saya mengambil bahagian dalam kaji selidik ini dan membenarkan gambar gigi anak saya digunakan untuk rekod pergigian kajian ini. ☐

________________________ ________________________

Nama murid Hubungan dengan murid

________________________ ________________________ ____________

Nama ibubapa/penjaga Tandatangan Tarikh

Versi:1 .1 Tarikh:30-04-14

Appendix 20 Example of signed consent form

BORANG KEBENARAN

Tajuk: Status kesihatan pergigian selepas penyelarasan kepekatan fluorida di dalam bekalan air di Malaysia

Nama Penyelidik: Dr. Nor Azlida Mohd Nor

Sila tandakan (/)

1. Saya telah membaca dan memahami risalah maklumat bertarikh 30 April 2014 (versi 1.1) untuk kaji selidik ini. ☑
2. Saya memahami bahawa penyertaan anak saya adalah secara sukarela dan boleh menarik diri pada bila-bila masa, tanpa sebarang sebab. ☑
3. Saya bersetuju anak saya mengambil bahagian dalam kaji selidik ini dan membenarkan gambar gigi anak saya digunakan untuk rekod pergigian kajian ini. ☑

AHMAD FAREEZ b. ADDRIZAL
Nama murid

Ibu
Hubungan dengan murid

NORADNIZAWATI bt. ABDULLAH
Nama ibubapa/penjaga

Tandatangan

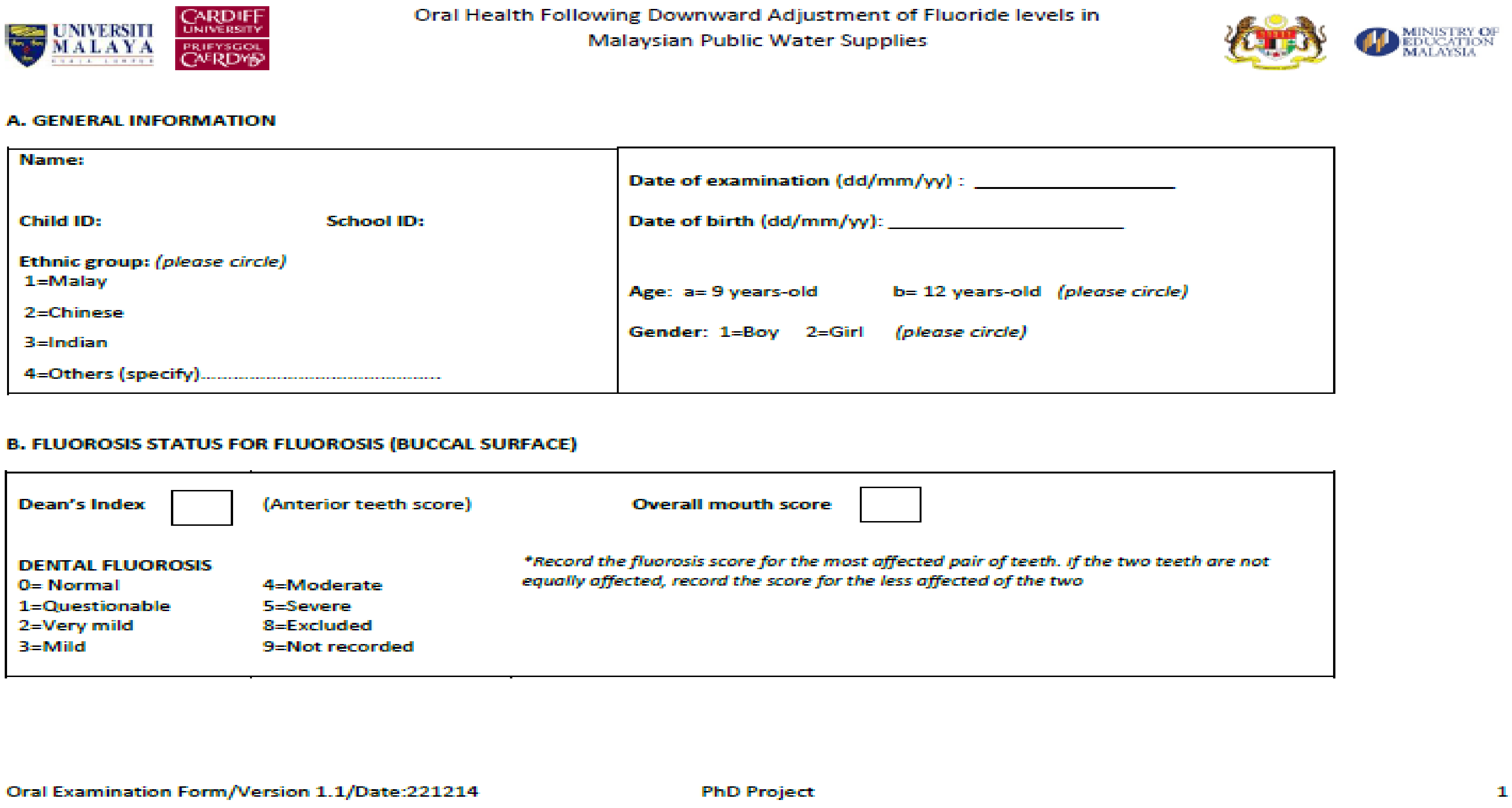
22.1.2015
Tarikh

Borang Kebenaran/ Versi:1 .1/ Tarikh:30-04-14

Appendix 21 Clinical examination room and procedure

[redacted]

Appendix 22 Clinical examination form



Oral Health Following Downward Adjustment of Fluoride levels in
Malaysian Public Water Supplies

A. GENERAL INFORMATION

Name:

Child ID: **School ID:**

Ethnic group: *(please circle)*
1=Malay
2=Chinese
3=Indian
4=Others (specify)……………………………………

Date of examination (dd/mm/yy) : ____________________

Date of birth (dd/mm/yy): ________________________

Age: a= 9 years-old b= 12 years-old *(please circle)*

Gender: 1=Boy 2=Girl *(please circle)*

B. FLUOROSIS STATUS FOR FLUOROSIS (BUCCAL SURFACE)

Dean's Index ☐ (Anterior teeth score) **Overall mouth score** ☐

DENTAL FLUOROSIS
0= Normal
1=Questionable
2=Very mild
3=Mild
4=Moderate
5=Severe
8=Excluded
9=Not recorded

**Record the fluorosis score for the most affected pair of teeth. If the two teeth are not equally affected, record the score for the less affected of the two*

Oral Examination Form/Version 1.1/Date:221214 PhD Project 1

NOTE: A 2-digit code should be used.
The first digit=restoration/sealant code.
Second digit= Caries Code
Φ Score for all sound teeth only

C. CHART FOR DENTAL CARIES (ALL SURFACES)- ICDAS Epidemiology Index

	Upper Right		55	54	53	52	51		61	62	63	64	65	Upper Left		
	17	16	15	14	13	12	11		21	22	23	24	25	26	27	
D								D								D
O					■	■	■	O	■	■	■					O
M								M								M
B								B								B
L								L								L
Φ								Φ								Φ
D								D								D
O					■	■	■	O	■	■	■					O
M								M								M
B								B								B
L								L								L
Φ								Φ								Φ
	47	46	45	44	43	42	41		31	32	33	34	35	36	37	
	Lower Right		85	84	83	82	81		71	72	73	74	75	Lower Left		

Appendix 23 Criteria for Dean's fluorosis index

Code	Classification	Criteria
0	Normal	The enamel represents the usual translucent semivitriform type of structure. The surface is smooth, glossy and usually of pale creamy white colour.
1	Questionnable (<10% of surface)	The enamel discloses slight (<10% of surface) aberrations from the translucency of normal enamel, ranging from a few white flecks to occasional white spots. This classification is utilised in those instances where a definite diagnosis is not warranted and a classification of "normal" not justified.
2	Very mild (10-25% of surface)	Small, opaque, paper white areas scattered irregularly over the tooth but not involving as much as approximately 25 per cent of the tooth surface. Frequently included in this classification are teeth showing no more than about 1 – 2mm of white opacity at the tip of the summit of the cusps, of the bicuspids or second molars.
3	Mild (25-50% of surface)	The white opaque areas in the enamel of the teeth are more extensive but do involve as much as 50 percent of the tooth.
4	Moderate	All enamel surfaces of the teeth are affected and surfaces subject to attrition show wear. Brown stain is frequently a disfiguring feature
5	Severe	All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is discrete or confluent pitting. Brown stains are widespread and teeth often present a corroded-like appearance.

Appendix 24 Example of the intra-oral photographs of fluorosis

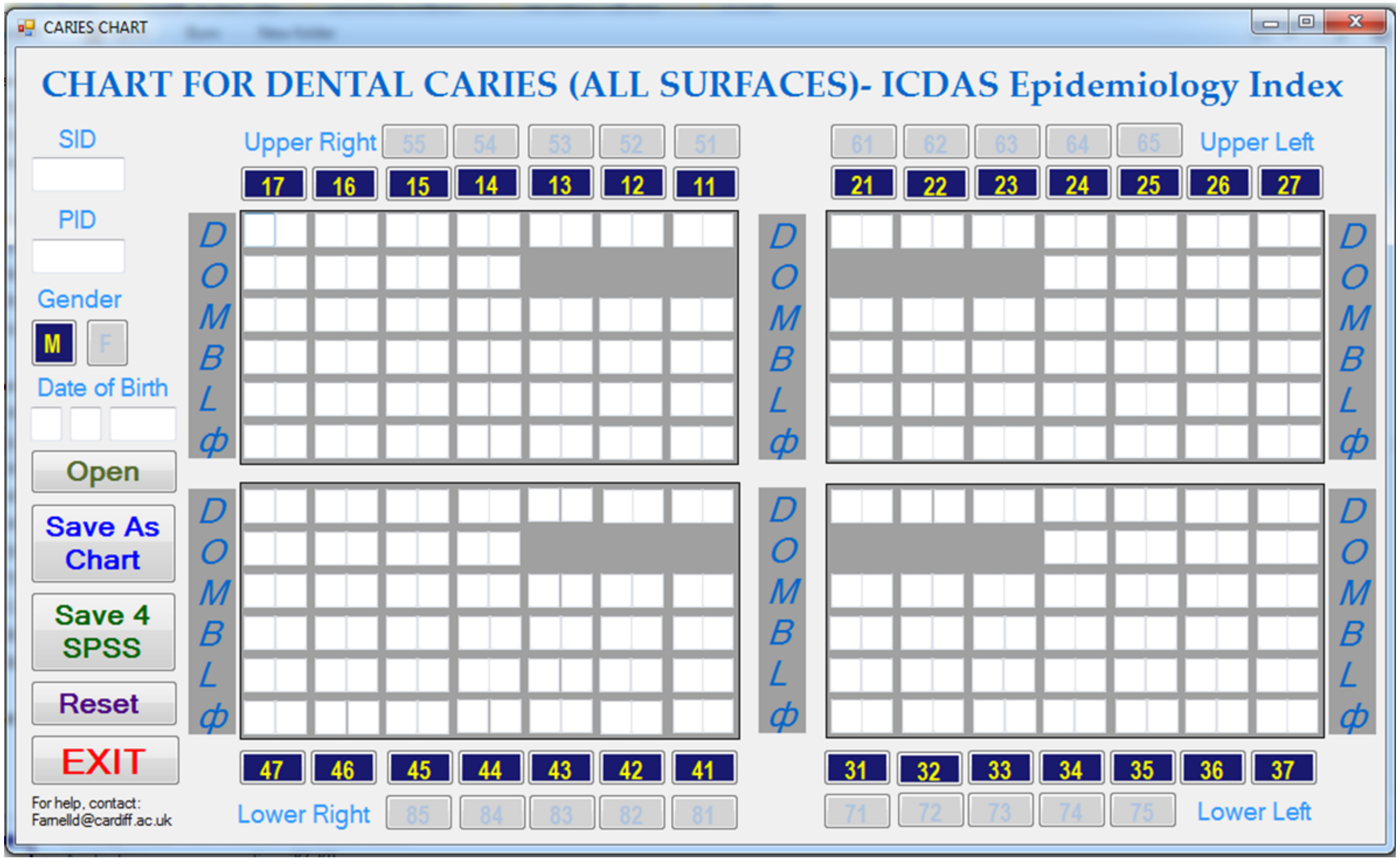
Normal

Very mild

Mild

Moderate

Appendix 25 Visual basic interface for ICDAS caries data



Appendix 26 Feedback from an expert committee of the questionnaire following face-validation

Original Questionnaire	Committee Feedback	Pre-questionnaire after modification
Question 4 Your child's Identity Card (IC) number	Remove this question as it is considered confidential and not relevant for the research.	Question 4 was removed
Question 7 Was your child given the following milk at the infant stage? Breast feeding ☐ How long? _____ months ____ (years)/ Formula milk ☐ Since what age? _____ months ____ (years)/ Both the above ☐	Consider to rephrase this question and reorganize the answer options to meet the objectives of the study.	The changes have been made as follows: Q9. At what age did your child begin breast feeding? _____ years / _____months Q10. At what age did your child finish breast feeding? _____ years / _____months Q12. At what age did your child start an infant formula? _____ years / _____months Q13. At what age did your child finish an infant formula? _____ years / _____months

Original Questionnaire	**Committee Feedback**	**Pre-questionnaire after modification**
Question 15 How did you usually prepare the infant formula for your child? Tap water Reverse osmosis/ filtered tap water Bottled water Question 15 (Malay version) *Jenis air yang digunakan untuk membancuh susu formula anak anda?* *Air masak (dimasak)* *Air paip ditapis/ reverse osmosis* *Air mineral/air minuman di dalam botol*	Require rephrasing answer option of Question 15 from "tap water" to "boiled tap water" as the word tap water alone may have a different meaning to Malaysian population. This is due to the norm practice of the society to boil tap water before drinking or preparing infant formula. The term "reverse osmosis" is considered a common term among lay population in Malaysia and this term remained as an answer option in the Malay version. Direct translation for "bottled water" to Malay is *"air botol"* may give different meaning to respondents. The committee suggested to change "bottled water" to "mineral water" for the English version. The Malay term that have same conceptual meaning to original version as *"air mineral/ air minuman di dalam botol"*	The changes have been made as follows: How did you usually prepare the infant formula for your child? Tap water (boiled) Reverse osmosis/ filtered tap water Mineral water *Jenis air yang digunakan untuk membancuh susu formula anak anda?* *Air masak (dimasak)* *Air paip ditapis/ reverse osmosis* *Air mineral/air minuman di dalam botol*

Original Questionnaire	Committee Feedback	Pre-final questionnaire after modification
Question 19 Does your child brush their teeth? Yes, everyday Yes, sometimes Never Not sure	To remove the term “yes” for answer options in Question 19 Yes, everyday Yes, sometimes………..	The changes have been made as follows: Does your child brush their teeth? Everyday Sometimes Never Not sure

Appendix 27 Article in the Community Dental Health Journal

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Examiner reliability in fluorosis scoring: a comparison of photographic and clinical methods

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Objective: To assess examiner reliability when scoring dental fluorosis in Malaysian children using clinical (Dean's Index) and photographic methods. ***Method:*** The upper central incisors of 111 children were examined both clinically and photographically for fluorosis status using Dean's index. Twenty children were re-examined after a two-week interval for intra-examiner reliability by a single examiner. In addition, two independent examiners and the clinical examiner scored 111 photographic images of the same children in a standardized manner. Fluorosis scores were compared individually between examiners for both clinical and photographic scoring. Examiner reliability was assessed using both simple and weighted kappa statistics at tooth level. Sensitivity, specificity, positive-negative predictive values and a Receiver Operating Characteristic (ROC) curve were also calculated to determine the accuracy of the test. ***Results:*** Across the three examiners, the prevalence of fluorosis (Dean's score ≥2) using photographs was lower (ranged from 23% to 26%) than the prevalence recorded by clinical examination (30%). The kappa score for intra-examiner reliability for the duplicate clinical examination was excellent (0.89). Inter-examiner reliability between the photographic method and the clinical examination (gold standard) for each examiner was substantial with weighted kappa values ranging from 0.74 to 0.77. The photographic method indicated higher specificity (99%) than sensitivity (79%) and the area under the ROC curve was also high (0.89) which suggests good accuracy of the diagnostic test. ***Conclusion:*** These results suggest that photographic examination of fluorosis on central incisors can be recorded with good examiner reliability. The recorded fluorosis prevalence was lower using the photographic scores.

Key words: *Dean's index, dental fluorosis, Malaysia, reliability*

Introduction

Dental fluorosis is defined as hypomineralization of tooth enamel resulting from exposure to excess levels of fluoride during tooth formation (Dean, 1934). Clinically, dental fluorosis presents as white striations or diffuse parchment-like areas on the enamel. In more severe cases, fluorosed enamel appears pitted and yellowish-brown in colour (Browne *et al*., 2005; Buzalaf and Levy, 2011; Mascarenhas, 2000).

Several epidemiological indices have been developed to describe the clinical appearance of dental fluorosis. No one index has emerged as the agreed standard method, the index of choice to a degree depending on the objective of the study. Dean's Index (Dean, 1934) was the first index reported in the literature. In the intervening years, other indices were developed, the aim being to improve Dean's Index criteria: Thylstrup and Fejerskov Index (TFI; Fejerskov *et al*., 1977), Tooth Surface Index of Fluorosis (TSIF; Horowitz *et al*., 1984) and the Fluorosis Risk Index (Pendrys, 1990). These indices were classified as aetiological indices that specifically measure fluoride induced enamel changes. In contrast, descriptive indices such as the Developmental Defects of Enamel (DDE) index, record enamel defects, based on descriptive criteria, without assuming the aetiology of the defects (FDI, 1982).

Regardless of which index is used in the clinical assessment of fluorosis, the diagnosis can be affected by many factors such as variation in the method of examination, tooth condition (wet or dry), lighting conditions, examiner bias and intra and inter-examiner reliability (Whelton *et al*., 2004). A potential way of overcoming these shortcomings is to use a standardized photographic method for capturing a permanent record of the appearance of the enamel.

There are however, advantages and disadvantages in photographic assessment compared with direct clinical recording of dental fluorosis. The major benefits of photographs are that they capture a permanent record and allow blind scoring. Photography also enables scoring by multiple examiners in multi-site studies and allows repeated assessments of the same images (Cochran *et al*., 2004a; Ellwood *et al*., 1994; Fejerskov *et al*., 1977; Soto-Rojas *et al*., 2008).

The disadvantages of using photographs are firstly variation in photographic technique between studies such as differences in equipment, lens, lighting system and the quality of the image produced. Secondly, difficulties in capturing teeth images due to lack of accessibility especially for posterior teeth mean that photographs have only been used to record the anterior teeth, mainly incisors and canines. This could result in under reporting of the prevalence of dental fluorosis. In contrast, the greater detail provided by photographs may well result in over reporting prevalence (Cochran *et al*., 2004a; Soto-Rojas *et al*., 2008).

There are a number of studies that have assessed the prevalence of fluorosis using photographs alone (Cochran *et al.*, 2004b; Ellwood *et al.*, 1994; Tavener *et al.*, 2007) and clinical examinations compared with photographs (Stephen *et al.*, 2002). Several studies have compared photographic methods with clinical examination using the (DDE) Index (Nunn *et al.*, 1993; Wong *et al.*, 2005), the Fluorosis Risk Index (Cruz-Orcutt *et al.*, 2012; Soto-Rojas *et al.*, 2008) and the TF index (Ellwood *et al.*, 1996). Some studies compared several indices against each other using both clinical and photographic methods (Pretty *et al.*, 2012; Sabieha and Rock, 1998). However, information is scarce on how Dean's Index compares with photographic methods. Therefore, the present study aims to compare examiner reliability and the relative prevalence and severity scores resulting from clinical and photographic assessment of fluorosis using Dean's index in Malaysian children.

Method

Data from this study were obtained from a larger on-going Malaysian fluoridation study. The 111 children studied in this exercise were aged 9 and 12 years old and were lifetime residents in a fluoridated community (0.5-0.7mg/L) located in Shah Alam, Selangor. To be included in the study, the children were required to have no medical contraindications to undergoing a clinical dental examination and have had informed written consent provided by their parent or guardian.

Fluorosis was scored by three examiners. Examiner 1 undertook both clinical and photographic assessment, whilst examiners 2 and 3 participated only in the photographic assessment. Examiner 1 received extensive training in the use of Dean's Index as part of the Malaysian National Oral Health Survey. The training for fluorosis assessment involved an online training module (Whelton *et al.*, 2014), theoretical explanation and clinical assessment on clinical subjects. The same online training module was used by the two photographic only examiners (2 and 3). This online training generated kappa scores for intra-examiners reliability and was repeated until each examiner reached very good to excellent kappa values. Prior to conduct of the study, Examiner 1 repeated the online training module and received intra-oral photographic technique training from the Audio-Visual Department, School of Dentistry, Cardiff University.

Clinical examinations were conducted by a trained and calibrated examiner (examiner 1). Clinical recording of fluorosis was conducted under natural light with the subject sitting on a chair in the upright position using a disposable mirror, CPITN probe and gauze for plaque removal (if necessary). Maxillary central incisors were evaluated using Dean's Index in a wet condition (0, normal; 1, questionable; 2, very mild; 3, mild; 4, moderate; 5, severe). If fluorosis was present, diagnosis was based on the condition of the maxillary central incisors. If the two central incisors were not equally affected, the condition of the least affected tooth was recorded. Twenty children were re-examined after a two-week interval to assess intra-examiner agreement.

Immediately after the clinical examination, digital images of the maxillary incisors were taken with a digital SLR camera, Nikon 90D body, sigma 105mm f/2.8 macro lens and sigma macro ring flash E140. The photographic technique used in this study followed the method described by Cochran and colleagues (2004a). A cheek retractor was inserted into the child's mouth and they were instructed to keep their head still and place their teeth edge to edge. If it was not possible to maintain edge to edge incisal contact, the child was instructed to bring their upper and lower central incisors into the same vertical plane as far as possible. The photographs were taken while the teeth were still wet. Children were asked to moisten their teeth before the photograph was taken. If this was not possible damp cotton wool was used to keep the teeth moist. Most of the photographs only involved one exposure per child. However on occasion, where the examiner was not satisfied with the first photograph (such as issues with specular reflection), further exposures were attempted.

None of the images contained any identifying aspects of the subject's face. A photographic log form enabled the digital images to be linked to a subject identifying code. The digital images were downloaded to a computer for storage and viewing. In those cases where more than one exposure had been taken, the best quality image was selected.

Photographic image scoring took place in Cardiff University, 45 days after clinical examinations in Malaysia. Each photograph was assigned a unique identifying number. The photographs were then mixed randomly for blind fluorosis scoring. All 111 images were included in the assessment and projected onto a white screen using Microsoft PowerPoint in a darkened room. The size of the image projected on screen was approximately 69cm by 38cm. In terms of magnification of the image approximately five times linear magnification from standard photo print size 12.5cm by 7.5cm. All three examiners were seated approximately three metres from the screen and scored the photographs at the same time under identical lighting conditions. Following individual assessment, all examiners re-examined all photographs and discussed scores thoroughly to achieve consensus agreement on the final photographic score. The consensus photographic score was based on the agreement of at least two of the examiners. In the blind scoring protocol, a specific code of 'unable to score image' was also included alongside with Dean's Index code. Any issues with the images such as presence of light reflection or excess camera-flash were noted during the evaluation of each photograph.

This study was reviewed and approved by Cardiff University Dental School Research Ethics Committee (DSREC 14/17a). In addition, permission to conduct the study was obtained from the relevant Ministries in Malaysia namely the Ministry of Health, the Ministry of Education and the State Education Department.

Data were entered analysed using SPSS and STATA software. The tooth-level Dean's score was compared between the same examiner (clinical versus duplicate clinical score; clinical versus photographic score) and different examiners (individual photographic score versus clinical score, Table 1); individual photographic score versus other examiner, Table 2) and individual photographic score versus consensus photographic score, Table 2). The clinical score was used as the gold standard.

Table 1. Inter-examiner agreement of dental fluorosis by clinical examination

	Unweighted data		*Weighted data*	
Clinician Examiners	*Kappa*	*Agreement*	*Kappa*	*Agreement*
Examiner 1 clinical versus Examiner 2 photographs	0.82	93%	0.77	90%
Examiner 1 clinical versus Examiner 3 photographs	0.72	89%	0.74	87%

Table 2. Inter-examiner agreement of dental fluorosis between individual photographic score and consensus photographic score

	Unweighted data		*Weighted data*	
Clinicians	*Kappa*	*Agreement (%)*	*Kappa*	*Agreement (%)*
Examiner 1 vs Examiner 2	0.78	92	0.80	95
Examiner 1 vs Examiner 3	0.72	90	0.85	96
Examiner 2 vs Examiner 3	0.85	95	0.75	89
Examiner 1 vs Consensus	0.83	94	0.91	96
Examiner 2 vs Consensus	0.91	96	0.87	94
Examiner 3 vs Consensus	0.90	96	0.82	92

Note: Consensus photographic score based on the agreement of at least two of the three examiners. Examiner 1 was both a clinical and a photographic examiner, Examiners 2 and 3 were photographic examiners only

Table 3. Weighting matrix used for computing the weighted kappa statistic

	Normal	*Questionable*	*Very mild*	*Mild*	*Moderate*
Normal	1	½	0	0	0
Questionable	½	1	½	0	0
Very mild	0	½	1	½	0
Mild	0	0	½	1	½
Moderate	0	0	0	½	1

A positive diagnosis of fluorosis was based on very mild or greater (Dean's score ≥2) or as no fluorosis (Dean's score 0 or 1). For statistical analysis the data were dichotomised into fluorosis or no fluorosis as simple kappa analysis. A weighted kappa value was generated for inter-examiner reliability using STATA Software in order to utilize the full range of Dean's Index. The weighted matrix used for computing weighted kappa statistics is shown in Table 3. A weight of 1 was given for exact agreement, a weight of 0.5 was given when examiner disagreed by only one severity level and a weight of 0 was given when examiners disagreed by more than one severity level. Descriptive analysis was used to describe the prevalence of fluorosis. McNemar's Test was used to determine if there were statistically significant differences between the prevalence of fluorosis using clinical and photographic methods (Altman, 1990). Percentage agreement and kappa statistics were used to assess examiner reliability at tooth level. Kappa interpretation was based on the definition by Landis and Koch (1977). Kappa values 0.81 to 1.0 indicate excellent agreement, 0.61 to 0.80 indicate substantial agreement, 0.41 to 0.60 indicate moderate agreement, 0.21 to 0.40 indicate fair and less than 0.20 indicate poor agreement. In addition, sensitivity, specificity, positive-negative predictive values and a Receiver Operating Characteristic (ROC) curve between clinical and consensus photo score were also calculated to determine the accuracy of the diagnostic test.

Results

A total of 111 participants were examined clinically and 111 images of these same participants were examined photographically for fluorosis on central maxillary incisors. It was possible to score all 111 images and none were excluded because of poor image quality.

Following re-assessment of 20 children, intra-examiner clinical examination reliability by a single examiner (examiner 1) indicated substantial agreement (89.6%) with a weighted kappa value of 0.89. Intra-examiner agreement between all 111 photographs and corresponding clinical examinations by a single examiner (examiner 1) also indicated substantial agreement with a weighted kappa value of 0.87. Although there was good intra-examiner reliability, Examiner 1 identified a significantly higher prevalence of fluorosis in clinical scores (30%) than photographic scores (23%) (p=0.02).

Table 4 shows the fluorosis prevalence and frequency distribution of Dean's scores for individual examinations of clinical and photographic methods. Most fluorosis cases fell into very mild and mild categories. The prevalence of fluorosis (Dean's score ≥2) using clinical examination was higher than the consensus photographic score (30% vs. 24%, p=0.07).

Table 1 shows inter-examiner reliability between clinical and photographic methods. Inter-examiner reliability between photographic examiners (2 and 3) versus clinical examiner (examiner 1) was found to have substantial agreement using both weighted and simple kappa statistics.

Table 2 shows all examiners demonstrated substantial to excellent inter-examiner reliability for photographic scoring with weighted kappa values ranging from 0.72 to 0.91. There was little difference found between weighted and simple kappa analysis.

Further analysis was carried out using the consensus photographic score versus the clinical examination (gold standard) score. Sensitivity, specificity, positive-negative predictive values and the Receiver Operating Characteristic (ROC) curve were calculated. Table 5 shows that the diagnosis of fluorosis using the photographic method had a higher specificity (99%) than sensitivity (79%); positive predictive value 96%; negative predictive value 92% likelihood ratios (+LR=39;-LR=0.22).

Table 4. Fluorosis prevalence and frequency distribution of Dean's scores for individual examinations for clinical and photographic methods

Method of examination	*Dean's Index score, n (%)*						*Fluorosis**
	0	*1*	*2*	*3*	*4*	*5*	*Prevalence*
	Normal	*Questionable*	*Very mild*	*Mild*	*Moderate*	*Severe*	*n (%)*
Clinical	72 (65)	6 (5)	21 (19)	10 (9)	2 (2)	0	33 (30)
Photographic:							
Examiner 1	73 (66)	12 (11)	15 (14)	9 (8)	2 (2)	0	26 (23)
Examiner 2	77 (69)	5 (5)	21 (19)	6 (5)	2 (2)	0	29 (26)
Examiner 3	75 (68)	11 (10)	10 (9)	14 (13)	1 (1)	0	25 (23)
Consensus photo score	73 (69)	11 (10)	16 (14)	9 (8)	2 (2)	0	27 (24)

Note: *A positive diagnosis of fluorosis is based on a Dean's classification score of very mild or greater. Consensus photographic score based on the agreement of at least two of the three examiners. Examiner 1 was both a clinical and a photographic examiner, Examiner s 2 and 3 are photographic examiner only.

Table 5. Level of agreement in the diagnosis of dental fluorosis between clinical score (gold standard) and consensus photographic score

Photographic	*Clinical Examination*		
Scores	*Fluorosis*	*No fluorosis*	*Totals*
Fluorosis	26 (79%)	1 (1%)	27 (24%)
No fluorosis	7 (21%)	77 (99%)	84 (76%)
Totals	33 (100%)	78 (100%)	111(100%)

Sensitivity 0.79 (79%); specificity 0.99 (99%); accuracy 0.93 (93%); positive predictive value 96%; negative predictive value 92%; likelihood ratio of positive test (+LR) 39; likelihood ratio of the negative test (-LR) 0.22.

The area under the curve (AUC) was high (0.89) when consensus photographic score was compared to clinical score. The AUC results closer to maximum value of 1 suggest good accuracy of the diagnostic test in the differentiation between fluorosis and non-fluorosis.

Discussion

The present study's analysis focused on examiner reliability on fluorosis assessment and is part of larger on-going research into Malaysian fluoridation. The key findings of this study were fluorosis prevalence was higher using clinical examinations than the photographic method and both intra- and inter-examiner reliability was good for photographic assessment.

It is difficult to compare the data from the current study, with other published studies because of the differences in the clinical examination method, photographic technique and indices used in previous studies. In the following comparisons these limitations should be borne in mind.

Findings from this study can be compared with the one previous study assessing agreement of fluorosis diagnosis between clinical against photographic methods using Dean's Index (Pretty *et al.*, 2012). That study compared a specific photographic techniques (traditional digital technique versus polarized white light versus quantitative light fluorescence) using two indices (Dean's and TF Indices). These authors reported a higher fluorosis prevalence (Dean's Index) with all photographic methods than with clinically recorded scores. The difference in findings may have been due their drying of teeth prior to taking the photograph, whereas in the present study the teeth were photographed wet. By drying the teeth, the contrast between normal and abnormal enamel may be enhanced which allows a more detailed examination. In contrast, measuring fluorosis with the teeth wet may obscure some of the subtleties of fluorosis (Cochran *et al.*, 2004a; Ellwood *et al.*, 1994; Thylstrup and Fejerskov, 1978), but it could be argued more nearly reflects conditions of everyday life. In addition, Pretty and coworkers used two different indices in their study and whether the teeth were dried or not during the clinical examination was not clearly discussed. The differences in clinical examination method used for each index may also account for some of the differences.

When comparing the results of the present study with other studies of different indices for measuring fluorosis, the present findings were similar to some (Martins *et al.*, 2009; Soto-Rojas *et al.*, 2008). Soto Rojas and colleagues reported a higher prevalence of fluorosis by clinical examination (22%) than by the photographic method (18%) using TSFI criteria. The later study by Martins *et al.* (2009) reported that the prevalence of fluorosis was higher when assessed by clinical examination (49%) compared to a photographic method (37%) among 49 Brazilian children, however the fluorosis criteria used was not made clear. However, other studies reported higher fluorosis prevalence using a photographic method (Cruz-Orcutt *et al.* 2012; Wong *et al.*, 2012). Cruz-Orcutt *et al.* (2012) dried teeth with the effect described earlier. While, the difference observed by Wong *et al.* (2012) may be explained by the difference in photographic method. Although the authors examined and photographed teeth in a wet condition, they used conventional photographs and not the digital photographs of the present study.

Overall, all the study examiners demonstrated good intra-examiner and inter-examiner reliability between clinical and photographic scoring. There was not much different found between simple kappa or weighted kappa analysis. The substantial to excellent agreement between diagnosis of fluorosis using the photographic method and clinical assessment is in accordance with other studies (Ellwood *et al.*, 1996; Martins *et al.*, 2009; Sabieha and Rock, 1998; Soto-Rojas *et al.*, 2008; Wong *et al.*, 2005).

This could be attributed to the standardized photographic technique employed. For example the use of a ring flash reduced shadows in the photographs. In addition, images were viewed at a standard distance from the screen and scored at the same time by all examiners under similar conditions, which aimed to reduce magnification effects and examiner bias during assessment. The viewing conditions during photographic assessment are considered one of the key factors that affect examiner agreement (Tavener *et al*., 2007). That study reported low examiner reliability (kappa value less than 0.60) among ten examiners in fluorosis assessment of 120 images when assessed remotely using different computer monitors. The resultant different lighting, contrast and brightness affected the viewing conditions. In addition, the used of photographs avoids the inherent limitations with a clinical examination such as uncooperative patient and non-uniform lighting.

Although there was good intra-examiner reliability, examiner 1 demonstrated significantly higher prevalence in clinical score than photographic score. The major difference lay in differentiating between the questionable and very mild categories of fluorosis. The possible explanation may be due to the limitations of Dean's Index that the diagnostic category for the mildest form of fluorosis is unclear, imprecise and lacks sensitivity (Clarkson 1989; Horowitz 1986). Despite these limitations, Dean's Index has been used extensively because of the simplicity of the index. It also allows historical comparison with previous studies. Other specific fluorosis indices such as TFI and TSIF were found to be more sensitive to detecting the mildest form of fluorosis than Dean's Index (Rozier, 1994). In addition, another possible factor is that drying may have occurred during clinical examination. It is challenging to keep the teeth moist throughout the clinical examination. In the present study, the teeth were re-wetted prior to photography, which may explain the reason for the lower fluorosis score using photographs.

The findings from the present study support results from other studies that the photographic method is a valid and reliable method for assessing fluorosis. Although the study was able to suggest good reliability for photographic assessment, it had some limitations. Firstly, there was potential bias of foreknowledge of the clinical situation by Examiner 1 in photographic scoring. Effort was made to overcome this type of bias, by assessing the photographs 45 days after the clinical examination and the photographs were mixed randomly for blind scoring. Secondly, the distribution of fluorosis distribution within the studied population was based on index teeth scores among selected samples and should not be confused with population prevalence. Lastly, caution should also be taken when interpreting the reliability results, as the variation of prevalence and severity of fluorosis would affect the agreement. For example, the agreement levels will be greater if the sample examined has more people free of fluorosis and the agreement level will be lower when more categories were used in fluorosis classification. The overall distribution of fluorosis score is reported in Table 4. In this study all children examined irrespective of fluorosis status were included in determining the reliability scores.

Conclusion

The results suggest that photographic examination of fluorosis on central incisors can be recorded with good examiner reliability. The reported fluorosis prevalence was lower when using the photographic scoring method.

References

Altman, D.G. (1990): Practical statistics for medical research. London: Chapman & Hall/ CRC.

Browne, D., Whelton, H. and O'Mullane, D. (2005): Fluoride metabolism and fluorosis. *Journal of Dentistry* **33**, 177-186.

Buzalaf, M.A. and Levy, S.M. (2011): Fluoride intake of children: considerations for dental caries and dental fluorosis. *Monograph in Oral Science* **22**, 1-19.

Cochran, J.A., Ketley, C. E., Sanches, L., Mamai⊠Homata, E., Oila, A.M., Arnadottir, I.B. and O'Mullane, D.M. (2004a): A standardized photographic method for evaluating enamel opacities including fluorosis. *Community Dentistry and Oral Epidemiology* **32**(sup), 19-27.

Cochran, J.A., Ketley, C.E., Arnadottir, I.B., Fernandes, B., Koletsi-Kounari, H., Oila, A.M., and O'Mullane, D.M. (2004b): A comparison of the prevalence of fluorosis in 8-year-old children from seven European study sites using a standardized methodology. *Community Dentistry and Oral Epidemiology* **32** (Sup), 28-33.

Cruz-Orcutt, N., Warren, J.J., Broffitt, B., Levy, S.M. and Weber-Gasparoni, K. (2012): Examiner reliability of fluorosis scoring: a comparison of photographic and clinical examination findings. *Journal of Public Health Dentistry* **72**, 172-175.

Dean, H.T. (1934): Classification of mottled enamel diagnosis. *Journal of the American Dental Association* **21**, 1421-1426.

Ellwood, R., O'Mullane, D., Clarkson, J. and Driscoll, W. (1994): A comparison of information recorded using the Thylstrup Fejerskov index, Tooth Surface Index of Fluorosis and Developmental Defects of Enamel index. *International Dental Journal* **44**, 628-636.

Ellwood, R.P., Cortea, D.F. and O'Mullane, D.M. (1996): A photographic study of developmental defects of enamel in Brazilian school children. *International Dental Journal* **46**, 69-75.

F.D.I Commission on Oral Health, Research and Epidemiology (1982): An epidemiological index of developmental defects of dental enamel (D.D.E Index). *International Dental Journal* **32**, 159-167.

Fejerskov, O., Thylstrup, A. and Larsen, M.J. (1977): Clinical and structural features and possible pathogenic mechanisms of dental fluorosis. *Scandinavian Journal of Dental Research* **85**, 510-534.

Horowitz, H.S., Driscoll W.S., Meyers R.J., Heifetz S.B. and Kingman A. (1984): A new method for assessing the prevalence of dental fluorosis--the Tooth Surface Index of Fluorosis. *Journal of the American Dental Association* **109**, 37-41.

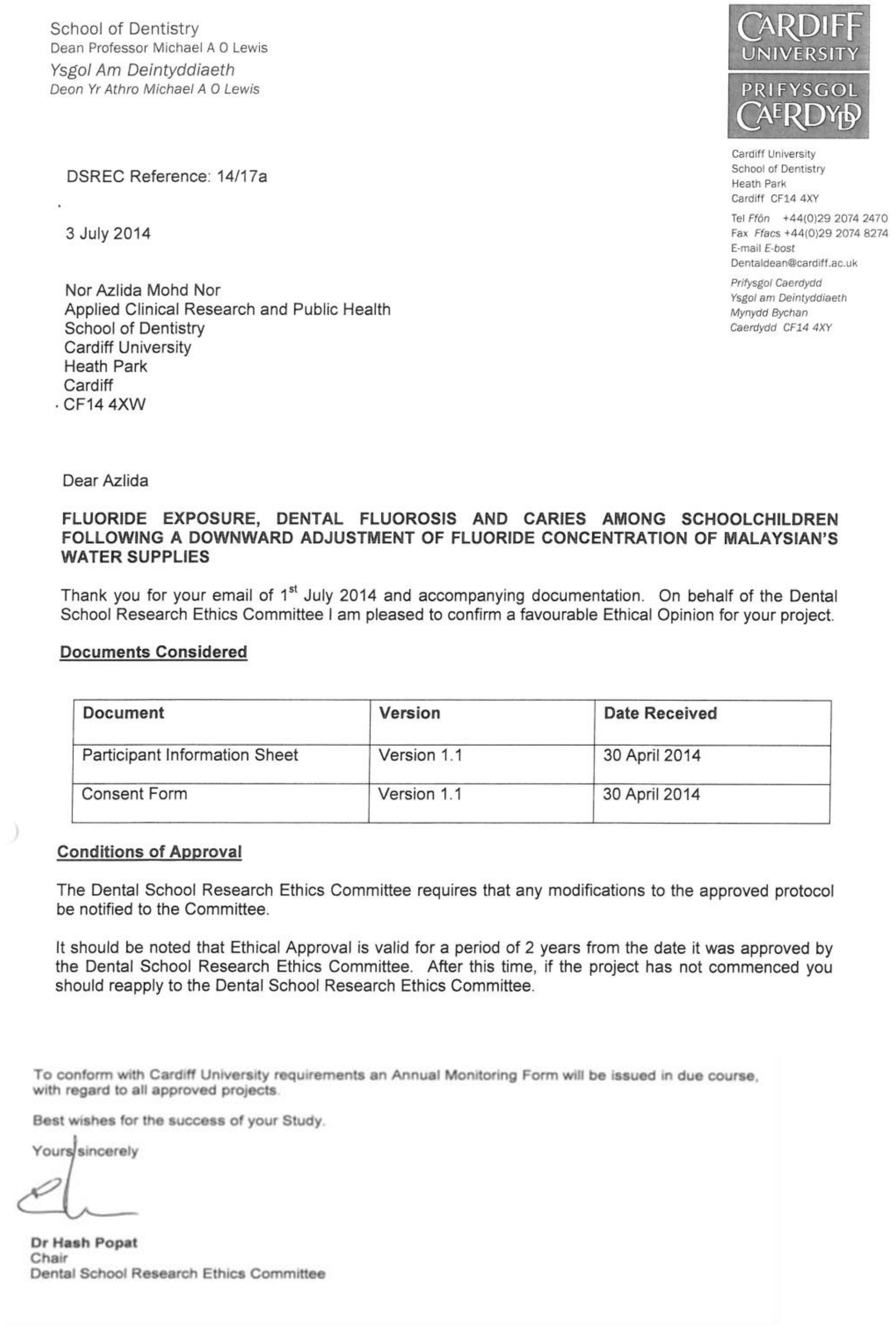
Horowitz, H.S. (1986): Indexes for measuring dental fluorosis. *Journal of Public Health Dentistry* **46**, 179-183.

Landis, J.R. and Koch, G.G. (1977): The measurement of observer agreement for categorical data. *Biometrics* **33**, 159-174.

Martins, C.C., Chalub, L., Lima-Arsati, Y.B., Pordeus, I. A. and Paiva, S.M. (2009): Agreement in the diagnosis of dental fluorosis in central incisors performed by a standardized photographic method and clinical examination. *Cadernos de Saude Publica* **25**, 1017-1024.

Mascarenhas, A.K. (2000): Risk factors for dental fluorosis: a review of the recent literature. *Pediatric Dentistry* **22**, 269-277.

Appendix 28 Ethical approval from Cardiff University



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DSREC Reference: 14/17a

3 July 2014

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Dear Azlida

FLUORIDE EXPOSURE, DENTAL FLUOROSIS AND CARIES AMONG SCHOOLCHILDREN FOLLOWING A DOWNWARD ADJUSTMENT OF FLUORIDE CONCENTRATION OF MALAYSIAN'S WATER SUPPLIES

Thank you for your email of 1st July 2014 and accompanying documentation. On behalf of the Dental School Research Ethics Committee I am pleased to confirm a favourable Ethical Opinion for your project.

Documents Considered

Document	**Version**	**Date Received**
Participant Information Sheet	Version 1.1	30 April 2014
Consent Form	Version 1.1	30 April 2014

Conditions of Approval

The Dental School Research Ethics Committee requires that any modifications to the approved protocol be notified to the Committee.

It should be noted that Ethical Approval is valid for a period of 2 years from the date it was approved by the Dental School Research Ethics Committee. After this time, if the project has not commenced you should reapply to the Dental School Research Ethics Committee.

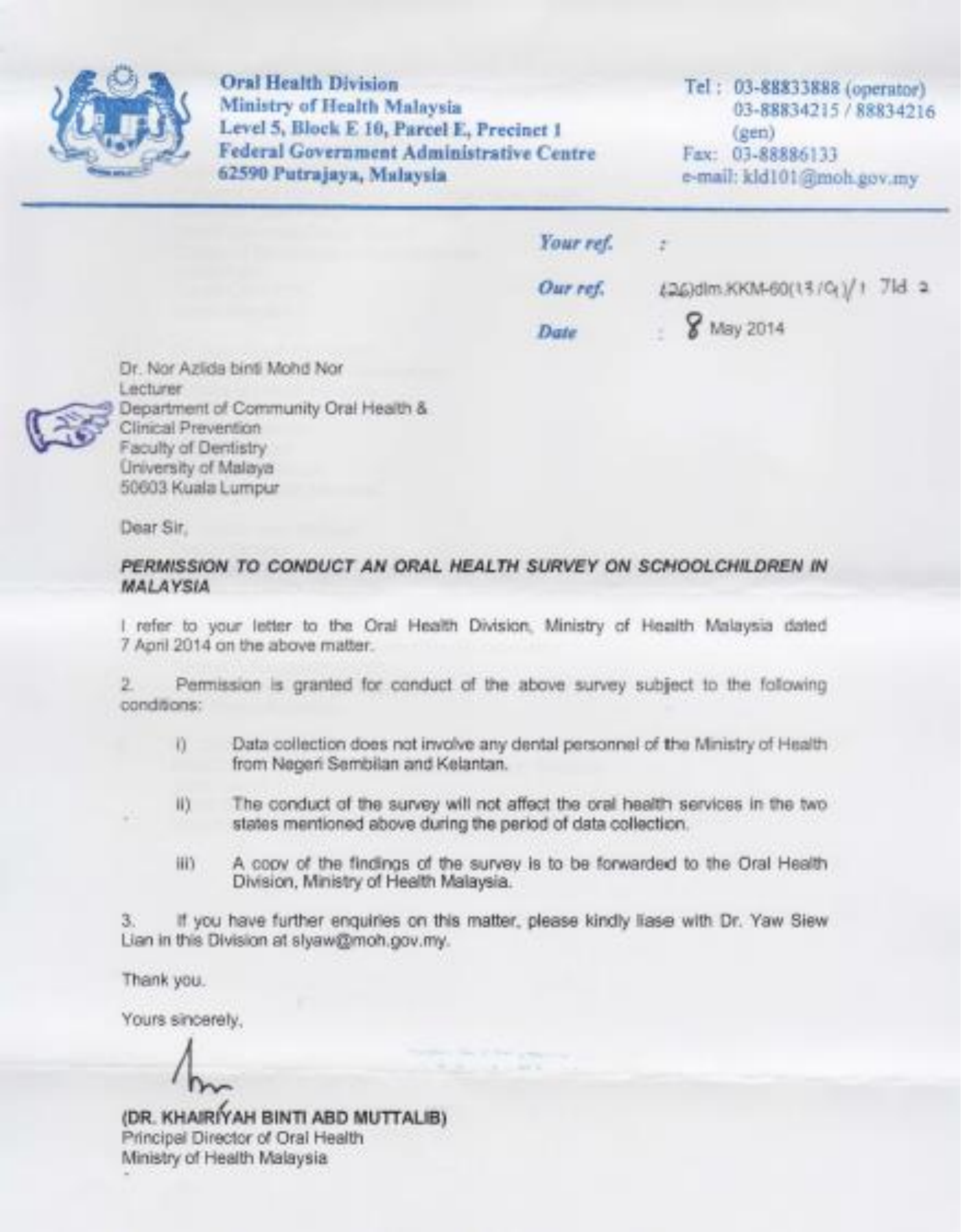
To conform with Cardiff University requirements an Annual Monitoring Form will be issued in due course, with regard to all approved projects.

Best wishes for the success of your Study.

Yours sincerely

Dr Hash Popat
Chair
Dental School Research Ethics Committee

Appendix 29 Approval from the Ministry of Health, Malaysia



Oral Health Division
Ministry of Health Malaysia
Level 5, Block E 10, Parcel E, Precinct 1
Federal Government Administrative Centre
62590 Putrajaya, Malaysia

Tel : 03-88833888 (operator)
03-88834215 / 88834216
(gen)
Fax: 03-88886133
e-mail: kld101@moh.gov.my

Your ref. :
Our ref. (26)dlm.KKM-60(13/09)/1 Jld 2
Date : 8 May 2014

Dr. Nor Azlida binti Mohd Nor
Lecturer
Department of Community Oral Health &
Clinical Prevention
Faculty of Dentistry
University of Malaya
50603 Kuala Lumpur

Dear Sir,

PERMISSION TO CONDUCT AN ORAL HEALTH SURVEY ON SCHOOLCHILDREN IN MALAYSIA

I refer to your letter to the Oral Health Division, Ministry of Health Malaysia dated 7 April 2014 on the above matter.

2. Permission is granted for conduct of the above survey subject to the following conditions:

i) Data collection does not involve any dental personnel of the Ministry of Health from Negeri Sembilan and Kelantan.

ii) The conduct of the survey will not affect the oral health services in the two states mentioned above during the period of data collection.

iii) A copy of the findings of the survey is to be forwarded to the Oral Health Division, Ministry of Health Malaysia.

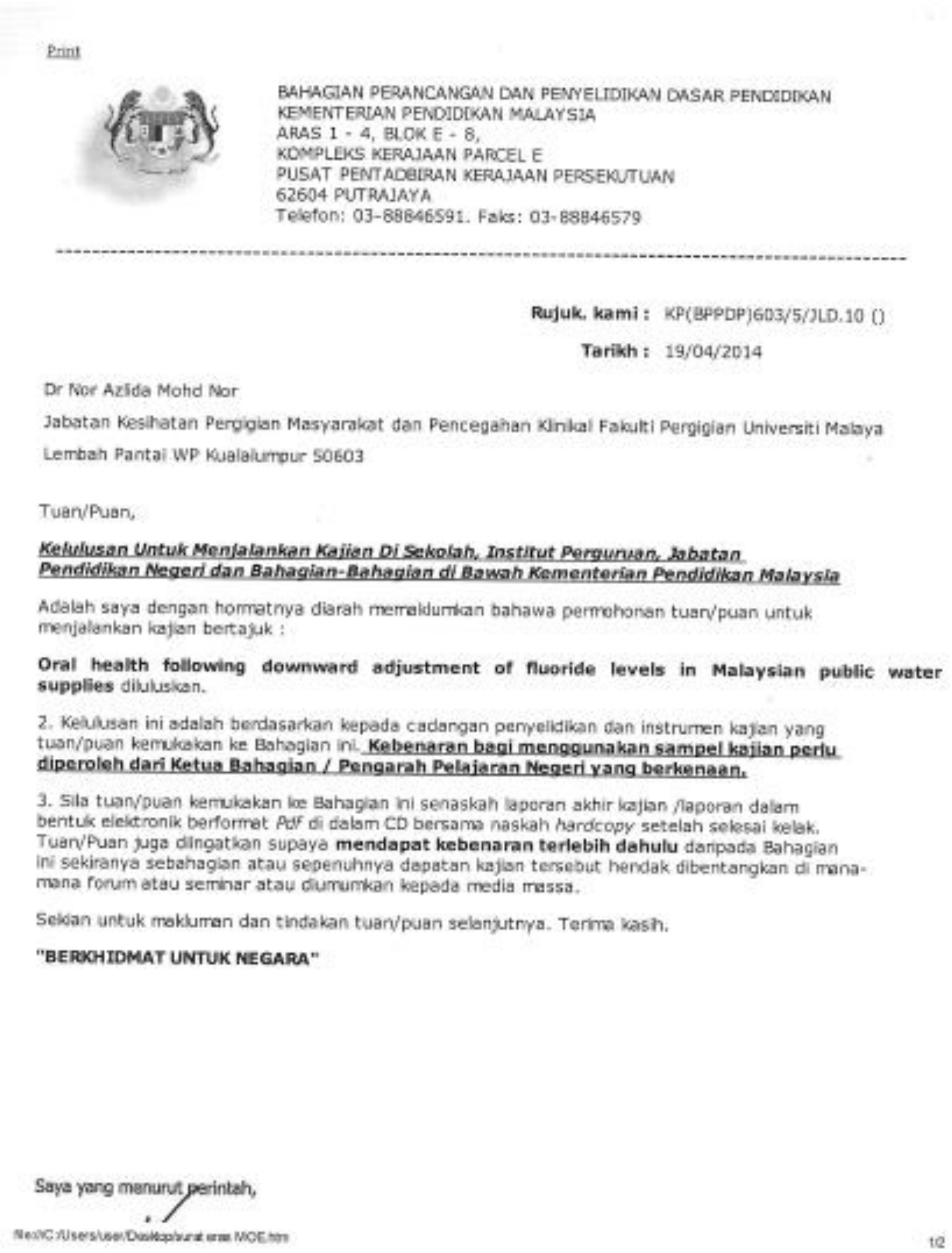
3. If you have further enquiries on this matter, please kindly liase with Dr. Yaw Siew Lian in this Division at slyaw@moh.gov.my.

Thank you.

Yours sincerely,

(DR. KHAIRIYAH BINTI ABD MUTTALIB)
Principal Director of Oral Health
Ministry of Health Malaysia

Appendix 30 Approval from the Ministry of Education, Malaysia



Print

BAHAGIAN PERANCANGAN DAN PENYELIDIKAN DASAR PENDIDIKAN
KEMENTERIAN PENDIDIKAN MALAYSIA
ARAS 1 - 4, BLOK E - 8,
KOMPLEKS KERAJAAN PARCEL E
PUSAT PENTADBIRAN KERAJAAN PERSEKUTUAN
62604 PUTRAJAYA
Telefon: 03-88846591. Faks: 03-88846579

Rujuk. kami : KP(BPPDP)603/5/JLD.10 ()
Tarikh : 19/04/2014

Dr Nor Azlida Mohd Nor
Jabatan Kesihatan Pergigian Masyarakat dan Pencegahan Klinikal Fakulti Pergigian Universiti Malaya
Lembah Pantai WP Kualalumpur 50603

Tuan/Puan,

Kelulusan Untuk Menjalankan Kajian Di Sekolah, Institut Perguruan, Jabatan Pendidikan Negeri dan Bahagian-Bahagian di Bawah Kementerian Pendidikan Malaysia

Adalah saya dengan hormatnya diarah memaklumkan bahawa permohonan tuan/puan untuk menjalankan kajian bertajuk :

Oral health following downward adjustment of fluoride levels in Malaysian public water supplies diluluskan.

2. Kelulusan ini adalah berdasarkan kepada cadangan penyelidikan dan instrumen kajian yang tuan/puan kemukakan ke Bahagian ini. **Kebenaran bagi menggunakan sampel kajian perlu diperoleh dari Ketua Bahagian / Pengarah Pelajaran Negeri yang berkenaan.**

3. Sila tuan/puan kemukakan ke Bahagian ini senaskah laporan akhir kajian /laporan dalam bentuk elektronik berformat *Pdf* di dalam CD bersama naskah *hardcopy* setelah selesai kelak. Tuan/Puan juga diingatkan supaya **mendapat kebenaran terlebih dahulu** daripada Bahagian ini sekiranya sebahagian atau sepenuhnya dapatan kajian tersebut hendak dibentangkan di mana-mana forum atau seminar atau diumumkan kepada media massa.

Sekian untuk makluman dan tindakan tuan/puan selanjutnya. Terima kasih.

"BERKHIDMAT UNTUK NEGARA"

Saya yang menurut perintah,

Appendix 31 Approval from State Education Department, Malaysia

a. Approval from State Education Selangor for pilot study

JABATAN PENDIDIKAN SELANGOR
Jalan Jambu Bol 4/3E, Seksyen 4,
40604 SHAH ALAM,
SELANGOR DARUL EHSAN,
MALAYSIA

JPS

Tel : 03 - 5518 6500
Faks : 03 - 5510 2133
Laman Web : http://jpnselangor.moe.gov.my

Rujukan Kami : JPNS.PPN 600-1/49 JLD.33(60)
Tarikh : 6/05/2014

DR NOR AZLIDA BINTI MOHD NOR
JABATAN KESIHATAN PERGIGIAN MASYARAKAT DAN PENCEGAHAN FIZIKAL
FAKULTI PERGIGIAN
UNIVERSITI MALAYA
50603 LEMBAH PANTAI
WP KUALA LUMPUR

Tuan,

ORAL HEALTH FOLLOWING DOWNWARD ADJUSTMENT OF FLURIDE LEVELS IN MALAYSIAN PUBLIC WATER SUPPLIES

Perkara di atas dengan segala hormatnya dirujuk.

2. Jabatan ini tiada halangan untuk pihak tuan menjalankan kajian/penyelidikan tersebut di sekolah-sekolah dalam Negeri Selangor seperti yang dinyatakan dalam surat permohonan.

3. Pihak tuan diingatkan agar mendapat persetujuan daripada Pengetua/Guru Besar supaya beliau dapat bekerjasama dan seterusnya memastikan bahawa penyelidikan dijalankan hanya bertujuan seperti yang dipohon. Kajian/Penyelidikan yang dijalankan juga tidak mengganggu perjalanan sekolah serta tiada sebarang unsur paksaan.

4. Tuan juga diminta menghantar senaskah hasil kajian ke Unit Perhubungan dan Pendaftaran Jabatan Pendidikan Selangor sebaik selesai penyelidikan/kajian.

Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"

Saya yang menurut perintah,

(HAJI MOHD MAHMUDI BIN BAKRI)
Penolong Pendaftar Institusi Pendidikan dan Guru
Jabatan Pendidikan Selangor
b.p. Ketua Pendaftar Institusi Pendidikan dan Guru
Kementerian Pendidikan Malaysia

s.k. - Fail

b. Approval from State Education Negeri Sembilan for main study

PENDIDIKAN
جابتن ڤلاجرن نڬري سمبيلن دارا الخصوص
JABATAN ~~PELAJARAN~~ NEGERI
NEGERI SEMBILAN DARUL KHUSUS
JALAN DATO' HAMZAH KARUNG BERKUNCI No. 6
70990 SEREMBAN, NEGERI SEMBILAN DARUL KHUSUS
Tel : 06-7653100 Fax : 06-7639969

"1MALAYSIA : RAKYAT DIDAHULUKAN PENCAPAIAN DIUTAMAKAN"

Ruj. Kami : JPNS.SPS.PP.100-1/7 Jld.8(79)
Tarikh : 4 MEI 2014

Dr. Nor Azlida Mohd Nor
Jabatan Kesihatan Pergigian Masyarakat dan Pencegahan Klinikal
Fakulti Pergigian Universiti Malaya Lembah Pantai WP
50603 Kuala Lumpur

Tuan/Puan,

Kebenaran Menjalankan Kajian Ke Sekolah-Sekolah Di Negeri Sembilan Darul Khusus Di Bawah Kementerian Pendidikan Malaysia

Saya dengan hormatnya di arah memaklumkan bahawa permohonan tuan/puan untuk menjalankan kajian bertajuk:- **"Oral Health Following Downward Adjusment of Fluoride Levels In Malaysian Public Water Supplies"** telah **diluluskan.**

2. Tuan/Puan hendaklah berjumpa terus dengan Pengetua atau Guru Besar sekolah berkenaan untuk meminta persetujuan dan membincangkan kajian tersebut di tempat seperti berikut:

1) **Sekolah Rendah di Negeri Sembilan**

3. Dimaklumkan bahawa kebenaran ini diberi berdasarkan surat kelulusan dari pihak Kementerian Pendidikan Malaysia, Bahagian Perancangan Dan Penyelidikan Dasar Pendidikan, nombor rujukan KP(BPPDP)603/5/JLD.10() bertarikh 19 April 2014.

4. Tuan/Puan hendaklah menghantar satu naskah hasil kajian ke Jabatan Pendidikan Negeri Sembilan (u.p: Unit Perhubungan, Pendaftaran & Pelajaran Swasta).

Sekian untuk makluman dan tindakan tuan/puan selanjutnya.

Terima kasih.

"BERKHIDMAT UNTUK NEGARA"

Saya yang menurut perintah,

DATO' HAJAH KALSOM BINTI KHALID
Pengarah Pendidikan
Negeri Sembilan Darul Khusus

s.k. Pengetua atau Guru Besar sekolah berkenaan.

Nota: -Sila beri satu salinan surat kelulusan semasa membuat kajian di sekolah.

b. Approval from State Education Negeri Kelantan for main study

JABATAN PENDIDIKAN KELANTAN

~~جابتن ڤلاجارن كلنتن~~

~~JABATAN PELAJARAN KELANTAN~~
JALAN DOKTOR,
15000 KOTA BHARU,
KELANTAN DARUL NAIM.

TELEFON
Pejabat Am : 09-741 8000
Pengarah : 09-741 8001
Timb Pengarah : 09-741 8002

FAKS : 09-748 2554
Web : [illegible]

Ruj. Kami : JPKn/SPS/UPP.600-5/4 Jld. 3 (23)
Tarikh : 5 Mei 2014

Dr. Nor Azlida Mohd Nor
Jabatan Kesihatan Pergigian Masyarakat dan
Pencegahan Klinikal Fakulti Pergigian Universiti Malaya
50603 Lembah Pantai
Kuala Lumpur

Tuan/Puan,

KEBENARAN MENJALANKAN KAJIAN / PENYELIDIKAN DI SEKOLAH KERAJAAN / BANTUAN KERAJAAN DI NEGERI KELANTAN

Adalah saya dengan hormatnya diarah merujuk surat permohonan tuan / puan mengenai perkara di atas.

2. Surat kebenaran dari Pengarah Bahagian Perancangan & Penyelidikan Dasar Pendidikan, Kementerian Pendidikan Malaysia Rujukan: KP(BPPDP)603/5/Jld.10( ) bertarikh 19 April 2014 berkaitan.

3. Jabatan Pendidikan Kelantan tiada halangan bagi tuan/puan menjalankan kajian/penyelidikan seperti tajuk:

"Oral health following downward adjustment of fluoride levels in Malaysian public water supplies" diluluskan.

4. Kelulusan ini adalah dihadkan berdasarkan kepada tajuk kajian / penyelidikan yang dikemukakan ke Jabatan ini bagi tempoh: **01 Mei 2014 hingga 31 Disember 2014.**

5. Sekolah-sekolah yang terlibat adalah: **Sekolah-Sekolah Di Negeri Kelantan.**

6. Tuan/Puan dinasihatkan supaya terlebih dahulu berbincang dengan Pengetua/Guru Besar sekolah-sekolah berkenaan sebelum kajian /penyelidikan dijalankan.

Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"

Saya yang menurut perintah,

(CHE HANUNI BT CHE ALI)
Penolong Pendaftar Institusi Pendidikan Dan Guru
b.p Pendaftar Institusi Pendidikan Dan Guru
Jabatan Pendidikan Kelantan

s.k
- i. Pengarah Pendidikan Kelantan
- ii. Pengarah, Bahagian Perancangan & Penyelidikan Dasar Pendidikan Kementerian Pendidikan Malaysia.
- ii. Pegawai Pendidikan Daerah: PPD berkenaan.
- iv. Pengetua / Guru Besar Sekolah berkenaan

CHCA/nh/Borang Kajian

Appendix 32. Bivariate analyses between risk factors and prevalence of fluorosis (Deans≥2) in fluoridated and non-fluoridated areas (Tables 1 to 8)

Table 1. Bivariate analysis of fluorosis prevalence and demographic characteristics of study participants (by area) in a fluoridated

Fluoridated	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
Demographic	Yes	No		
Gender				
Boys	89 (34.1)	172 (65.9)	Ref	
Girls	124 (37.0)	211 (63.0)	1.14 (0.81-1.59)	0.461
Father Education				
College/University	50 (34.2)	96 (65.8)	Ref	
High school	131 (36.1)	232 (63.9)	0.82 (0.39-1.76)	0.614
≤Primary school	12 (30.0)	28 (70.0)	1.08 (0.72-1.62)	0.695
Mother Education				
College/University	60 (33.7)	118 (66.3)	Ref	
High school	124 (37.1)	210 (62.9)	0.73 (0.36-1.48)	0.385
≤Primary school	13 (27.1)	35 (72.9)	1.16 (0.79-1.70)	0.443
Father monthly income				
≥ MYR 4000	54 (39.1)	84 (60.9)	Ref	
MYR 1000-3999	54 (35.8)	97 (64.2)	0.21 (0.05-0.95)	**0.042**
<MYR 1000	87 (32.1)	184 (67.9)	1.02 (0.71-1.45)	0.936
Mother monthly income				
≥ MYR 4000	79 (35.6)	143 (64.4)	Ref	
MYR 1000-3999	111 (35.9)	198 (64.1)	0.74 (0.48-1.13)	0.158
<MYR 1000	2 (10.5)	17 (89.5)	0.87 (0.54-1.40)	0.554

Ref: reference category

Table 2. Bivariate analysis of fluorosis prevalence and infant feeding practices among study participants in fluoridated area

Fluoridated	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
Infant feeding practices	Yes	No		
Use of infant formula				
No	71 (74.7)	24 (25.3)	Ref	
Yes	188 (37.6)	312 (62.4)	1.78 (1.09-2.93)	**0.023**
Breast feeding				
No	374 (64.4)	207 (35.6)	Ref	
Yes	6 (40.0)	9 (60.0)	0.83 (0.29-2.37)	0.728
Age finished breast feeding				
>12 months	84 (29.0)	206 (71.0)	Ref	
≤12 months	124 (42.2)	170 (57.8)	1.79 (0.27-2.52)	**0.001**
Age started formula				
>12 months	58 (32.0)	123 (68.0)	Ref	
≤12 months	135 (41.8)	188 (58.2)	1.52 (1.04-2.23)	**0.031**
Age finished formula				
>48 months	116 (38.3)	187 (61.7)	Ref	
≤48 months	76 (38.0)	124 (62.0)	0.99 (0.68-1.43)	0.949
Type of water used to prepare formula				
Bottled water	1 (5.6)	17 (94.4)	Ref	
Tap water	144 (40.7)	210 (59.3)	11.66 (1.53-88.57)	**0.018**
Filtered tap water	46 (35.9)	82 (64.1)	9.54 (1.23-73.99)	**0.031**
Duration of formula use				
>48 months	80 (41.2)	114 (58.8)	Ref	
≤48 months	109 (35.9)	195 (64.1)	0.80 (0.55-1.15)	0.228
Feeding method				
Formula only	6 (40.0)	9 (60.0)	Ref	
Combine breast & formula	182 (37.5)	303 (62.5)	0.90 (0.32-2.57)	0.846
Breast only	24 (25.3)	71 (74.7)	0.51 (0.16-1.57)	0.240

Ref: reference group

Table 3. Bivariate analysis of fluorosis prevalence and oral hygiene habits at age less than six years among study participants in fluoridated area

Fluoridated	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
Oral hygiene habits at age less than 6 years	Yes	No		
Frequency of toothbrushing				
Twice/day or more	119 (31.9)	254 (68.1)	Ref	
Once /day or less	93 (42.3)	127 (57.7)	1.13 (0.61-2.09)	0.697
Supervised toothbrushing				
Never	3 (42.9)	4 (57.1)	Ref	
Yes	206 (35.9)	368 (64.1)	0.79 (0.12-5.24)	0.810
Habits after toothbrushing				
Spat	198 (35.8)	355 (64.2)	Ref	
Swallowed	12 (35.3)	22 (64.7)	2.01 (0.68-5.95)	0.210
Eating/ licking toothpaste				
Never	94 (38.2)	152 (61.8)	Ref	
Yes	117 (34.0)	227 (66.0)	1.24 (0.80-1.93)	0.329
Amount of toothpaste used				
Medium to large	118 (34.4)	225 (65.6)	Ref	
Small	93 (37.5)	155 (62.5)	0.84 (0.54-1.30)	0.432
Type of toothpaste used				
Non-fluoridated toothpaste	24 (29.3)	58 (70.7)	Ref	
Fluoridated toothpaste	184 (36.8)	316 (63.2)	0.50 (0.24-0.99)	0.048*
Age started toothbrushing				
After 2 years	142 (36.9)	243 (63.1)	Ref	
Before 2 years	71 (33.6)	140 (66.4)	1.13 (0.61-2.09)	0.697
Age started toothbrushing with toothpaste				
After 2 years	150 (36.7)	259 (63.3)	Ref	
Before 2 years	60 (33.1)	121 (66.9)	1.23 (0.65-2.35)	0.523

Ref: reference group

Table 4. Bivariate analysis of fluorosis prevalence and exposure to fluoride gel/varnish among study participants in a fluoridated area

Fluoridated	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
	Yes	No		
Exposure to fluoride varnish/gel				
No	22 (30.1)	51 (69.9)	Ref	0.317
Yes	131 (36.3)	230 (63.7)	0.76 (0.44-1.31)	

Ref: reference group

Table 5. Bivariate analysis of fluorosis prevalence and demographic characteristics of study participants in a non-fluoridated area

Non-fluoridated	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
Demographic	Yes	No		
Gender				
Boys	9 (3.9)	221 (96.1)	Ref	0.174
Girls	21 (6.6)	296 (93.4)	1.74 (0.78-3.88)	
Father Education				
College/University	10 (9.6)	94 (90.4)	Ref	
High school	20 (6.0)	314 (94.0)	0.60 (0.27-1.32)	0.205
≤Primary school	0	62 (100)	-	-
Mother Education				
College/University	8 (7.1)	105 (92.9)	Ref	
High school	21 (6.1)	325 (93.9)	0.22 (0.03-1.79)	0.157
≤Primary school	1 (1.6)	60 (98.4)	0.85 (0.37-1.97)	0.702
Father monthly income				
≥ MYR 4000	9 (8.9)	92 (91.1)	Ref	
MYR 1000-3999	20 (5.1)	371 (94.9)	0.55 (0.24-1.25)	0.154
<MYR 1000	0	8 (100)	-	-
Mother monthly income				
≥ MYR 4000	7 (9.7)	65 (90.3)	Ref	
MYR 1000-3999	6 (5.0)	115 (95.0)	0.49 (0.20-1.24)	0.132
<MYR 1000	17 (5.0)	320 (95.0)	0.48 (0.16-1.50)	0.210

Ref: reference group

Table 6. Bivariate analysis of fluorosis prevalence and infant feeding practices among study participants in a non-fluoridated area

Non-fluoridated	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
Infant feeding practices	Yes	No		
Use of infant formula				
No	8 (3.7)	206 (96.3)	Ref	
Yes	22 (6.6)	309 (93.4)	1.83 (0.80-4.20)	0.151
Breast feeding				
No	2 (16.7)	10 (83.3)	Ref	
Yes	28 (5.2)	507 (94.8)	0.28 (0.07-1.32)	0.107
Age finished breast feeding				
>12 months	20 (5.2)	368 (94.8)	Ref	
≤12 months	8 (5.4)	139 (94.6)	1.06 (0.46-2.46)	0.894
Age started formula				
>12 months	12 (7.7)	144 (92.3)	Ref	
≤12 months	10 (5.5)	171 (94.5)	0.70 (0.30-1.67)	0.424
Age finished formula				
>48 months	13 (6.3)	195 (93.8)	Ref	
≤48 months	9 (7.2)	116 (92.8)	0.86 (0.36-2.07)	0.736
Type of water used to prepare formula				
Bottled water	2 (14.3)	12 (85.7)	Ref	
Tap water	18 (6.5)	259 (93.5)	0.28 (0.09-2.01)	0.417
Filtered tap water	1 (2.6)	38 (97.4)	0.15 (0.01-1.90)	0.158
Duration of formula use				
>48 months	5 (7.7)	60 (92.3)	Ref	
≤48 months	16 (6.1)	248 (93.9)	0.77 (0.27-2.20)	0.631
Feeding method				
Formula only	2 (6.7|)	10 (83.3)	Ref	
Combine breast & formula	20 (6.3)	299 (93.7)	0.33 (0.07-1.63)	0.175
Breast only	8 (3.7)	206 (96.3)	0.19 (0.04-1.04)	0.055

Ref: reference group

Table 7. Bivariate analysis of fluorosis prevalence and oral hygiene habits at age less than six years among study participants in a non-fluoridated area

Non-fluoridated	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
Oral hygiene habits at age less than 6 years	Yes	No		
Frequency of toothbrushing				
Twice/day or more	11 (4.2)	252 (95.8)	Ref	
Once /day or less	19 (6.8)	262 (93.2)	1.56 (0.60-4.07)	0.364
Supervised toothbrushing				
Never	1 (7.7)	12 (92.3)	Ref	
Yes	28 (5.6)	473 (94.4)	3.94 (0.37-41.41)	0.254
Habits after toothbrushing				
Spat	29 (5.7)	476 (94.3)	Ref	
Swallowed	1 (2.9)	33 (97.1)	0.61 (0.07-5.25)	0.649
Eating/ licking toothpaste				
Never	16 (6.8)	220 (93.2)	Ref	
Yes	14 (4.5)	294 (95.5)	1.72 (0.66-4.47)	0.265
Amount of toothpaste used				
Medium to large	16 (5.6)	272 (94.4)	Ref	
Small	14 (5.5)	241 (94.5)	1.21 (0.48-3.07)	0.691
Type of toothpaste used				
Non-fluoridated toothpaste	4 (7.0)	53 (93.0)	Ref	
Fluoridated toothpaste	26 (5.5)	447 (94.5)	1.09 (.23-5.12)	0.918
Age started toothbrushing				
After 2 years	19 (4.8)	375 (95.2)	Ref	
Before 2 years	11 (7.2)	141 (92.8)	0.50 (0.15-1.68)	0.263
Age started toothbrushing with toothpaste				
After 2 years	22 (5.2)	398 (94.8)	Ref	
Before 2 years	8 (6.5)	116 (93.5)	1.74 (0.47-6.47)	0.406

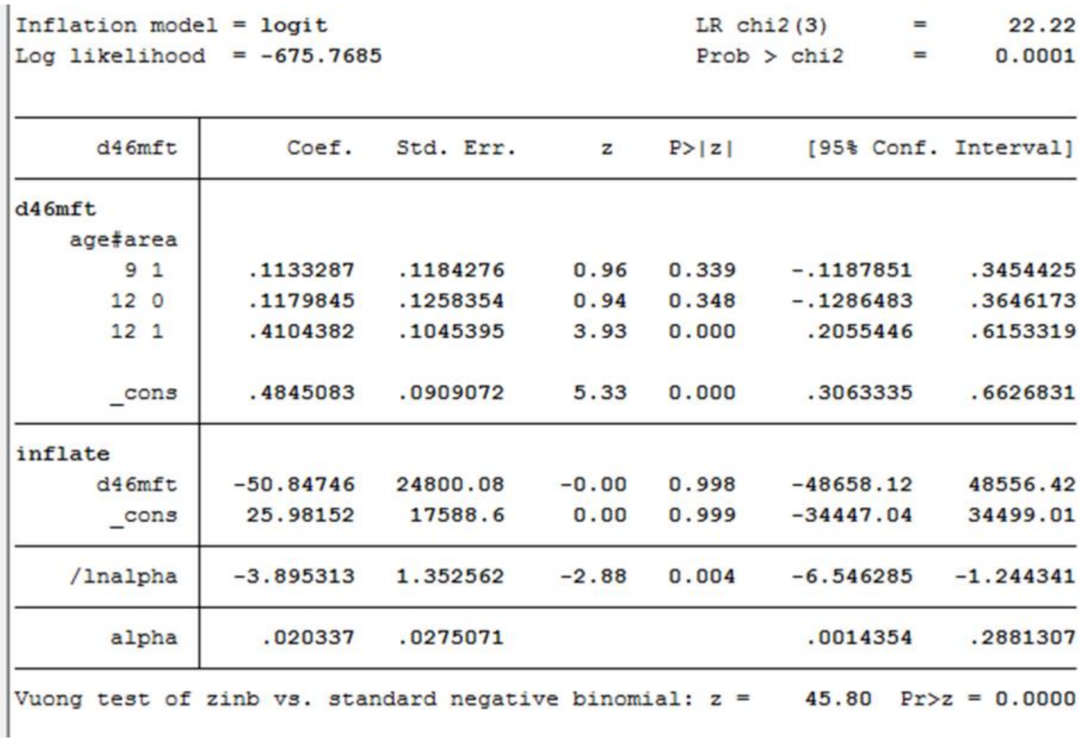
Ref: reference category

Table 8. Bivariate analysis of fluorosis prevalence and exposure to fluoride gel/varnish among study participants in a non-fluoridated area

Non-fluoridated	Fluorosis (Deans≥2) n (%)		Odds ratio 95% CI	p value
	Yes	No		
Exposure to fluoride varnish/gel				
No	16 (5.5)	273 (94.5)	Ref	
Yes	6 (7.4)	75 (92.6)	1.37 (0.52-3.61)	0.531

Ref: reference group

Appendix 33 Vuong test to compare caries Model 1 vs Model 2



```
Inflation model = logit                           LR chi2(3)      =     22.22
Log likelihood  = -675.7685                       Prob > chi2     =    0.0001

------------------------------------------------------------------------------
      d46mft |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
d46mft       |
    age#area |
        9 1  |   .1133287   .1184276     0.96   0.339    -.1187851    .3454425
       12 0  |   .1179845   .1258354     0.94   0.348    -.1286483    .3646173
       12 1  |   .4104382   .1045395     3.93   0.000     .2055446    .6153319
             |
       _cons |   .4845083   .0909072     5.33   0.000     .3063335    .6626831
-------------+----------------------------------------------------------------
inflate      |
      d46mft |  -50.84746   24800.08    -0.00   0.998    -48658.12    48556.42
       _cons |   25.98152    17588.6     0.00   0.999    -34447.04    34499.01
-------------+----------------------------------------------------------------
    /lnalpha |  -3.895313   1.352562    -2.88   0.004    -6.546285   -1.244341
-------------+----------------------------------------------------------------
       alpha |    .020337   .0275071                      .0014354    .2881307
------------------------------------------------------------------------------
Vuong test of zinb vs. standard negative binomial: z =    45.80  Pr>z = 0.0000
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Appendix 34 Bivariate analyses between risk factors and prevalence of caries at dentine level (D_{4-6}MFT>0) and caries at all levels (D_{1-6}MFT>0) in a fluoridated and non-fluoridated areas (Tables 9 to 18)

Table 9. Bivariate analysis between demographic characteristics and prevalence of caries at dentine level (D_{4-6}MFT>0) and caries at all levels (D_{1-6}MFT>0) in a fluoridated area

Fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Demographic characteristics	D_{4-6}MFT>0 N (%)		Odds ratio 95% CI	p value	D_{1-6}MFT>0 N (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Gender								
Boys	61 (23.0)	204 (77.0)	Ref		156 (58.9)	109 (41.1)	Ref	
Girls	91 (26.6)	251 (73.4)	1.21 (0.84-1.76)	0.312	215 (62.9)	127 (37.1)	1.18 (0.85-1.64)	0.316
Father Education								
College/University	39 (26.0)	111 (74.0)	Ref		98 (65.3)	52 (34.7)	Ref	
High school	89 (24.1)	281 (75.9)	1.04 (0.41-2.61)	0.932	220 (59.5)	150 (40.5)	0.65 (0.28-1.53)	0.326
≤Primary school	12 (30.0)	28 (70.0)	0.80 (0.46-1.38)	0.415	26 (65.0)	14 (35.0)	0.63 (0.38-1.03)	0.064
Mother Education								
College/University	48 (25.9)	137 (74.1)	Ref		119 (64.3)	66 (35.7)	Ref	
High school	82 (24.3)	256 (75.7)	1.36 (0.55-3.41)	0.507	206 (60.9)	132 (39.1)	0.85 (0.37-1.94)	0.690
≤Primary school	13 (27.1)	35 (72.9)	1.25 (0.68-2.28)	0.472	28 (58.3)	20 (41.7)	0.90 (0.54-1.52)	0.698
Father monthly income								
≥ MYR 4000	55 (24.3)	171 (75.7)	Ref		135 (59.7)	91 (40.3)	Ref	
MYR1000-3999	80 (25.4)	235 (74.6)	1.35 (0.39-4.67)	0.639	196 (62.2)	119 (37.8)	1.73 (0.54-5.56)	0.358
<MYR 1000	6 (31.6)	13 (68.4)	1.34 (0.81-2.24)	0.256	12 (63.2)	7 (36.8)	1.50 (0.96-2.35)	0.073
Mother monthly income								
≥ MYR 4000	43 (30.3)	99 (69.7)	Ref		92 (64.8)	50 (35.2)	Ref	
MYR 1000-3999	28 (18.2)	126 (81.8)	0.65 (0.35-1.19)	0.160	86 (55.8)	68 (44.2)	0.93 (0.53-1.62)	0.795
<MYR 1000	72 (26.2)	203 (73.8)	0.38 (0.19-0.76)	**0.007**	173 (62.9)	102 (37.1)	0.73 (0.40-1.33)	0.305

Ref: reference category

Table 10. Bivariate analysis between infant feeding practices and prevalence of caries at dentine level ($D_{4-6}MFT>0$) and caries at all levels ($D_{1-6}MFT>0$) in a fluoridated area

Fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Infant feeding practices	$D_{4-6}MFT>0$ n (%)		Odds ratio 95% CI	p value	$D_{1-6}MFT>0$ n (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Use of infant formula								
No	29 (29.4)	68 (70.1)	Ref		56 (57.7)	41 (42.3)	Ref	
Yes	123 (24.2)	386 (75.8)	0.75 (0.46-1.21)	0.234	314 (61.7)	195 (38.3)	1.18 (0.76-1.83)	0.464
Breast feeding								
No	3 (20.0)	12 (80.0)	Ref		12 (80.0)	3 (20.0)	Ref	
Yes	149 (25.2)	443 (74.8)	1.35 (0.38-4.83)	0.649	359 (60.6)	233 (39.4)	0.39 (0.11-1.38)	0.143
Age finished breast feeding								
>12 months	69 (23.3)	227 (76.7)	Ref		175 (59.1)	121 (40.9)	Ref	
≤12 months	80 (26.8)	219 (73.2)	1.20 (0.83-1.74)	0.332	187 (62.5)	112 (37.5)	1.15 (0.83-1.61)	0.393
Age started formula								
>12 months	40 (21.6)	145 (78.4)	Ref		114 (61.6)	71 (38.4)	Ref	
≤12 months	85 (25.9)	243 (74.1)	1.27 (0.83-1.95)	0.277	202 (61.6)	126 (38.4)	1.00 (0.69-1.45)	0.994
Age finished formula								
>4 months	84 (27.3)	224 (72.3)	Ref		200 (64.9)	108 (35.1)	Ref	
≤48 months	40 (19.6)	164 (80.4)	0.65 (0.42-0.99)	**0.048**	115 (56.4)	89 (43.6)	0.70 (0.49-1.00)	**0.052**
Type of water used to prepare formula								
Bottled water	1 (5.6)	17 (94.4)	Ref		11 (61.1)	7 (38.9)	Ref	
Tap water	85 (23.6)	275 (76.4)	5.26 (0.69-40.07)	0.109	221 (61.4)	139 (38.6)	1.01 (0.38-2.67)	0.981
Filtered tap water	37 (28.2)	94 (71.8)	6.69 (0.86-52.10)	0.069	80 (61.1)	51 (38.9)	1.0 (0.36-2.74)	0.997

Duration of formula								
>48 months	53 (27.2)	142 (72.8)	Ref		126 (64.6)	69 (35.4)	Ref	
≤48 months	70 (22.4)	242 (77.6)	0.78 (0.51-1.17)	0.226	185 (59.3)	127 (40.7)	0.80 (0.55-1.16)	0.232
Feeding method								
Formula only	3 (20.0)	2 (80.0)	Ref		12 (80.0)	3 (20.0)	Ref	
Combine breast & formula	120 (24.3)	372 (75.7)	1.28 (0.36-4.62)	0.703	302 (61.1)	192 (38.9)	0.39 (0.11-1.41)	0.152
Breast only	29 (29.9)	68 (70.1)	1.71 (0.45-6.50)	0.434	56 (57.1)	41 (42.3)	0.34 (0.09-1.29)	0.113

Ref: reference category

Table 11. Bivariate analysis between oral hygiene habits at the time of study (2015) and prevalence of caries at dentine level ($D_{4-6}MFT>0$) and caries at all levels ($D_{1-6}MFT>0$) in fluoridated area

Fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
(Oral hygiene habits, in 2015)	$D_{4-6}MFT>0$ n (%)		Odds ratio 95% CI	p value	$D_{1-6}MFT>0$ n (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Frequency of toothbrushing								
Twice/day or more	132 (26.6)	365 (73.4)	Ref		305 (61.4)	192 (38.6)	Ref	
Once /day or less	20 (19.0)	85 (81.0)	0.65 (0.38-1.10)	0.109	64 (61.0)	41 (39.0)	0.98 (0.64-1.51)	0.937
Supervise toothbrushing								
Never	11 (25.0)	33 (75.0)	Ref		33 (75.0)	11 (25.0)	Ref	
Yes	135 (25.0)	406 (75.0)	1.00 (0.49-2.03)	0.995	325 (60.1)	216 (39.9)	0.50 (0.25-1.01)	0.055
Habits after brushing								
Spat	150 (25.2)	445 (74.8)	Ref		363 (61.0)	232 (39.0)	Ref	
Swallowed	2 (40.0)	3 (60.0)	1.98 (0.33-11.95)	0.457	4 (80.0)	1 (20.0)	2.56 (0.28-23.01)	0.402
Eating/ licking toothpaste								
Never	121 (23.7)	389 (76.3)	Ref		310 (60.8)	200 (39.2)	Ref	
Yes	28 (31.5)	61 (68.5)	1.48 (0.90-2.41)	0.121	56 (62.9)	33 (37.1)	1.10 (0.69-1.74)	0.703
Amount of toothpaste used								
Medium to large	145 (25.9)	415 (74.1)	Ref		348 (62.1)	212 (37.9)	Ref	
Small	7 (18.4)	31 (81.6)	0.65 (0.28-1.50)	0.309	20 (52.6)	18 (47.4)	0.68 (0.35-1.31)	0.246
Type of toothpaste								
Non-fluoridated	6 (21.4)	22 (78.6)	Ref		16 (57.1)	12 (42.9)	Ref	
Fluoridated	146 (25.8)	420 (74.2)	1.28 (0.51-3.21)	0.606	363 (62.4)	213 (37.6)	1.24 (0.58-2.68)	0.579

Ref: reference category

Table 12. Bivariate analysis between oral hygiene habits (at age less than six years) and prevalence of caries at dentine level (D_{4-6}MFT>0) and caries at all levels (D_{1-6}MFT>0) in a fluoridated area

Fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Oral hygiene habits at age less than 6 years	D_{4-6}MFT>0 n (%)		Odds ratio 95% CI	p value	D_{1-6}MFT>0 n (%)		Odds ratio 95% CI	P value
	Yes	No			Yes	No		
Frequency of toothbrushing								
Twice/day or more	96 (25.5)	281 (74.5)	Ref		235 (62.3)	142 (37.7)	Ref	
Once /day or less	56 (24.7)	171 (75.3)	0.96 (0.66-1.40)	0.827	136 (59.9)	91 (40.1)	0.90 (0.64-1.27)	0.554
Supervised toothbrushing								
Never	1 (14.3)	6 (85.7)	Ref		4 (57.1)	3 (42.9)	Ref	
Yes	149 (25.5)	436 (74.5)	2.06 (0.25-17.17)	0.508	361 (61.7)	224 (38.3)	1.21 (0.27-5.45)	0.805
Habits after brushing								
Spat	138 (24.5)	426 (75.5)	Ref		346 (61.3)	218 (38.7)	Ref	
Swallowed	13 (38.2)	21 (61.8)	1.91 (0.93-3.92)	0.077	23 (67.6)	11 (32.4)	1.32 (0.63-2.76)	0.464
Eating/ licking toothpaste								
Never	62 (24.9)	187 (75.1)	Ref		154 (61.8)	95 (38.2)	Ref	
Yes	88 (25.0)	264 (75.0)	1.00 (0.69-1.46)	0.978	215 (61.1)	137 (38.9)	0.97 (0.69-1.35)	0.849
Amount of toothpaste used								
Medium to large	81 (23.3)	266 (76.7)	Ref		214 (61.7)	133 (38.3)	Ref	
Small	71 (27.8)	184 (72.2)	1.27 (0.88-1.83)	0.210	157 (61.6)	98 (38.4)	1.00 (0.71-1.39)	0.980

Type of toothpaste								
Non-fluoridated	20 (23.8)	64 (76.2)	Ref		57 (67.9)	27 (32.1)	Ref	
Fluoridated	131 (25.7)	378 (74.3)	1.11 (0.65-1.90)	0.707	312 (61.3)	197 (38.7)	0.75 (0.45-1.23)	0.252
Age started toothbrushing								
After 2 years	106 (27.0)	287 (73.0)	Ref		241 (61.3)	152 (38.7)	Ref	
Before 2 years	46 (21.5)	168 (78.5)	0.74 (0.50-1.10)	0.138	130 (60.7)	84 (39.3)	0.98 (0.69-1.37)	0.889
Age started toothbrushing with toothpaste								
After 2 years	115 (27.7)	300 (72.3)	Ref		269 (64.8)	146 (35.2)	Ref	
Before 2 years	36 (19.5)	149 (80.5)	0.63 (0.41-0.96)	**0.032**	99 (53.5)	86 (46.5)	0.63 (0.44-0.89)	**0.009**

Ref: reference category

Table 13. Bivariate analysis between exposure to fluoride varnish/gel and prevalence of caries at dentine level (D_{4-6}MFT>0) and caries at all levels (D_{1-6}MFT>0) in a fluoridated area

Fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
	D_{4-6}MFT>0 N (%)		Odds ratio 95% CI	p value	D_{1-6}MFT>0 N (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Exposure to Fluoride varnish/gel								
No	90 (24.3)	280 (75.7)	Ref		215 (58.1)	155 (41.9)	Ref	
Yes	15 (20.5)	58 (79.5)	0.81 (0.44-1.49)	0.489	42 (57.5)	31 (42.5)	0.98 (0.59-1.62)	0.928

Table 14. Bivariate analysis between demographic characteristics and prevalence of caries at dentine level ($D_{4-6}MFT>0$) and caries at all levels ($D_{1-6}MFT>0$) in a non-fluoridated area

Non-fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Demographic characteristics	$D_{4-6}MFT>0$ N (%)		Odds ratio 95% CI	p value	$D_{1-6}MFT>0$ N (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Gender								
Boys	108 (46.8)	123 (53.2)	Ref		159 (68.8)	72 (31.2)	Ref	
Girls	152 (47.9)	165 (52.1)	1.05 (0.75-1.47)	0.782	243 (76.7)	74 (23.3)	1.49 (1.02-2.18)	**0.041**
Father Education								
College/University	46 (44.2)	58 (55.8)	Ref		75 (72.1)	29 (27.9)	Ref	
High school	159 (47.5)	176 (52.5)	1.25 (0.57-2.76)	0.584	248 (74.0)	87 (26.0)	1.20 (0.47-3.04)	0.706
≤Primary school	32 (51.6)	30 (48.4)	1.09 (0.60-1.97)	0.784	49 (79.0)	13 (21.0)	0.90 (0.46-1.76)	0.763
Mother Education								
College/University	45 (39.8)	68 (60.2)	Ref		79 (69.9)	34 (30.1)	Ref	
High school	172 (49.6)	175 (50.4)	1.52 (0.68-3.42)	0.312	257 (74.1)	90 (25.9)	1.07 (0.42-2.73)	0.894
≤Primary school	32 (52.5)	29 (47.5)	1.41 (0.77-2.60)	0.268	47 (77.0)	14 (23.0)	0.92 (0.46-1.81)	0.802
Father monthly income								
≥ MYR 4000	47 (46.5)	54 (53.5)	Ref		71 (70.3)	30 (29.7)	Ref	
MYR1000-3999	184 (46.9)	208 (53.1)	1.02 (0.22-4.70)	0.978	294 (75.0)	98 (25.0)	1.48 (0.26-8.57)	0.661
<MYR 1000	4 (50.0)	4 (50.0)	0.95 (0.49-1.81)	0.868	6 (75.0)	2 (25.0)	1.57 (0.77-3.20)	0.213
Mother monthly income								
≥ MYR 4000	33 (45.8)	39 (54.2)	Ref		52 (72.2)	20 (27.8)	Ref	
MYR 1000-3999	57 (47.1)	64 (52.9)	0.83 (0.38-1.78)	0.624	86 (71.1)	35 (28.9)	0.83 (0.35-1.94)	0.664
<MYR 1000	164 (48.5)	174 (51.5)	0.73 (0.32-1.67)	0.458	253 (74.9)	85 (25.1)	0.71 (0.28-1.76)	0.454

Ref: reference category

Table 15. Bivariate analysis between infant feeding practices and prevalence of caries at dentine level ($D_{4\text{-}6}$MFT>0) and caries at all levels ($D_{1\text{-}6}$MFT>0) in a non-fluoridated area

Non-fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Infant feeding practices	$D_{4\text{-}6}$MFT>0 n (%)		Odds ratio 95% CI	p value	$D_{1\text{-}6}$MFT>0 n (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Use of infant formula								
No	115 (53.5)	100 (46.5)	Ref		164 (76.3)	51 (23.7)	Ref	
Yes	143 (43.2)	188 (56.8)	0.66 (0.47-0.93)	**0.019**	236 (71.3)	45 (28.7)	0.77 (0.52-1.15)	0.200
Breast feeding								
No	6 (50.0)	6 (50.0)	Ref		9 (75.0)	3 (25.0)	Ref	
Yes	254 (47.4)	282 (52.6)	0.901 (0.29-2.83)	0.858	393 (73.3)	143 (26.7)	0.92 (0.25-3.43)	0.897
Age finished breast feeding								
>12 months	182 (46.9)	206 (53.1)	Ref		111 (75.0)	37 (25.0)	Ref	
≤12 months	72 (48.6)	76 (51.4)	1.07 (0.73-1.57)	0.718	282 (72.7)	106 (27.3)	1.13 (0.73-1.74)	0.587
Age started formula								
>12 months	70 (44.9)	86 (55.1)	Ref		42 (26.9)	114 (73.1)	Ref	
≤12 months	78 (43.1)	103 (56.9)	0.93 (0.60-1.43)	0.743	127 (70.2)	54 (29.8)	0.7 (0.54-1.40)	0.555
Age finished formula								
>48 months	56 (44.8)	69 (55.2)	Ref		145 (69.7)	63 (30.3)	Ref	
≤48 months	91 (43.8)	117 (56.3)	0.96 (0.61-1.50)	0.852	95 (76.0)	30 (24.0)	0.73 (0.44-1.21)	0.216

Type of water used to prepare formula								
Bottled water	4 (28.6)	10 (7.4)	Ref		10 (71.4)	4 (28.6)	Ref	
Tap water	128 (46.2)	149 (53.8)	2.15 (0.66-7.01)	0.206	202 (72.9)	75 (27.1)	1.08 (0.33-3.54)	0.902
Filtered water	14 (35.9)	25 (64.1)	1.40 (0.37-5.30)	0.620	25 (64.1)	14 (35.9)	0.71 (0.19-2.70)	0.602
Duration of formula								
>48 months	26 (40.0)	39 (60.0)	Ref		45 (69.2)	20 (30.8)	Ref	
≤48 months	118 (44.7)	146 (55.3)	1.21 (0.70-2.11)	0.494	188 (71.2)	76 (28.8)	1.10 (0.61-1.98)	0.753
Feeding method								
Formula only	6 (50.0)	6 (50.0)	Ref		9 (75.0)	3 (25.0)	Ref	
Combine breast & formula	137 (42.9)	182 (57.1)	0.75 (0.24-2.39)	0.629	227 (71.2)	92 (28.8)	0.82 (0.22-3.11)	0.773
Breast only	115 (53.5)	100 (46.5)	1.15 (0.36-3.68)	0.814	164 (76.3)	51 (23.7)	1.07 (0.28-4.11)	0.919

Ref: reference category

Table 16. Bivariate analysis between oral hygiene habits at the time of study (2015) and prevalence of caries at dentine level ($D_{4\text{-}6}MFT>0$) and caries at all levels ($D_{1\text{-}6}MFT>0$) in a non-fluoridated area

Non-fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Oral hygiene habits, in 2015	$D_{4\text{-}6}MFT>0$ n (%)		Odds ratio 95% CI	p value	$D_{1\text{-}6}MFT>0$ n (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Frequency of toothbrushing								
Twice/day or more	210 (48.3)	225 (51.7)	Ref		327 (75.2)	108 (24.8)	Ref	
Once /day or less	50 (44.6)	62 (55.4)	0.86 (0.57-1.31)	0.493	75 (67.0)	37 (33.0)	0.67 (0.43-1.05)	0.080
Supervise toothbrushing								
Never	28 (46.7)	32 (53.3)	Ref		42 (70.0)	19 (30.0)	Ref	
Yes	217 (47.5)	240 (52.5)	1.03 (0.60-1.77)	0.905	337 (73.7)	120 (26.3)	1.20 (0.67-2.17)	0.538
Habits after brushing								
Spat	248 (46.9)	281 (53.1)	Ref		387 (73.2)	142 (26.8)	Ref	
Swallowed	4 (50.0)	4 (50.0)	1.13 (0.28-4.68)	0.861	6 (75.0)	2 (25.0)	1.10 (0.22-5.52)	0.907
Eating/ licking toothpaste								
Never	232 (48.2)	249 (51.8)	Ref		353 (73.4)	128 (26.6)	Ref	
Yes	28 (42.4)	38 (57.6)	0.79 (0.47-1.33)	0.376	49 (74.2)	17 (25.8)	1.05 (0.58-1.88)	0.883
Amount of toothpaste used								
Medium to large	236 (47.1)	265 (52.9)	Ref		366 (73.1)	135 (26.9)	Ref	
Small	23 (51.1)	22 (48.9)	1.17 (0.64-2.16)	0.607	35 (77.8)	10 (22.2)	1.29 (0.62-2.68)	0.493
Type of toothpaste								
Non-fluoridated	13 (68.4)	6 (31.6)	Ref		16 (84.3)	3 (15.8)	Ref	
Fluoridated	240 (46.3)	278 (53.7)	0.40 (0.15-1.06)	0.067	376 (72.6)	142 (27.4)	0.50 (0.14-1.73)	0.272

Ref: reference category

Table 17. Bivariate analysis between oral hygiene habits (at age less than six years) and prevalence of caries at dentine level (D_{4-6}MFT>0) and caries at all levels (D_{1-6}MFT>0) in a non-fluoridated area

Non-fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
Oral hygiene habits at age less than 6 years	D_{4-6}MFT>0 n (%)		Odds ratio 95% CI	p value	D_{1-6}MFT>0 n (%)		Odds ratio 95% CI	P value
	Yes	No			Yes	No		
Frequency of toothbrushing								
Twice/day or more	130 (46.3)	151 (53.7)	Ref		211 (75.1)	70 (24.9)	Ref	
Once /day or less	129 (48.9)	135 (51.1)	1.11 (0.79-1.55)	0.544	190 (72.0)	74 (28.0)	0.85 (0.58-1.25)	0.409
Supervised toothbrushing								
Never	5 (38.5)	8 (61.5)	Ref		10 (76.9)	3 (23.1)	Ref	
Yes	244 (48.6)	258 (51.4)	1.51 (0.48-4.69)	0.473	373 (74.3)	129 (25.7)	0.88 (0.24-3.20)	0.831
Habits after brushing								
Spat	240 (47.4)	266 (52.6)	Ref		371 (73.3)	135 (26.7)	Ref	
Swallowed	16 (47.1)	18 (52.9)	0.99 (0.49-1.98)	0.966	26 (76.5)	8 (23.5)	1.18 (0.52-2.68)	0.687
Eating/ licking toothpaste								
Never	114 (48.3)	122 (51.7)	Ref		175 (74.2)	61 (25.8)	Ref	
Yes	145 (46.9)	164 (53.1)	0.95 (0.67-1.33)	0.749	226 (73.1)	83 (26.9)	0.95 (0.65-1.40)	0.790
Amount of toothpaste used								
Medium to large	135 (46.9)	153 (53.1)	Ref		208 (72.2)	80 (27.8)	Ref	
Small	123 (48.0)	133 (52.0)	1.05 (0.75-1.47)	0.785	192 (75.0)	64 (25.0)	1.15 (0.79-1.69)	0.464

Type of toothpaste								
Non-fluoridated	28 (49.1)	29 (50.9)	Ref		37 (64.9)	20 (35.1)	Ref	
Fluoridated	224 (47.3)	250 (52.7)	0.93 (0.54-1.61)	0.790	353 (74.5)	121 (25.5)	1.58 (0.88-2.82)	0.125
Age started toothbrushing								
After 2 years	186 (47.2)	208 (52.8)	Ref		283 (71.8)	111 (28.2)	Ref	
Before 2 years	74 (48.4)	79 (51.6)	1.05 (0.72-1.52)	0.808	119 (77.8)	34 (22.2)	1.37 (0.88-2.13)	0.158
Age started toothbrushing with toothpaste								
After 2 years	200 (47.6)	220 (52.4)	Ref		306 (72.9)	114 (27.1)	Ref	
Before 2 years	58 (46.4)	67 (53.6)	0.95 (0.64-1.42)	0.811	94 (75.2)	31 (24.8)	1.18 (0.52-2.68)	0.687

Ref: reference category

Table 18. Bivariate analysis between exposure to fluoride varnish/gel and prevalence of caries at dentine level ($D_{4-6}MFT>0$) and caries at all levels ($D_{1-6}MFT>0$) in a non-fluoridated area

Non-fluoridated	Dentine caries		Unadjusted		Caries at all levels		Unadjusted	
	$D_{4-6}MFT>0$ N (%)		Odds ratio 95% CI	p value	$D_{1-6}MFT>0$ N (%)		Odds ratio 95% CI	p value
	Yes	No			Yes	No		
Exposure to Fluoride varnish/gel								
No	132 (45.5)	158 (54.5)	Ref		307 (71.4)	83 (28.6)	Ref	
Yes	35 (43.2)	46 (56.8)	0.91 (1.55-1.50)	0.712	59 (72.8)	22 (27.2)	1.08 (0.62-1.87)	0.795

Ref: reference category

Appendix 35 List of conferences attended

1. ‘Clinician agreement on fluorosis scoring: a comparison of photographic and clinical methods’ at Malaysia International Dental Exhibition and Conference, Kuala Lumpur, 12-14th June 2015. (Oral presentation).
2. The British Society for Oral and Dental Research (BSODR) Scientific Meeting 2015, Cardiff City Hall, 14-16th September 2015. (Participant).
3. ‘Caries experience among Malaysian children in fluoridated and non-fluoridated areas using ICDAS II criteria’ at the British Association for the Study of Community Dentistry Conference, Spring Scientific Meeting, Windermere, Cumbria, United Kingdom, 14-15th April 2016. (Poster presentation).
4. ‘Fluorosis following reduction of fluoride level in the water supply’ at the 95th General Session & Exhibition of the International Association for Dental Research (IADR), San Francisco, US, 22-25th March 2017. (Oral presentation).
5. ‘The effects of stopping the addition or reducing the level of fluoride level in the public water supply: a systematic review’ at the British Association for the Study of Community Dentistry (BASCD) Spring Scientific Conference, Oxford, United Kingdom, 6-7th April 2017. (Poster presentation).