The Effect of 10% Povidone-Iodine on *S. Mutans* Levels in Children with "Early Childhood Caries"

by

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Abstract

An antibacterial agent that is effective and acceptable to young children will be a useful supplement to behaviour modification techniques for the prevention of early childhood caries. Objective: The objective of the present study was to determine the effect of 10% povidone-iodine solution (betadine) on *S. mutans* in children with dental caries. Methods: The study was designed as a randomized single blind, no treatment control trial. Twenty-five children between 2 and 6.8 years of age with unremarkable medical history were recruited from a private pediatric dental practice in Burnaby, BC. All subjects were scheduled for dental rehabilitation under general anesthesia. Parents completed a consent form and a variety of survey instruments. Before intubation, height and weight of all participants were recorded and these measurements were repeated at all subsequent study appointments for children in experimental group. Dental examination data and the plaque sample were collected after intubation. Following dental treatment, children were randomly assigned to the experimental group (N=13) or the control group (N=12). Experimental children had betadine applied 3 times at 2-month intervals and no treatment was employed for control children. After 6 months, both experimental and control children received another dental examination and a mouth swab was taken from all subjects. Plaque samples were cultured for total bacteria and *S. mutans*. Results: Betadine application was well-accepted by all experimental children. No adverse effect was reported. At baseline, experimental and control children had similar dietary habits, dmfs, and *S. mutans* levels. All children's *S. mutans* counts decreased significantly at 6-months (P=0.0004). Although the overall *S. mutans* decrease was greater for experimental children than for control children, this difference was not significant (P=0.58). Ten of 13 experimental children had a ≥ 1 $\log_{10}$ decrease in *S. mutans* over 6 months,

compared with 7 of 12 control children. The number of children with new cavitated carious lesions at 6 months was the same for both groups. For this group of children, the effect of rehabilitation under general anesthesia may have made a major contribution to decreasing plaque *S. mutans* at 6-months post-treatment that overwhelmed the effect of betadine. Conclusion: Results suggest that betadine may have an effect on *S. mutans*, but additional research with more subjects and a longer time period after application are indicated.

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Dedicated to

my dear husband, Shahram

and

my sweet son, Nima

Chapter 1

Introduction

Although the prevalence of dental caries in most developed countries has markedly declined in recent years, it still remains unacceptably high in some populations. In some areas the incidence of caries is starting to rise again (Johnson 1991, Federation Dentaire Internationale 1994). According to the 1993 national survey of children's dental health in the UK, almost 50% of 5-year-old children had evidence of caries (Downer 1994). Furthermore, surveys in developing countries have also demonstrated an increase in the prevalence of dental caries over the last two decades (Cleaton-Jones *et al.* 1989, Al Ghanim *et al.* 1998). Evidence indicates that children who have caries in their primary teeth as infants or toddlers will develop additional dental decay in their primary teeth (O'Sullivan and Tinanoff 1996) and are more likely to develop caries in their permanent teeth (Kaste *et al.* 1992). Therefore, the importance of a healthy primary dentition cannot be overstated.

Apart from the job of initiating the digestion of food, primary teeth serve multiple roles such as preserving adequate spacing for permanent teeth, assistance in the development of early speech and contributing to the development of healthy social skills and confidence (Valaitis *et al.* 2000). Early childhood caries (ECC), describing any form of caries in infants and preschool children (Kaste and Gift 1995), is a significant childhood disease. It is a painful condition that interferes with eating, sleeping, and proper growth. Furthermore, it is not unusual for ECC to progress to a point where teeth become nonvital, a situation which may affect the underlying developing permanent dentition (Billings 1996). In addition to its serious consequences, treatment of ECC is expensive, often requiring extensive restorative treatment and extraction of teeth at an early age. The deep sedation or general

anesthesia that is often employed to treat very young children is extremely costly (Tinanoff 1997). Therefore, preventive programs are important for both infants (up to 1 year of age) and toddlers (ages 1 to 3) (Wendt 1995).

Researchers, who struggle with determining appropriate effective methods to manage ECC and concentrate on health education programs alone, have achieved only limited success (Horowitz 1998). Therefore, there is a compelling need for research to develop other preventive strategies to manage this devastating problem. A promising approach to prevention of ECC is to evaluate various chemotherapeutic products that have shown promise as primary preventive agents. Previous studies have demonstrated positive outcomes for antimicrobial agents such as fluoride (Twetman 1996), chlorhexidine (Achong *et al.* 1999), and povidone-iodine (Lopez *et al.* 1999) for reducing caries.

The ability of iodine solutions to suppress oral *S. mutans* has been demonstrated when topically applied to the teeth (Gibbons *et al.* 1974, Caufield and Gibbons 1979). The use of betadine, an iodine-containing solution, has also been successful to control the incidence of new carious lesions in high-risk 12 to 19 months of age infants in Puerto Rico (Lopez *et al.* 1999, Lopez *et al.* 2002). However, no studies have reported on the effect of betadine on the number of oral *S. mutans* in children with ECC. The purpose of the present study is to investigate the effectiveness of povidone-iodine as an agent to decrease the numbers of bacteria that are known to cause early childhood caries.

Chapter 2

Review of the literature

2.1. Early Childhood Caries

2.1.1. Definition

Early childhood caries (ECC) is the current term used to describe rampant dental caries in infants and toddlers (Kaste and Gift 1995). For many children the condition is initiated and exacerbated by prolonged and inappropriate feeding with a baby bottle (Johnsen 1982). The condition, when associated with a bottle habit, has been described as first affecting the primary maxillary anterior teeth, followed by the primary molars. Mandibular incisors generally are not affected (Milnes 1996). Terms to describe this condition have evolved during the last two decades to include "*nursing caries*", "*nursing bottle caries*", "*baby bottle caries*" and "*baby bottle tooth decay*" (Tinanoff 1997). In 1994, a conference at the Centers for Disease Control and Prevention in Atlanta, Georgia, recommended the use of the less specific term, "*early childhood caries*", because it was the consensus of the attendees that the relationship between bottle habits and caries was not absolute (CDCP 1994).

Although the term ECC is not descriptive in terms of risk factors, characteristics of the condition, and prevention, it is the currently used terminology.

2.1.2. Prevalence

The prevalence of ECC reported by different investigators demonstrates a wide variation. This variation may be because of the lack of a universally-accepted definition for ECC (Milnes 1996) and the fact that young children are not always cooperative for a thorough clinical examination (Twetman *et al.* 2000). In European countries such as the UK and Sweden, the prevalence of ECC has been reported to

range from 0.5% at 12 months to 30-40% at 5 years (Hinds and Gregory 1995, Wendt 1995). A similar range in prevalence has been reported in areas as diverse as the Middle East and South Africa (Raadal *et al.* 1993, Al Mahammadi *et al.* 1997). Differences among these studies likely reflect the difficulty in controlling the many variables that are related to ECC (Milnes 1996).

Significant differences have also been shown in the prevalence of ECC in various ethnic, cultural or socioeconomic segments of Canadian and American societies. An estimated 80% of caries is said to be found in just 25% of children (Kaste *et al.* 1996). The rate of ECC in North America ranges from 1% to 70%, depending on the group of children studied (Milnes 1996). A recent report by the BC Ministry of Health Dental Staff stated that the prevalence of nursing bottle tooth decay in the province's 5-year olds was, on average, 11.3% (Bassett *et al.* 1999). This statistic means that more than one in ten BC children entering kindergarten in 1998 had obvious decay on their top front primary teeth.

While the prevalence of early childhood caries has been widely studied, the variety of definitions used in the numerous studies makes comparison of data difficult. What is clear is that ECC is a serious world wide health problem that demonstrates an alarming prevalence among children in British Columbia.

2.1.3. Etiology

2.1.3.1. Biological risk factors

The oral ecology is a balanced system involving bacteria, the host and the diet. Like many other diseases, caries is the result of an ecologic imbalance in the oral cavity. The etiology of ECC is probably similar to other types of coronal caries but the biology may differ in some respects (Seow 1998). The basic process is that

cariogenic microorganisms act on fermentable carbohydrate to produce acids. A long-term low pH environment will demineralize tooth enamel and result in dental caries (Keyes 1960).

Several factors are unique to children in early childhood. These factors include: early colonization by mutans streptococci, behavioral habits associated with feeding and oral hygiene, immature specific and non-specific defence systems, and newly erupted and immature teeth (Seow 1998). These factors may modify the biology of ECC and complicate its etiology.

2.1.3.1.1. Microflora

2.1.3.1.1.1. Mutans Streptococci (MS)

Mutans streptococci (MS) are implicated as the primary bacteria initiating caries in humans (Keyes 1960). While several different MS species exist, *S. sobrinus* and especially *S. mutans* are the species most commonly isolated from the human's oral cavity (Bratthall 1972). *S. sobrinus*, generally found in association with *S. mutans*, is thought to be principally responsible for the development of smooth surface caries (Lindquist and Emilson 1991). In children with rampant caries, the dental plaque concentration of *S. mutans* has been reported to range from an average of 30%-40% to over 50% of the total cultivable plaque flora and 10% of the salivary flora (Van Houte *et al.* 1982, Boue *et al.* 1987). Such high numbers of acidogenic microorganisms in the presence of a frequent carbohydrate intake produces abundant acid that lowers plaque pH for extended periods and demineralizes a child's teeth.

Virulence of MS: MS have certain properties that enable them to predominate in dental plaque and induce the development of caries. The organisms produce water-insoluble glucan polymers, uniquely from the sucrose, through the action of glucosyltransferases (Tanzer *et al.* 1984). In addition to the mediation of

irreversible adhesion and colonisation of MS to teeth, these glucans increase the thickness of plaque, and result in enhanced rates of sugar diffusion and acid production at the deeper plaque layers (Van Houte *et al.* 1989). MS can ferment various sugars to produce large quantities of lactic acid. Lactic acid, the most important acid involved in the etiology of dental caries, is potent in the demineralizing of tooth enamel and inducing dental caries (Johnson *et al.* 1980).

In addition, mutans streptococci can grow at low pH values, some strains even growing at pH of less than 4. These streptococci produce large amounts of a membrane-associated ATPase, capable of functioning at low pH, which helps to pump hydrogen ions from the cell and thus reduces intracellular acidification (Carlsson 1989). In the presence of carbohydrates (not necessarily sucrose) some MS synthesize intracellular polysaccharide (ICP), which typically resembles glycogen. When there is no exogenous carbohydrate, ICP can be metabolized leading to continue acid production and maintenance of acidogenicity (Spatafora *et al.* 1995). And finally, MS produce dextranase, which assists the bacterium in its invasion of dextran-containing early dental plaque (Tanzer 1989).

Colonization of MS in dental plaque: Adhesion of MS to the teeth occurs as a two step process. Initial attachment of MS is reversible and mediated by surface components of the bacteria interacting with the salivary proteins, which form the tooth surface pellicle (Bowen *et al.* 1991). Reversible attachment is followed by sucrose-dependent irreversible attachment. In the presence of fermentable carbohydrates, especially sucrose, MS irreversibly adhere to the pellicle through the synthesis of glucans mediated by glucosyltransferases produced by the bacteria (Loesche 1986).

Establishment of MS in infants: The age at which MS first colonize a child's teeth is a factor in predicting the caries activity at a later age. Children whose teeth are colonized earlier by MS show higher caries experience than those colonized later or not at all (Tenovuo *et al.* 1990). Most studies including pre-dentate children suggest that the initial acquisition of MS coincides with the emergence of the first primary tooth (Mohan *et al.* 1998). The reason may be related to the fact that MS generally require a non-shedding surface to colonize (Loesche 1986). MS colonization, prior to tooth eruption may be associated with oral developmental mucosal nodules. This association shows that in infants with nodules, the overall oral environment is favourable to both the occurrence of nodules and MS infection (Wan *et al.* 2001). The infection rate of MS increases with age because of the increasing number of retentive sites (teeth) for bacterial colonization (Grindefjord *et al.* 1996).

Transmission of MS: The strong correlation between salivary MS counts in mothers and in their children demonstrates that MS are transmitted from the primary caregiver, usually the mother, to the infant via the mother's saliva (Brown *et al.* 1985). This exchange can occur, for example, through kissing or saliva-contaminated food (Li and Caufield 1995).

2.1.3.1.1.1. Lactobacilli

Since lactobacilli produce large amounts of acid in the presence of sugars and are able to survive at very low pH values (Van Houte 1980), this bacteria was originally thought to be the primary etiological agent of dental caries. However, it is now believed that lactobacilli probably play a more important role in the progression of dental caries rather than in initiation of the disease (Van Houte 1980). Clinical studies in preschool children have also demonstrated a low frequency of isolation of lactobacilli (Roeters *et al.* 1995); however, both mutans streptococci and lactobacilli

have usually been isolated from the children with high caries experience (Bratthall 1991).

2.1.3.1.2. Host

2.1.3.1.2.1. Salivary factors

One of the main host defenses against caries is saliva. In addition to clearance of the foods, saliva can neutralize the acids produced by bacteria. The pH buffering capacity of saliva is mainly mediated by bicarbonate ions or by sialin that is metabolized by bacteria to generate ammonia and raise the plaque pH (Loesche 1982). Saliva also contains antimicrobial proteins such as lysozyme, lactoferrin, peroxidase enzymes, agglutinins, and histidine-rich proteins, which may have a significant role in protection against dental caries (Scannapieco 1994). Oral clearance, buffering capacity, and antimicrobial activities of saliva largely depend on its flow rate (Tenovuo 1991). Therefore, infants and young children who feed frequently during sleep, when the flow rate of saliva is the lowest (Scheneyer *et al.* 1956), are at high-risk to early childhood caries.

2.1.3.1.2.2. Immunological factors

Specific immune mechanisms against cariogenic mutans streptococci in dental caries are centered on the inhibition of adherence, and the pathogenic activity of cariogenic bacteria (Smith and Taubman 1991). These mechanisms include specific immune factors derived from saliva or serum and gingival crevicular fluid, non-specific antimicrobial systems derived mainly from saliva, and phagocytic cells which transudate through the gingival crevice (Tenovuo *et al.* 1987).

Among host factors, secretary IgA (SIgA), the predominant immunoglobulin in whole saliva, constitutes the main specific immune defence mechanism in the oral cavity (Russell *et al.* 1999). This system is functional in newborn infants, who

develop SIgA antibodies as they become colonized by oral microorganisms (Smith *et al.* 1990). The mechanisms of action of SIgA antibodies include interference with sucrose-independent (Hajishengallis *et al.* 1992) and sucrose-dependent (Smith *et al.* 1990) attachment of MS to tooth surfaces, as well as possible inhibition of bacterial metabolic activities by neutralizing enzymes, toxins and virus (Fukui *et al.* 1973, Funakoshi *et al.* 1982).

In addition to secretary IgA, the oral cavity receives immunoglobulins derived from the circulation by transudation through the gingival crevice. These comprise IgM, IgG, and IgA (Russell *et al.* 1999). Serum antibodies to MS, mainly belong to the IgG class, can activate the complement system and facilitate phagocytosis of MS by neutrophils (Scully *et al.* 1980). While the volume of crevicular fluids is small, the transudation is heavy during the eruption of the teeth (Tenovuo 1991), therefore, serum antibodies may also have a significant protective role against caries-induced bacteria in infants during the early stages of microbial establishment. However, the overall anti-caries effect of these immunological factors is still unknown.

2.1.3.1.2.3. Tooth maturation and defects

Final maturation and hardening of the enamel of primary teeth are complete some time after eruption, when ions such as fluoride are incorporated (Fejerskov and Clarkson 1966). Therefore, the immediate period after eruption and before final maturation of the teeth is when teeth are most susceptible to caries (Carlos and Gittlesohn 1965). In ECC, high concentrations of MS and a frequent intake of fermentable carbohydrates combined with immature newly erupted teeth, increases an infant susceptibility to dental caries (Seow 1998). Developmental enamel defects such as enamel hypoplasia may also predispose the primary teeth to plaque retention and

caries risk (Seow 1991). An increased prevalence of hypoplasia in some children with ECC has been demonstrated (Li *et al.* 1996). However, whether enamel hypoplasia present at the subclinical level in infants poses a significant risk factor for ECC needs further confirmation.

2.1.3.1.3. Substrate (Carbohydrates)

2.1.3.1.3.1. Cariogenicity

Diet plays a major role in the development of dental caries. Observations in humans (Moynihan and Holt 1996) and in animals (Firestone *et al.* 1984) have clearly shown that frequent and prolonged oral exposure to fermentable carbohydrates is fundamental to caries activity. The mechanism by which diet affects dental caries is simple. The bacteria on the teeth utilize carbohydrates in their glycolytic pathways to produce energy, and acid is a byproduct of this metabolism (Nyvad and Fejerskov 1996). Consequently, the dental plaque pH falls to a point, which leads to demineralization of enamel (Johnson *et al.* 1980). Abundant epidemiological evidence from groups who have consumed low quantities of sugars as well as from those who have consumed high quantities shows that sugar, especially sucrose, is a major dietary factor affecting dental caries prevalence and progression (Rugg-Gunn 1996, Karjalainen *et al.* 2001).

Several dietary characteristics of early childhood suggest substrate as one of the most significant caries risk factors in ECC. In early childhood, children generally have increased and frequent carbohydrate consumption from juice, milk, infant formulas, and solid food (Tsubouchi *et al.* 1995). Many young children are accustomed to prolonged bottle feeding during bedtime or naptime. In addition, toddlers often consume carbohydrate in the form of sticky, sweetened, between-meal-

snacks, which are associated with a marked increase in caries risk, and caries activity (Gustafson *et al.* 1954)

In addition to sucrose, other sugars such as glucose and fructose, found in fruit and honey, also have the ability to drop the plaque pH and to demineralize enamel (Imfeld and Muhlemann 1978). Another predominant dietary carbohydrate of significance in ECC is starch. While the relationship between sucrose and dental caries development is indisputable, the relationship between food starch and dental caries continues to be debated. Collectively, evidence suggests that raw starch causes only a small drop in plaque pH. However, soluble starch and refined starch in different foods such as bread or crackers causes a drop of pH which may be as large as that caused by sugars (Lingstorm *et al.* 1989).

2.1.3.1.3.2. Frequency

A strong correlation between the frequency of carbohydrate ingestion and the prevalence of dental caries has been demonstrated (Firestone *et al.* 1984). Frequent exposure to carbohydrates maintains the plaque pH below the critical point of pH 5.5. These repeated acid attacks over a period of time, cause tooth demineralization which leads to dental caries. One example of high-frequency carbohydrate consumption is prolonged or nighttime bottle feeding which is a common ECC feeding behavior.

2.1.3.1.3.3. Retentiveness and Clearance

The form of the carbohydrate is another critical factor in the cariogenicity of foodstuff. Products that are sticky and retained for long periods in the mouth have a higher cariogenicity than foods that are eliminated quickly. Previous investigators who have examined the effects of the consistency (retentiveness) of the sugar on dental caries rate have demonstrated that the addition of sugar to the diet caused

increased caries activity, but the degree was dependent on the consistency of the sugar (Gustafsson *et al.* 1954). In addition to retentiveness, oral clearance of carbohydrates by saliva also makes a significant contribution to ECC. During sleep, the flow rate of saliva is low, increasing the length of contact time between plaque and substrate. Therefore, the cariogenicity of the substrate increases significantly (Firestone 1982).

In summary, the major biological factors that contribute to ECC are a combination of high concentration of MS, immature newly erupted teeth, and frequent intake of fermentable carbohydrates often at night, when the clearance effect of saliva is lowest.

2.1.3.2. Non-biological risk factors

2.1.3.2.1. Infant feeding patterns

Children with ECC often have a history of sleeping with a bottle or using a bottle or breast beyond the normal weaning time (Dilley *et al.* 1980), but some children who sleep with a bottle do not develop ECC (Kaste and Gift 1995). In one US study involving children in a Head Start infants program, 86% of children with caries of the maxillary incisors were reported to have taken a bottle to bed. However, surprisingly, 69% of those who did not have maxillary anterior caries also repeatedly took a bottle to bed (O'Sullivan and Tinanoff 1993). In another study, 90% of children were bottle-fed between 12 and 18 months of age, yet the prevalence of ECC was only 20% (Serwint *et al.* 1993).

Another issue related to infant feeding pattern is the contents of the nursing bottle. Bovine milk is the most common fluid placed in a baby's bottle (Seow 1987), however, from the literature; there is no evidence to suggest that bovine milk is cariogenic. On the contrary, there is much evidence that milk is cariostatic (Bowen and Pearson 1993). In fact, it has been demonstrated that the risk of MS colonization

appears lower among infants who consume milk rather than sweetened beverages in the bottle (Mohan *et al.* 1998). The same result has been reported for milk-based formulas. The main mechanism of protection afforded by milk is increasing the calcium and phosphate concentrations in the plaque and enhancing the remineralization of enamel (Reynolds 1987). Unlike milk, the cariogenicity of fruit juices and soft drinks is well documented (Smith 1987). Acids found in juices and soft drinks decrease the oral pH and cause profound enamel demineralization.

In addition to the contents of the nursing bottle, physical presence of the nipple in the mouth may have negative consequences. The flow rate of the saliva on the palatal surfaces of maxillary teeth may be restricted by the nipple (Bowen 1997).

Another controversial caries risk is the potential cariogenicity of prolonged or night time breastfeeding. Several case reports have suggested a relationship between night time or prolonged breastfeeding and ECC (Derkson and Ponti 1982, Curzon and Drumond 1987). In contrast, some studies demonstrate no association between ECC and breastfeeding, while others suggest that breastfeeding may have a protective effect against dental caries (Silver 1992, Roberts *et al.* 1994). Overall, the relationship between breastfeeding and ECC is likely to be complex and confounded by many other biological and non-biological variables (Valaitis *et al.* 2000).

In summary, these findings suggest that the role of bottle and breastfeeding in caries development is not as clear as previously thought and further clarification of the association of infant feeding patterns and caries is required.

2.1.3.2.2. Demographic characteristics

2.1.3.2.2.1. Socioeconomic status

Most investigators have demonstrated that ECC is particularly prevalent in children from low-income families (Tang *et al.* 1997, Ramos-Gomez *et al.* 1999).

Socioeconomic status may influence caries risk in several ways. These families may have lower perceived need for health care, resulting in less attention to preventive strategies and long delays in attending for treatment (Chen 1995). Poverty also effects the ability to obtain a stable income and to provide adequate nutrition. Furthermore, for those families of low-socioeconomic status, the financial cost of treating the disease is prohibitively expensive and access to dental care is limited (Evans *et al.* 1996).

Although a strong relationship between socioeconomic background and dental health has been clearly documented (Evans *et al.* 1996, Gibson and Williams 1999), since most studies include ethnic minority groups of lower income, it is difficult to separate the influence of ethnicity from the influence of low socioeconomic status on ECC (Reisine and Douglas 1998).

2.1.3.2.2.2. Race and ethnicity

Most studies of children from different cultural groups have demonstrated differences in the prevalence of ECC. Investigators in the United States, Canada, and Europe have demonstrated that in ethnic minorities, prevalence of ECC is increased (Grindefjord *et al.* 1993, Harrison *et al.* 1997, Watson 1999). Native American and Canadian First Nations children also demonstrate an extremely high rate of ECC ranging from 70% to 80% (Milnes 1996). Overall, evidence suggests increased risk of ECC in ethnic minorities which could be associated with cultural norms including prenatal diet, feeding practices, care of primary teeth, and access to dental and medical services (Reisine and Douglas 1998).

2.1.3.2.2.3. Parenting style and family circumstances

Parenting beliefs and their subsequent behaviours have significant contribution to either protect or place a child at risk for dental disease. Some investigators believe that parenting practices such as feeding or oral hygiene habits are central to the etiology of ECC (Holbrook *et al.* 1993, Milnes 1996). Parenting behaviours vary with culture and socioeconomic status (Chen 1995).

The relationship between parental behaviours and ECC might be influenced by parental stress. A recent study exploring the relationship between parental stress and ECC concluded that a higher caries rate was found consistently among children whose parents experienced greater stress (Quinonez *et al.* 2001). Parental stress measures might be representative of family circumstance factors such as socioeconomic status, education level, marital status, and number of children in the family in a high-risk population (Quinonez *et al.* 2001). Therefore, caregivers preoccupied with more immediate and pressing issues may be less likely to follow preventive oral health behaviours for themselves or their children.

2.1.3.2.3. Toothbrushing

The data on the association of toothbrushing behaviour and ECC are equivocal. Several studies have demonstrated an inverse relationship between toothbrushing frequency and carious lesions on smooth surfaces (Paunio *et al.* 1993, Wendt *et al.* 1994), while others found that toothbrushing frequency had no significant effect on dental caries (Febres *et al.* 1997). In addition to the frequency of toothbrushing, parental involvement in toothbrushing is another variable. A recent study describing the relationship between oral hygiene behaviour and dental health of infants and toddlers reported that children who brushed their teeth themselves were more likely to have visible plaque compared with children whose teeth were cleaned by their parents (Habibian *et al.* 2001). The age toothbrushing is started is another

variable to explore, although no evidence exists to indicate an association between age of onset of toothbrushing and ECC (Serwint 1993). Overall, toothbrushing alone is not as effective in reducing caries rates as was previously believed. However, there is convincing evidence to support the decay preventive benefit of toothbrushing with fluoride-containing toothpaste (O'Mullane 1994).

In summary, from the non-biological point of view, children from different cultural and ethnic minority groups living in low socioeconomic environments with a history of inappropriate feeding pattern and parental stress are likely at higher risk of dental caries in childhood.

2.1.4. Prevention of Early Childhood Caries

Theoretically, dental caries can be prevented by a combination of community, professional, and individual strategies. While a variety of interventions to prevent dental caries in young children are available, more than one approach is probably needed to increase the potential of preventing the disease (Twetman *et al.* 2000).

2.1.4.1. Community-based strategies

2.1.4.1.1. Dental health education

The mainstay of many community-based programs has been education in the hope of increasing the knowledge of caregivers about the etiology of ECC and persuading them to adopt healthy behaviors and to discontinue unhealthy behaviors. However, many investigators have concluded that education alone is not effective in preventing early childhood caries (Reisine and Litt 1993, Tinanoff 1995). Conversely, investigators in the UK demonstrated that providing dental health education programs for mothers of young infants was successful in preventing the occurrence of nursing

caries (Kowash *et al.* 2000). Educational programs that encourage behavioural changes still continue to be the highlight of most preventive interventions.

2.1.4.1.2. Water fluoridation

The effectiveness of water fluoridation on the prevention of dental caries has been extensively documented and many studies have demonstrated a caries reduction of 40% to 50% in the primary dentition (WHO 1994, Evans *et al.* 1996, Olofsson and Bratthall 2000). The reduction was more apparent in children of low socioeconomic status. However, as yet no evidence confirms that water fluoridation has a positive effect on ECC (Ismail 1998). Because water fluoridation demands no effort from the recipients, and does not require a dental visit or parental motivation, it is likely to provide a potential benefit to infants and toddlers.

2.1.4.2. Professional office-based preventive methods

2.1.4.2.1. Early assessment and counselling

The American Academy of Pediatric Dentistry recommends that all infants receive an early dental examination at the age of 1 year or within 6 months of the eruption of the first tooth (AAPD 1996). By early screening, dental professionals are able to review feeding and oral hygiene practices and plan a program for further preventive interventions.

The first step of a screening examination is to determine those children who are at high risk for dental decay. Factors to consider include signs of early dental caries (white demineralized areas), heavy plaque accumulation, high mutans streptococci levels and socioeconomic status (Steiner *et al.* 1992, Alaluusua and Mamivirta 1994, Grindefjord *et al.* 1996). Based on a determination of caries risk, different preventive strategies including dietary modification and brushing with a

fluoride toothpaste can be employed for children in different risk categories. Because no strategy for predicting caries risk is absolutely accurate, a combination of methods should be used.

2.1.4.2.2. Preventive therapies

2.1.4.2.2.1. Fluoride

Fluoride applied in the office plays an important role in any intensive preventive program. The frequency of the fluoride application is directly dependent on the caries activity, so children with high carious activity require more frequent applications (Ismail 1998). The anticariogenic effect of fluoride occurs because it inhibits demineralization and enhances remineralization of the enamel (Chow 1990). Topical fluoride treatments have been used for children for many years, traditionally, consisting of a four-minute application of fluoride gel held in contact with the teeth. Fluoride varnish is an ideal form of topical fluoride for young children in the high-risk group. The advantages of this method include slow release of fluoride from the varnish and retarded enamel demineralization following a cariogenic challenge (Peyrone *et al.* 1992). Fluoride varnish is easy to apply and does not require a professional prophylaxis prior to application (Kaste *et al.* 1996). Potential of ingestion is lower than fluoride gel and it is effective on both smooth surfaces and pit and fissure sites (O'Sullivan *et al.* 1994).

2.1.4.2.2.2. Fissure sealants

Young children at risk of ECC may benefit from the application of sealant materials. Sealants are the most effective means to prevent caries in pit and fissure surfaces, so should be considered as an important component of any preventive

programs. However, in young children moisture control remains a major obstacle, which precludes sealant use on many pre-cooperative children (Twetman *et al.* 2000).

2.1.4.3. Home-based strategies

2.1.4.3.1. Dietary modification

Feeding pattern is a more important determinant of caries activity in the primary dentition than in the permanent dentition (Kalsbeek and Verrips 1994). When children are introduced to sucrose-containing food and drinks around the time of the eruption of the first tooth, repeated experience and parental influence shape their future preferences for the majority of foods. Modifications to the diet should be made over time, aided by repetition and reinforcement. The goal must be to help caregivers develop lifelong dietary habits, which promote general and oral health for themselves and for their children (Tinanoff and Palmer 2000).

2.1.4.3.2. Toothbrushing with fluoride dentifrice

Infants who have a plaque free dentition and have their teeth brushed with fluoridated dentifrice are less likely to develop dental caries (Wendt 1994). The role of oral hygiene as a caries preventive measure in young children is not fully clear, but the effectiveness likely depends on the attention and awareness of caregivers (Karjalainen et *al.* 1994). Furthermore, because toothpaste is a vehicle to apply fluoride to the teeth, the main benefit of regular toothbrushing is to introduce fluoride into the mouth (Kanellis 2000).

2.1.4.3.3. Fluoride supplements

Dietary fluoride supplements in the form of drops or tablets were introduced as a substitute for fluoridated water for children living in non-fluoridated areas. They should be recommended for infants and toddlers only on an individual basis, and all

potential fluoride sources should be assessed before making any prescription (Twetman *et al.* 2000). The effectiveness of supplements, which are highly dependent on parental compliance, is unpredictable. For children at risk to ECC, supplements may not be practical because of the social, economic and life dilemmas already facing the families (Ismail 1998).

2.1.4.4. Prevention of transmission of cariogenic bacteria

Strategies to reduce the transmission of cariogenic microorganisms from mothers to their infants have been studied as methods to prevent ECC (Kohler and Andreen1996, Soderling *et al.* 2000). Preventive programs targeting mothers have been implemented during pregnancy and before eruption of the first infant's tooth. The programs generally included dental treatment, oral health education, and counselling as well as topical treatments of the mothers with various antibacterial agents, for example chlorhexidine (Tenovuo *et al.* 1992). Depending on compliance, the results have been beneficial not only in terms of the decrease of the prevalence of mutans streptococci in the infants, but also in significantly less caries (30%-50%) in the primary dentition (Kohler and Andreen 1996).

2.1.5. Rehabilitation of ECC

2.1.5.1 General anesthesia and ECC

Treatment of ECC is expensive, often requiring extensive restorative treatment and extraction of teeth (Tinanoff 1997). Routinely, deep sedation or general anesthesia is required because young children generally lack the ability to cope with prolonged dental procedures (Berkowitz *et al.* 1997). It might be assumed that the experience of such extensive therapy would encourage change in the family's dietary

and oral hygiene habits to avoid future dental caries and subsequent treatments, but unfortunately such change does not always occur (Peretz *et al.* 2000).

Because of the high cost of treating ECC utilizing general anesthesia, only a minority of children can afford it, therefore, the cost is often born by publicly-funded insurance programs (Kanellis 2000). In British Columbia in the year 2000, 2,641 children under the age of 4 where treated in hospital under GA and 3,173 were treated in private facilities under GA for ECC. The estimated cost for this treatment was over $ 10.5 million (ADSBC Children's Dentistry Task Force Report, 2001).

2.1.5.2. Relapse

In addition to the cost of treatment, several investigators have reported the susceptibility of children with ECC to future caries development following comprehensive treatment under general anesthesia (Berkowitz *et al.* 1997, Almedia *et al.* 2000, Primosch *et al.* 2001). Retreatment rates have been reported to range from 17% to 59% within an eighteen to twenty four month period following treatment.

Therefore, to prevent the costly and potentially risky treatment of children with early childhood caries in hospital or private operating rooms, it is logical to explore a variety of strategies to prevent ECC. In addition to nutritional counselling, oral hygiene, and fluoride therapy, more aggressive antimicrobial therapies are required to prevent the future development of carious lesions (Almedia *et al.* 2000).

2.1.6 Chemotherapeutic approaches to ECC

It has been known for about 80 years that dental caries is an infectious disease of bacterial origin and *S. mutans* is the primary pathogen responsible for dental decay (Clarke 1924). However, most preventive strategies currently employed to prevent ECC do little to suppress this pathogen. The use of antimicrobial agents to

reduce or eliminate the bacteria associated with caries is a relatively new approach to the prevention of ECC.

2.1.6.1. Fluoride

The ability of fluoride to prevent and arrest caries has been researched extensively. The primary mode of action of fluoride in reducing dental caries is "post-eruptive" in that it inhibits demineralization and enhances remineralization of dental enamel during the caries process (Chow 1990). However, fluoride also acts directly as an enzyme inhibitor, for example, of the glycolytic enzyme enolase, which is inhibited in a quasi-irreversible manner. It can also reduce the production of extra-cellular polysaccharides by bacteria. Furthermore, fluoride has an effect on the cariogenic potential of *S. mutans* and it is bactericidal at high concentration (Van Louveren 1990).

Because the inhibitory effect of fluoride alone on cariogenic bacteria is limited, some investigators have explored a combination of fluoride with other agents such as benzoate (Davis *et al.* 2001) or hypothiocyanite (Lenander-Lumikari *et al.* 1997) to have an additive inhibitory effect on *S. mutans*. Results are promising but not conclusive for clinical use.

2.1.6.2. Chlorhexidine

Chlorhexidine gluconate is a cationic bis-biguanide, with a broad antimicrobial spectrum that suppresses mutans streptococci (Brecx *et al.* 1993). Chlorhexidine binds to soft and hard tissues in the mouth and interferes with metabolic pathways through the bacterial cell wall. The mechanism is based on adhesion of the positively-charged chlorhexidine digluconate molecule to the negatively-charged cellular wall. The chlorhexidine molecule binds mainly to the

phosphate groups in lipopolysaccharides and carboxyl groups localized in proteins. This reaction results in an interruption of the transmembranal transport and therefore, a loss of low molecular substances in the cell (Gjermo 1978). Furthermore chlorhexidine appears to inhibit adhesion of bacteria to surfaces by displacing calcium from its binding site and deactivating the enzyme, glycosyltransferase (Gjermo 1978). A general antibacterial effect has been demonstrated on gram-positive microorganisms, particularly MS, which is terminated within weeks after cessation of a chlorhexidine regimen and a re-growth of microorganisms occurs (Emilson 1994). A report based on eight studies published between 1975 and 1994 has demonstrated that the overall caries inhibiting effect of chlorhexidine treatment is 46 per cent (Van Rijkom *et al.* 1996). Children with early colonization and a high proportion of mutans streptococci in the plaque may benefit from this antibacterial agent. Investigators have demonstrated significant reduction in MS levels in caries active pediatric patients (Gisselsson *et al.* 1994, Achong *et al.* 1999). Data have suggested that a short-term MS suppression can be achieved in toddlers with home-based toothbrushing with 1% chlorhexidine gel (Twetman and Grindefjord 1999), and also chlorhexidine varnishes can significantly decrease the levels of MS in plaque and saliva (Pienihakkinen *et al.* 1995).

Chlorhexidine rinses, varnishes and gels have several disadvantages including reduction of non-pathogenic as well as pathogenic flora, staining of the teeth, disturbances of taste, increased calculus formation, minor irritations, and superficial desquamation of the oral mucosa (Florta *et al.* 1971, Fardal and Turnbull 1986). The main disadvantages of chlorhexidine are unpleasant taste and staining (Addy *et al.* 1985). In some cases, staining of the teeth is severe, and removal requires a professional prophylaxis (Hoyos *et al.* 1977). To overcome these problems some

investigators have suggested a lower concentration of chlorhexidine, however, decreasing the concentration provided no significant reduction in side effects (Ernst *et al.* 1998). Evidently, chlorhexidine itself has an unpleasant taste, and manufacturers do not seem to be able to improve the taste of chlorhexidine products.

Chlorhexidine has also been reported to have a toxic effect, causing major changes in the oral mucosa after extreme overdose of mouth rinsing. The changes included a thickening of the mucosa, resembling leukoplakia, which disappeared when the dose was reduced (Kenrad 1990). These side effects, besides the need for frequent application of chlorhexidine (e.g. daily), have stimulated the search for alternative agents with similar benefits but fewer side effects that can be applied only a few times yearly.

2.1.6.3. Povidone-iodine (PVP-I)

In 1811 Bernard Courtois, a chemist, discovered the natural element iodine and in 1880, Devaine described its bactericidal efficacy (Fleischer and Reimer 1997). However, iodine's clinical application was limited until it was determined that binding iodine to macromolecules helped detoxify this effective microbicide. Subsequently, povidone-iodine (PVP-I) was introduced as a disinfectant agent in the late 1960s (Fleischer and Reimer 1997). PVP-I is routinely used as a local anti-infective agent for hand disinfection, pre-operative skin disinfection, treatment of burns and different skin lesions, and prevention of oral mucositis in pediatric cancer patients (Berkowitz *et al.* 1987).

2.1.6.3.1. Pharmacology

PVP-I is a compound that consists of iodine plus a solubilizing agent (i.e., polyvinyl-pyrrolidone [povidone]) (Gennaro 1990). To create PVP-I, polyvinyl

pyrrolidone, a synthetic polymer, is heated with elemental iodine in the presence of water. Hydrogen bonds are formed between the PVP complex and molecular iodine. This compound contains approximately 10% bound iodine. The 10% povidone-iodine solution generally contains 90% water, 8.5% polyvinyl-pyrrolidone and 1% available iodine and iodide (Zamora 1986). Therefore, available iodine can be calculated by dividing the PVP-I concentration by 10, so povidone-iodine 10% actually contains 1% iodine (Gennaro 1990). This product has a reddish-brown colour, and has a pH of approximately 4.5 (Siggia 1957). Combining iodine with polyvinyl-pyrrolidone increases its ability to dissolve in water and alcohol, reduces irritability, and decreases staining caused by pure iodine (Gennaro 1990).

The PVP-I complex facilitates slow release of iodine in solution. Therefore, bound and available iodine are in equilibrium in solution, which means that bound iodine is released from the complex when the available iodine is used (Fleischer and Reimer 1997). The free iodine concentration in PVP-I solution is 1 part per million (ppm) or 0.0001 percent. The preparations of polyvinyl-pyrrolidone iodine commercially available are povidone-iodine solution, surgical scrub, ointment, mouthwash gargle (mostly used for sore throat), shampoo, and skin cleanser. Of these, the 10% povidone-iodine solution is the most commonly used (Zamora 1986).

2.1.6.3.2. Mechanism of microbial destruction

Povidone-iodine is a potent microbicidal agent that acts on a wide variety of microorganisms, including Gram-positive and Gram-negative bacteria, fungi, mycobacteria, viruses and protozoans (Schreier *et al.* 1997). PVP, the hydrophilic polymer that acts as a carrier in povidone-iodine, does not have any intrinsic antibacterial activity, but because of its affinity to cell membrane, it delivers free iodine (I_2) directly to the bacterial cell surface (Vratsanos 1983). Delivery of iodine to

the sensitive elements of the cell membrane is a crucial event of antibacterial action. Iodine targets are located in the bacterial cytoplasm and cytoplasmic membrane, and its killing action takes place in a matter of seconds (Rodeheaver *et al.* 1982). In contact with PVP-I, amino (NH-), thiol (SH-), and phenolic hydroxy (OH-) groups in amino acids and nucleotides are iodinated and oxidated by free iodine. PVP-I also reacts strongly with double bonds of unsaturated fatty acids (C=C) in cell walls and organelle cell membranes. This interaction causes a transient or permanent pore formation which results in loss of cytoplasmic material and inactivation of enzymes that are essential for biologic viability. PVP-I also has been found to cause coagulation of nuclear material without rupturing cell walls (Schreier *et al.* 1997).

2.1.6.3.3. Safety

Clinically administration of PVP-I through any route may result in topical or systemic adverse effects, which vary according to the concentration of the solution utilized, the number of applications, and the route of administration (Zamora 1986). Furthermore, physical status of subjects including age, sex, body size, previous iodine intake, thyroid health, and general health is another variable in the adverse effects associated with PVP-I administration (Pennington 1990). Six types of systemic responses to excess iodine have been identified that include thyroiditis, goiter, hypothyroidism, hyperthyroidism, sensitivity reactions, and acute toxic responses (Pennington 1989).

2.1.6.3.3.1. Endocrine reaction

Potential adverse effects of iodine absorbed from iodine containing PVP preparations on thyroid function have been tested. In no case was increased iodine incorporation observed, and pathological increase of thyroid hormone was not found.

No evidence of developing hyper- or hypothyroidism as the result of the additional iodine supply was detected (Berkowitz *et al.* 1987, Hasenau *et al.* 1988, Adamietz *et al.* 1998). The effect of long-term treatment with PVP-I on thyroid function was assessed. They reported that patients treated with PVP-I for a "long period of time" (multiple application for several weeks) should be observed carefully for any manifestation of thyroid dysfunction (Nobukuni *et al.* 1997).

2.1.6.3.3.2. Acute tissue reaction and allergy

The incidence of allergy and tissue irritation after topical application of PVP-I is extremely rare. In a three years observation of PVP-I application on intact skin or mucosa in normal subjects, only two allergic reactions in 5,000 applications were recorded (Bogash 1956). However, in patients with known allergy to iodine-containing foods or compounds the incidence of allergic reactions was two in 500 applications, with 16 cases of minor irritation (Dungeman and Rakoski 1978). Sensitization to PVP-I is also rare. Among 600 patients who underwent a routine patch test, only 0.73% showed epicutaneous sensitization (Neidner 1997).

2.1.6.3.3.3. Poisoning and toxic response

The response to excess iodine is variable. Some individuals tolerate large intakes without side effects, whereas others may respond to levels close to recommended intakes. Acute responses to the ingestion of a large dose of an iodine-containing solution include cardiovascular collapse, convulsion and asthma attacks (Weigen and Thomas 1958). The 10^{th} edition of the U.S. Recommended Dietary Allowances (National Academy of Sciences 1989) indicates that levels of iodine up to 1 mg/day for children and up to 2 mg/day for adults have no adverse effects (Pennington 1990). Therefore, for the majority of a population, an iodine intake less

than 1 mg/day is probably safe, but it may cause some adverse effects in some individuals with underlying thyroid disease or who live in an area where goiter is endemic (Pennington 1990).

2.1.6.3.3.4. Discoloration and taste disturbances

Taste disturbance and staining of the soft tissue, teeth and filling materials are the potential side effects of oral antimicrobial agents examined in previous studies. A clinical trial comparing the effects of two antiseptic mouthwashes on gingival inflammation reported that several patients who had used chlorhexidine-gluconate mouthwashes previously preferred the PVP-I because it did not stain their teeth or fillings. Therefore, PVP-I appears to have the advantages of chlorhexidine, without its most troubling drawbacks (Fine 1985). Other investigators have reported that PVP-I is an excellent antiseptic agent against oral bacterial and fungal infections (Kovesi 1999). Because it is water soluble, it does not stain the oral mucosa and has no other side effects.

In general, no untoward systemic or tissue reactions have been reported after use of 10% intraoral PVP-I (Rahn *et al.* 1995, Dajani *et al.* 1997, Grossi *et al.* 1997). This antiseptic is frequently used in the treatment of many medical conditions and is considered to be safe. However, multiple daily applications over several weeks are not currently recommended (Nobukuni *et al.* 1997, Burks 1998).

2.1.6.3.4. Previous studies on betadine and dental caries

Only a few studies have investigated the anticaries efficacy of iodine compounds. An early investigation demonstrated that a single application of 0.2% I_2-KI eliminated mutans streptococci from accessible human tooth sites for up to 13 weeks post treatment (Gibbons *et al.* 1974). A subsequent trial utilizing 2.0% I_2-KI

reported similar findings, which persisted for 20 to 24 weeks after treatment (Caufield and Gibbons 1979). Recently, investigators in Puerto Rico applied 10% PVP-I at two-month intervals to a group of 12-19 months of age children identified to be at high risk to caries. None of the 15 children in the PVP-I group developed caries in 8 months, but 5 of the 16 in the placebo group showed caries in 8 months. No children reported any observable negative response to the PVP-I (Lopez *et al.* 1999). A subsequent clinical trial consisted of 83 subjects has also been conducted in Puerto Rico by the same investigators. The findings of this study expanded and corroborated their earlier observations (Lopez *et al.* 2002). The response of oral bacteria to the 10% PVP-I was not measured in any of those studies.

While topical application of povidone-iodine has been demonstrated to be effective as an antiseptic agent, there are only limited data to assess the benefits of intraoral povidone-iodine in decreasing the *S. mutans* concentration and therefore potentially preventing dental caries. At present, the anticaries effect of povidone-iodine is unclear. Additional studies are needed to investigate its caries-preventive strategy especially for children at risk to ECC.

Chapter 3

Specific aims of the study

For a group of young children who have received treatment for ECC under general anesthesia, the aims of this study were:

1) To measure the change in oral *S. mutans* levels after regular 10% PVP-I application to the teeth.

2) To measure the incidence of new carious lesions after regular 10% PVP-I application to the teeth.

3) To explore the relationship between specific demographic factors, feeding and parenting practices, and *S. mutans* levels.

Chapter 4

Materials & Methods

4.1. Overview of study design

The study was designed as a randomized single blind, no treatment control trial with two outcome measures. The main outcome measure was the number of *S. mutans* before and after intervention, and the other measure was the number of new carious lesions at the same end-point. Children in the study were recruited from Monarch Pediatric Dental Center, a pediatric dental practice in Burnaby, BC. The clinic has an on-sight general anesthetic suite. At least 15-20 patients are scheduled each week from Thursday to Saturday for dental treatment under general anesthesia (GA).

Criteria for a child to be included in the study were:

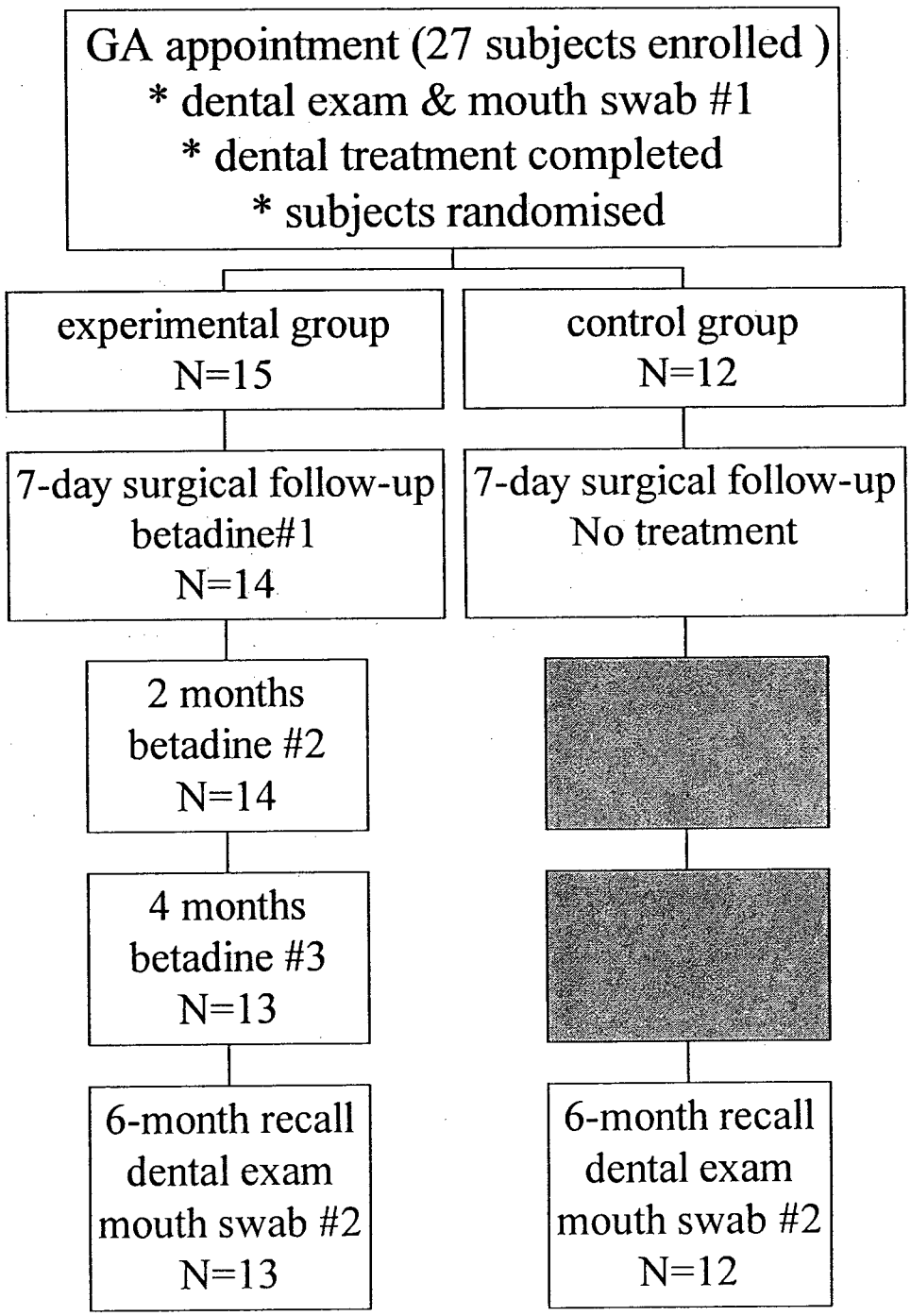
1) All treatment to be done under GA,
2) Unremarkable medical history, particularly no history of thyroid disease,
3) At least 15 teeth remaining after completion of treatment, and
4) Ability to return at two-month intervals.

Parents of children who fulfilled the criteria and were scheduled for GA as a consequence of ECC were approached to participate in the study. Using a random number table, subjects were assigned to experimental or control group (Figure 1).

The active agent employed was 10% povidone-iodine solution (betadine) applied at two-month intervals to the children in the experimental group. Parents of all participants received an honorarium at each visit. Telephone reminders and mail follow-up encouraged ongoing participation. At completion of study, parents were

interviewed briefly to determine if parents or children had any problems or after-effects related to betadine application.

Figure 1: *Overview of study design*



4.2. Clinical protocol

This study was a collaboration between the Department of Oral Health Sciences of UBC and the Departments of Dental Public Health Sciences and Microbiology of University of Washington, Seattle. All laboratory procedures were done at the University of Washington under the supervision of a microbiologist. This study was supported by the Comprehensive Center for Oral Health Research at the University of Washington, which is funded by the National Institute of Dental and Craniofacial Research, Washington, DC.

4.2.1. Ethics approval and consent

Ethics approval for the study was received from the Clinical Research Ethics Board of UBC (Appendix I). Parents of children on the GA waiting list, who fulfilled the inclusion criteria, were telephoned by Monarch staff to inform them of the study. An information sheet was also mailed to interested parents (Appendix II).

At the time of the GA appointment, the consent form was reviewed with interested parents and any questions about the study answered. Because of budget limitations, consent (Appendix III) and survey instruments (Appendix IV) were only available in English. For parents who did not understand English, multilingual staff members from Monarch were available to interpret and answer parents' questions.

4.2.2. Demographics, behavioural, and RAPIDD scale assessments

All survey instruments were modifications from earlier University of Washington studies, and had been tested previously for reliability and validity. Demographic and developmental data were obtained by an instrument that assessed child and caregiver characteristics. Data was also collected on feeding practices and

oral hygiene. Dietary information included the Even's snacking questionnaire to determine the frequency of intake of certain foods (Evens 1997) (Appendix IV).

In other study populations, parental readiness to alter parenting practices has previously been assessed by the RAPIDD (Readiness Assessment of Parents concerning Infant Dental Decay) Instrument (Weinstein and Riedy 2001). This instrument has been proposed as a tool to help determine parents' readiness to change behaviours affecting their children's oral health and was used in this study (Appendix IV).

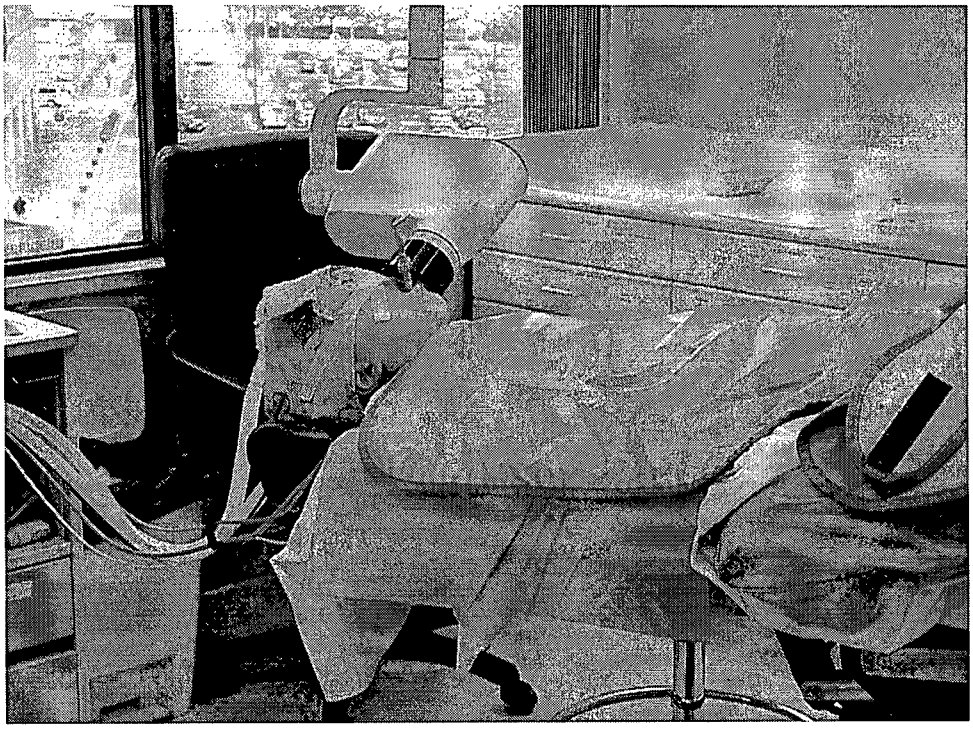
Parents completed the questionnaires with assistance, as needed, from Monarch staff prior to beginning of the surgery. All children received the standard dietary counselling and oral hygiene instructions that were part of the preventive program given to Monarch patients with extensive caries.

4.2.3. Dental examination and sampling:

After a child was enrolled in the study, an ID number was drawn at random from the random number table. Even ID numbers were assigned to the experimental group and odd numbers to the control group.

At the time of GA appointment, before intubation, height and weight of all participants were recorded. These measurements were repeated at all subsequent study appointments. Dental examination data and the plaque sample were collected following intubation. The dental examination was performed by the pediatric dentist treating the child with an explorer, mirror, air and dental operating light. Radiographs (2 bitewings and 6 periapicals) were exposed on all children. The project investigator (MA) was present for all treatment and recall appointments to ensure consistency among the examiners.

***Figure 2**: The operating room of Monarch Pediatric Dental Center*



Based on clinical and radiographic findings, teeth surfaces were scored as:

0=sound

1=decay (presence of caries in dentine)

2=restoration

3=decalcification "white spot"

4=missing (because of caries)

5=missing (because of natural exfoliation) (Appendix V).

A pooled plaque sample was obtained by swabbing the gingival third of buccal surfaces of all the teeth with a sterile cotton swab (BBL CultureSwab™ System, Becton & Dickinson, Sparks, MD). The swab was placed in the vial containing 2ml of reduced transport fluid with glass beads, which is part of the CultureSwab system. Routine restorative and surgical treatment, including application of fluoride varnish, were then completed.

4.2.4. Betadine application technique:

At the time of 7-day surgical follow-up visit, 10% PVP-I (Betadine solution, Purdue Frederick Inc.) was applied to the teeth of children in the experimental group by an available Monarch dentist. All dentists had been calibrated and trained in a standardised application technique. The project investigator (MA) was present at all visits.

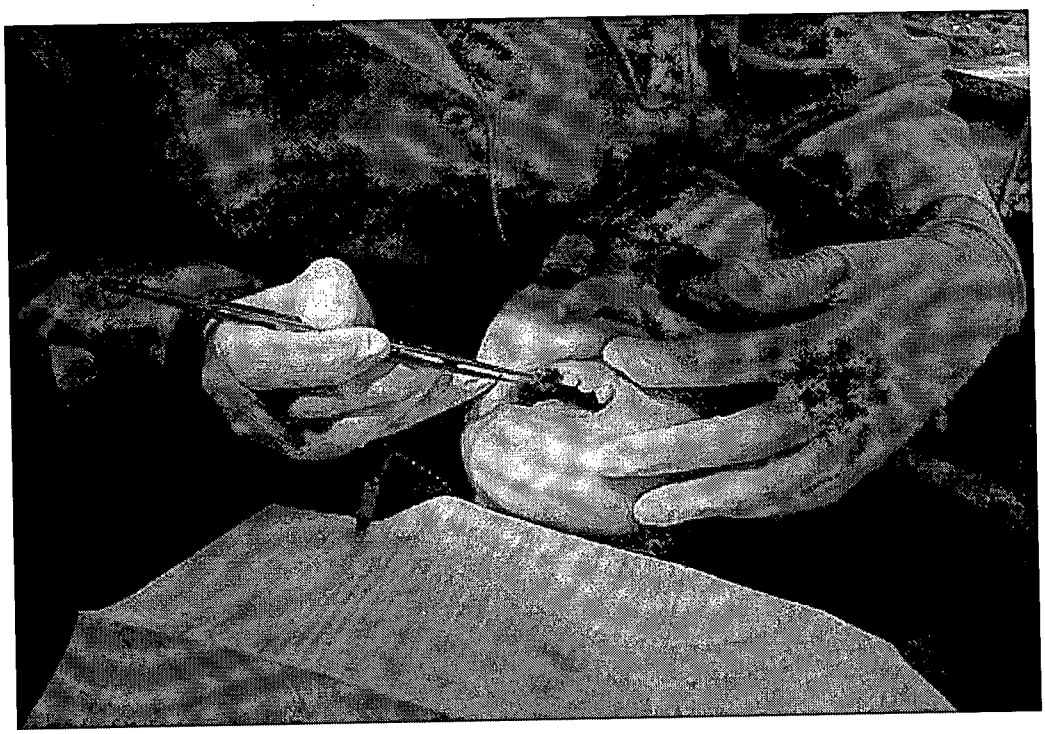


Figure 3: *Betadine application for experimental group*

Application was repeated at 2 and 4 months. First, the teeth were cleaned with a cotton roll and then the agent was applied by swabbing the dentition with a small sterile cotton ball that was saturated with the betadine solution and held in locking cotton pliers. The teeth were wiped off immediately with gauze after betadine application. Estimated time of application was 2-4 minutes, depending on the number of teeth present and the child's behaviour. Study dose for each application was approximately 0.20ml or 20mg of 10% PVP-I solution. After 6 months, both

experimental and control received another dental examination and a mouth swab was taken from all subjects along with regular preventive maintenance (Figure 1).

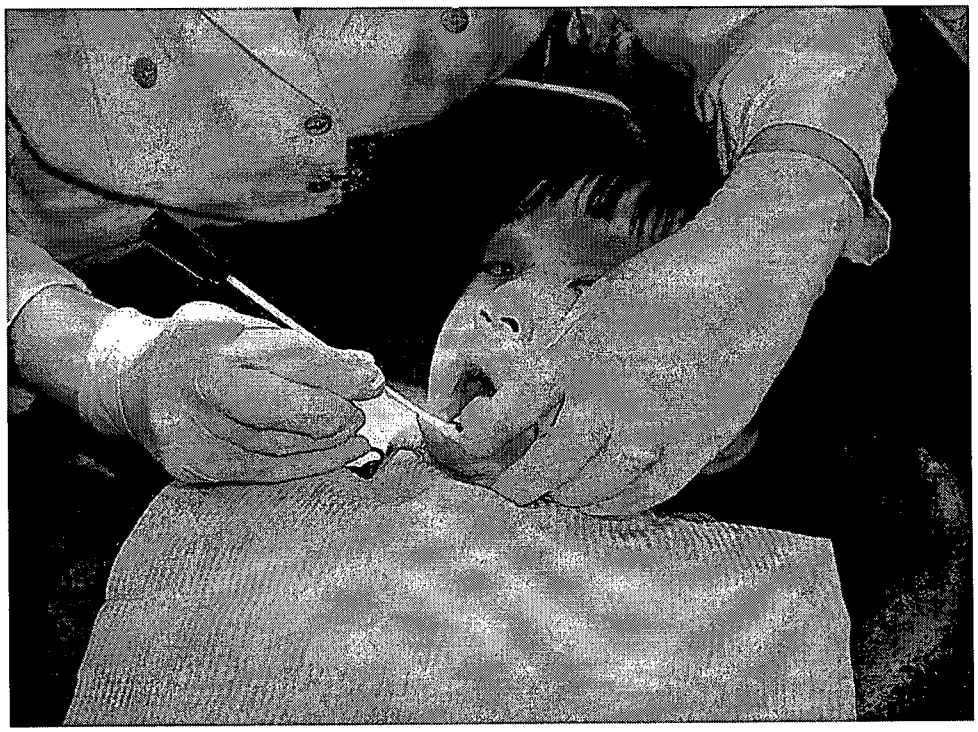


Figure 4: *Taking a plaque sample at 6 months*

4.3. Microbiology

All microbiological procedures were processed at the University of Washington in Seattle within 24 hours of collection. Culturing and analysis of the microbiological specimens were supervised by a microbiologist, Dr. Marilyn Roberts, Professor, University of Washington.

4.3.1. Sample plating and culture media

The swabs were suspended in 1ml of PRAS (0.038 M NaCl, 1.073 mM KCl, 2.05 mM resazurin, 23 mM L-cysteine), and then a dilution series from 10^{-1} to 10^{-5} was made in PRAS. For total aerobic counts, 10^{-3} to 10^{-5} dilutions of bacteria were

plated on blood agar (Columbia Base, Difco, Becton & Dickinson, Sparks, MD). 10^{-1} to 10^{-3} dilutions of bacteria were also plated on MSKB media (Columbia Base, Difco, Becton & Dickinson, Sparks, MD) to isolate *S. mutans*. Mitis Salivarius Kanamycin Bacitracin (MSKB) is a selective medium for *S. mutans*. This specific medium is composed of mitis salivarius agar base, 1% potassium tellurite, 20% sorbitol, 2 ug/ml kanamycin sulfate and 0.2 units/ml bacitracin (Kimmel and Tinanoff 1991). All plates were incubated at 36.5°C for 72 h and colonies were counted.

4.4. Data analysis

To ensure balanced arms of the trial, the randomization process was assessed by comparing baseline variables such as demographics, feeding information, and dental and bacterial measurements between the experimental and control groups. To determine any significant differences between the experimental and control groups, numerical data were compared by t-Test (two-sample assuming unequal variances). Nominal data were compared by a Fisher's exact test because some groups had less than 4 subjects. Significant differences were defined as $P < 0.05$.

To determine the effect of betadine, comparisons were made over time between the experimental and control groups for changes in *S. mutans* levels and total bacterial counts. Colony Forming Units (CFUs) per unit volume of *S. mutans* counts were transformed to $\log_{10}$ values to control variance for the purpose of applying parametric statistical tests to the data (Caufield and Gibbons 1979). Differences in $\log_{10}$ counts of *S. mutans* at baseline and 6 months were compared for the experimental and control groups using a t-Test and a reduction in the number of *S. mutans* of 1 $\log_{10}$ was considered to be a significant decrease in *S. mutans* levels. A

Fisher's exact test compared the number of children with new carious lesions at 6 months in the experimental and control groups.

To explore the possibility of other variables acting as confounding factors on the 6 month results, final dmfs, number of missing teeth (extracted or naturally exfoliated) and number of crowns placed (stainless steel or composite) were compared between the experimental and control groups. Finally, since such a large number of subjects from both the experimental and the control group demonstrated a 1 $\log_{10}$ or more decrease in *S. mutans* levels over time, we hypothesized that a factor other than the betadine intervention may have been responsible for this "change". Therefore, all the experimental and control subjects were pooled together and then separated into children with 1 $\log_{10}$ or more reduction in *S. mutans*, "change group", and those whose levels did not change by this amount or increased, "no change group". Comparisons for a variety of variables were made between the "change" and "no change" groups to determine any significant differences.

Chapter 5

Results

5.1. Study population

A total of 27 subjects were enrolled in the study. For the experimental group, one child withdrew before application of betadine and another one withdrew after two applications because of family move to another province. No control subject withdrew. Therefore, the final study number was 25 subjects: 13 in experimental and 12 in control group (Table 1). Children ranged in age from 2 to 6.8 years at the time of entry into the study. Characteristics of children, feeding habits and caries status at baseline are shown in Tables 1, 2, 3. Based on height and weight measurements recorded throughout the study, no significant changes were found in the normal physical growth and development of the children in experimental group.

5.2. Comparisons between experimental and control children at baseline

When the baseline measurements of demographics were compared (Table 1), no significant differences were found between experimental and control group, except for a significant difference in the gender distribution between the groups.

Table 1: Demographic characteristics of children in the study:

Demographic Variable	All children	Experimental	Control	P- value
Gender				
Female	13	4	9	0.03*
Male	12	9	3	
Child's Age (years)				
Mean (SD)	4.3 (1.1)	4.2(1.2)	4.3(0.6)	0.74**
Ethnicity				
Chinese	15	7	8	0.40*
Non-Chinese	10	6	4	
Mother's age (years)				
Mean (SD)	33.5(4.7)	33.7(5.1)	33.1(4.3)	0.75**
Marital status of mother				
Married	19	10	9	0.64*
Single	6	3	3	
Mother's education				
< High school	8	3	5	0.29*
≥ High school	17	10	7	
Length of time mother in North America				
< 6 years	8	4	4	0.61*
> 6 years	17	9	8	

* P-value, determined by Fisher's Exact Test, measure differences between experimental and control group.

** P-value determined by t-Test.

When the baseline measurements of child's feeding habits were compared (Table 2) no statistically significant differences were found between the experimental and control groups. However, twice as many children in the experimental group (N=10) as control group (N=5) had a history of sleep-time or nap-time bottle. The difference approached significance (P =0.08).

Table 2: *Bottle-feeding information:*

Bottle-feeding habit	**All children**	**Experimental**	**Control**	**P- value**
Still on bottle	7	5	2	0.22
History of sleeping with bottle	15	10	5	0.08
History of walking around with bottle	16	7	9	0.27

All children demonstrated a noticeable amount of dental decay, however, caries status and dmfs were similar between the groups. No statistically significant difference was detected between the baseline levels of *S. mutans* and total cultivable bacteria for experimental and control groups (Table 3).

Table 3*: Caries status and bacterial levels at baseline, mean (SD)*

Variable	**All children**	**Experimental**	**Control**	**P- value**
Decayed surfaces	25.1(10)	24.3(12.4)	26.8(8.1)	0.55
dmfs	27.9(11.4)	28.2(14.3)	27.7(7.8)	0.92
Sm $\log_{10}$	3.9(1.5)	4.2(1.4)	3.7(1.5)	0.38
BA $\log_{10}$	7.8(0.6)	7.8(0.6)	7.8(0.7)	0.79

Overall, the results demonstrated that except for gender distribution, the randomization was effective and experimental and control groups were similar.

5.3. Comparisons between experimental and control children after 6 months

When data from the final 6-month dental examination was compared between the experimental and control group, no significant differences were found for variables including dmfs, missing teeth because of extraction or natural exfoliation, and number of crowns placed (stainless steel and composite crowns) (Table 4).

Table 4: *Dental status at 6 months, mean (SD)*

Variable	All children	Experimental	Control	P- value
dmfs	44(14)	41.3(14.6)	47.3(12.6)	0.29
Number of missing (extractions + natural exfoliations) teeth	3.3(1.5)	3.2(1.3)	3.4(1.8)	0.82
Number of crowns (ssc + composite crown)	6.4(3)	5.8(2.2)	7(3.6)	0.36

Three subjects in the control group and one subject in the experimental group demonstrated caries at 6 months. Decalcified and carious surfaces for these 4 subjects are shown in Table 5.

Table 5: *Children with decay (caries or decalcification) at 6 months*

	Caries	**Decalcified**	***S. mutans***	**Comments**
Control				
Subject # 8	55 OL 65 OL	55 B, 63 B, 65 B, 75 BL, 74 B, 73 B, 83 B, 84 B, 85 B,	3.50×10^5	55 and 65 unerupted at baseline
Subject #17		63 B	*ND	
Subject #19		55 B, 63 B	*ND	
Experimental				
Subject #18	65 O		4.00×10^4	

*ND= Non Detectable

Numbers of total bacteria and *S. mutans* at visit 1 (baseline) and visit 2 (6 months) are shown for the experimental group in Table 6 and for the control group in Table 7. Individual changes in *S. mutans* expressed as $\log_{10}$ over time for each subject in both groups are visually shown in Figures 5 and 6. Comparisons of total and *S. mutans* counts over time for all children, the experimental and control groups are shown in Table 8.

Table 6:, Numbers of total cultivable aerobic bacteria and S. mutans at baseline and 6 months for the experimental group

		Total counts on blood agar (**BA)			Counts of *S. mutans* on MSKB		
ID#	***VISIT**	**BA**	**$\log_{10}$**	**log difference (BA2 - BA1)**	**sm**	**$\log_{10}$**	**log difference (sm2-sm1)**
3	1	5.60×10^7	7.74	-1.93	5.50×10^3	3.74	-3.47
	2	6.60×10^5	5.82		***ND	0.00	
4	1	1.40×10^8	8.15	-0.95	1.60×10^7	7.20	-1.09
	2	1.60×10^7	7.20		1.30×10^6	6.11	
6	1	1.10×10^7	7.04	-0.34	1.90×10^2	2.28	+0.94
	2	5.00×10^6	6.70		1.65×10^3	3.22	
7	1	8.10×10^7	7.91	-0.01	4.50×10^4	4.65	-4.65
	2	8.00×10^7	7.90		ND	0.00	
9	1	9.00×10^8	8.95	-2.41	4.20×10^5	5.62	-2.23
	2	3.45×10^6	6.54		2.45×10^3	3.39	
13	1	6.50×10^6	6.81	+0.14	2.40×10^4	4.38	-1.18
	2	9.00×10^6	6.95		1.60×10^3	3.20	
14	1	1.20×10^8	8.11	-0.90	1.70×10^4	4.23	-0.23
	2	1.60×10^7	7.20		1.00×10^4	4.00	
16	1	1.40×10^8	8.15	-2.14	3.80×10^4	4.58	-2.28
	2	1.00×10^6	6.00		2.00×10^2	2.30	
18	1	1.10×10^8	8.04	-0.86	2.80×10^5	5.45	-0.85
	2	1.50×10^7	7.18		4.00×10^4	4.60	
20	1	2.80×10^7	7.45	-2.37	5.00×10^3	3.70	-3.70
	2	1.20×10^5	5.08		ND	0.00	
24	1	2.90×10^7	7.46	-0.31	1.60×10^2	2.20	-2.20
	2	1.40×10^7	7.15		ND	0.00	
26	1	2.80×10^7	7.45	-0.44	1.60×10^4	4.20	-4.20
	2	1.10×10^7	7.04		ND	0.00	
27	1	6.60×10^7	8.82	-1.33	5.00×10^2	2.70	-2.70
	2	3.00×10^7	7.48		ND	0.00	

Table 7: *Numbers of total cultivable aerobic bacteria and S. mutans at baseline and 6 months for the control group.*

		Total counts on blood agar (**BA)			Counts of *S. mutans* on MSKB		
ID#	***VISIT**	**BA**	**$\log_{10}$**	**log difference (BA2 - BA1)**	**sm**	**$\log_{10}$**	**log difference (sm2-sm1)**
2	1	1.95×10^{7}	7.29	-0.25	2.00×10^{1}	1.30	+1.10
	2	1.10×10^{7}	7.04		2.50×10^{2}	2.40	
5	1	4.00×10^{6}	6.60	+0.47	3.50×10^{2}	2.54	-0.28
	2	1.20×10^{7}	7.08		1.80×10^{2}	2.26	
8	1	1.76×10^{8}	8.25	-0.38	2.80×10^{6}	6.45	-0.91
	2	7.40×10^{7}	7.87		3.50×10^{5}	5.54	
11	1	1.17×10^{7}	7.07	-0.07	2.60×10^{5}	5.41	-0.23
	2	1.00×10^{7}	7.00		1.50×10^{5}	5.18	
12	1	5.60×10^{7}	7.75	-1.05	1.40×10^{3}	3.15	-1.85
	2	5.00×10^{6}	6.70		2.00×10^{1}	1.30	
17	1	2.20×10^{8}	8.34	-1.49	4.00×10^{1}	1.60	-1.60
	2	7.00×10^{6}	6.85		***ND	0.00	
19	1	6.40×10^{8}	8.81	-1.69	2.50×10^{3}	3.40	-3.40
	2	1.30×10^{7}	7.11		ND	0.00	
21	1	2.40×10^{7}	7.38	-0.59	2.80×10^{4}	4.45	-4.45
	2	6.30×10^{6}	6.80		ND	0.00	
22	1	3.80×10^{8}	8.58	-1.25	1.00×10^{4}	4.00	-4.00
	2	2.10×10^{7}	7.32		ND	0.00	
23	1	6.20×10^{7}	7.79	+0.61	7.00×10^{3}	3.85	-0.24
	2	2.60×10^{8}	8.41		4.00×10^{3}	3.60	
25	1	5.30×10^{7}	7.72	-0.31	1.80×10^{5}	5.26	-2.65
	2	2.60×10^{7}	7.41		4.00×10^{2}	2.60	
28	1	4.50×10^{7}	7.65	-0.89	5.10×10^{2}	2.71	-2.71
	2	5.80×10^{6}	6.76		ND	0.00	

* Visit 1 = Baseline, Visit 2 = 6-month
** BA = Blood Agar (total count)
*** ND = Non Detectable

Figure 5: *The individual log_{10} S. mutans of baseline and 6 months for experimental group*

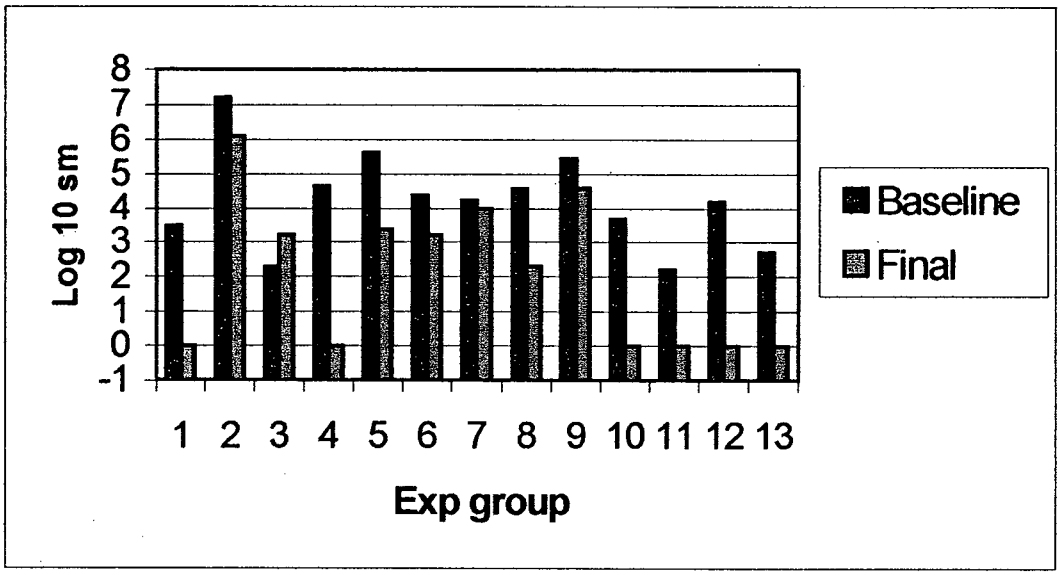


Figure 6: *The individual log_{10} S. mutans of baseline and 6 months for control group*

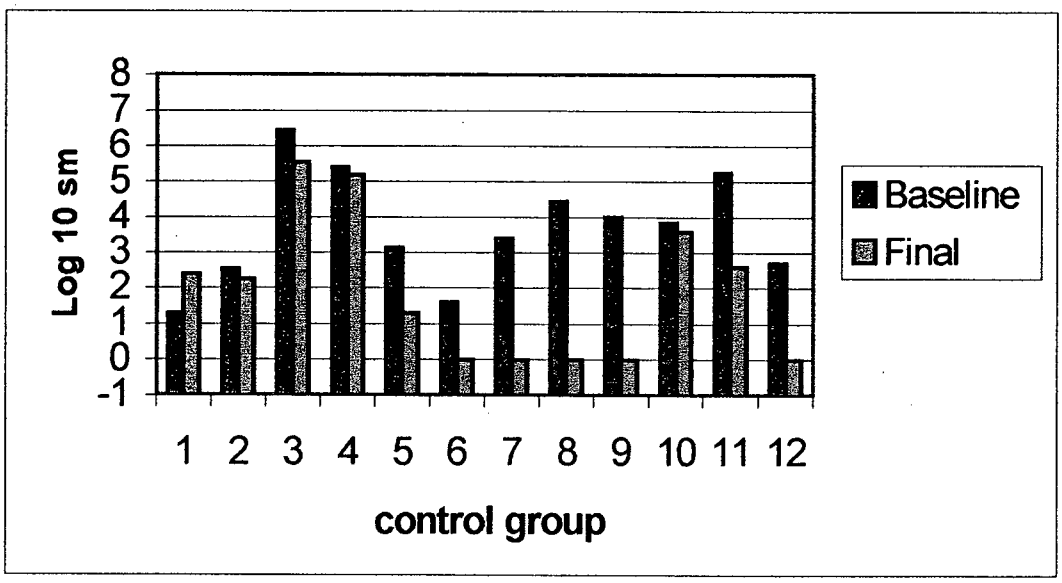


Table 8: *Comparisons of the mean log_{10} total counts and S. mutans at visit 1 (baseline) and visit 2 (6 months) for all subjects, experimental and control groups.*

	Total count	***S. mutans***
All subjects		
Visit 1	7.8(0.6)	3.95(1.5)
Visit 2	7(0.7)	1.99(2.1)
Difference	-0.8(0.8)	-2(2.1)
*p-value	0.00004	0.0004
Experimental group		
Visit 1	7.8(0.6)	4.2(1.4)
Visit 2	6.8(0.7)	2.1(2.2)
Difference	-1.1(0.9)	-2.1(1.6)
*p-value	0.001	0.0005
Control group		
Visit 1	7.8(0.7)	3.7(1.5)
Visit 2	7.2(0.5)	1.9(2)
Difference	-0.6(0.7)	-1.8(1.7)
*p-value	0.02	0.004

*P-value determined by t-Test measures the difference between visit 1 (baseline) and visit 2 (6 months).

When the total counts and *S. mutans* at 6 months were compared with baseline, the reductions of total counts and *S. mutans* for the experimental group were more than the control group. However, the reductions in counts for the experimental group were not significantly greater than that for the control group (Figure 7 and 8).

Figure 7: *Comparison of reduction in total counts (BA) over time between experimental and control groups.*

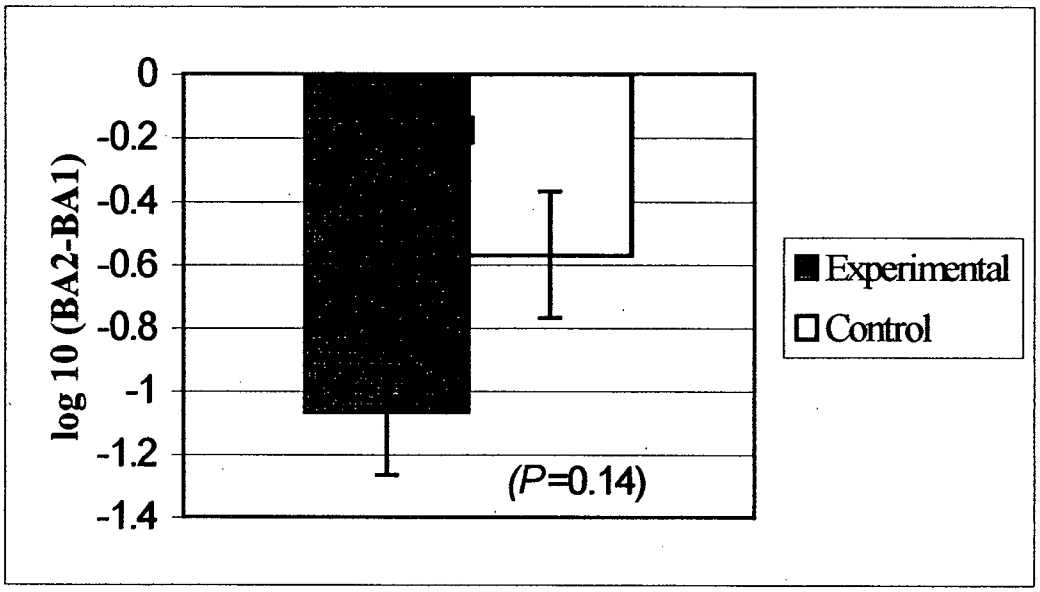
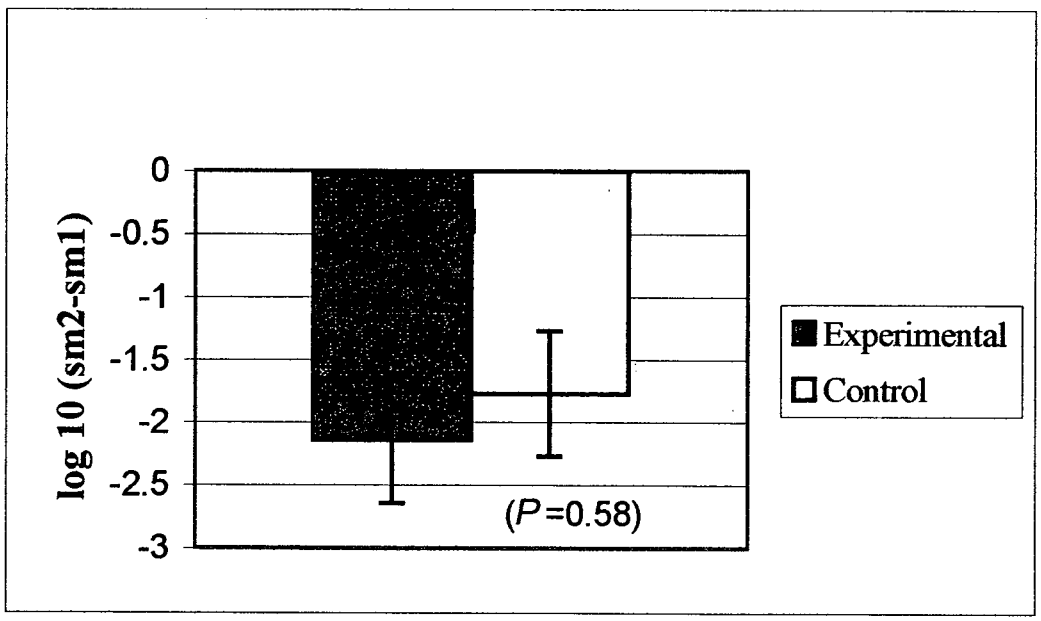


Figure 8: *Comparison of reduction in S. mutans counts over time between experimental and control groups.*



The following table displays caries status at visit 2 for all 4 subjects with follow-up longer than 6 months (Table 9).

Table 9: *Caries status and bacteria level for children with follow-up > 6months*

ID#	Group	visit 2 time	Decayed surfaces	$\log_{10}$ sm (baseline)	$\log_{10}$ sm (final)
7	experimental	9 mos	0	4.65	0
8	control	9 mos	10	6.45	5.54
23	control	8 mos	0	3.85	3.60
25	control	8 mos	0	5.26	2.60

5.4. Comparisons between "change" group and "no change" group

When the baseline demographics, feeding information, number of decayed surfaces and dmfs were compared between the "change" (children with 1 $\log_{10}$ or more reduction in *S. mutans*) and "no change" (children with less than 1 $\log_{10}$ reduction) groups, they were significantly different for the time that mother had been in North America (P=0.03). Furthermore, the 60% of the study children that were Chinese were unequally distributed between the "change" and "no change" groups. The difference approached significance (P=0.07). No significant differences were found between two groups for any other variables (Table 10).

Table 10: Comparison between "change" group and "no change" groups

Variable	"Change group" (sm diff >1 $\log_{10}$)	"No change" group (sm diff <1 $\log_{10}$)	P- value
Group			
Experimental	10	3	0.21
Control	7	5	
Gender			
Male	10	2	0.13
Female	7	6	
Bottle feeding			
Still on bottle	5	3	0.81
No bottle	12	5	
Age(years)			
Mean(SD)	4.4(1.2)	3.9(0.6)	0.14
Ethnicity			
Chinese	8	7	0.07
Non-Chinese	9	1	
Length of time mother in North America			
Mean(SD)	18.5(13.5)	9.1(7.5)	0.03
Number of decayed surfaces			
Mean(SD)	23.8(9)	27.9(12.7)	0.44
dmfs			
Mean(SD)	26.8(11.2)	30.4(12.2)	0.49
Number of crowns			
Mean (SD)	6.2(2.3)	6.9(4.3)	0.67
Number of missing teeth			
Mean(SD)	3.2(1.6)	3.6(1.5)	0.63

Chapter 6

Discussion

6.1. Summary of the results

Baseline comparisons demonstrated that, except for gender distribution, the experimental and control groups were similar based on demographics, feeding habits, caries status and bacterial levels (Table 1, 2, 3). Results demonstrated an overall reduction in total counts and *S. mutans* for all children in the experimental and control groups at 6 months following GA dental treatment (Table 8). The reductions of total counts and *S. mutans* for the experimental group were more than the control group However, the differences between two groups were not statistically significant (Figure 7, 8). Three children in the control group and one child in the experimental group demonstrated decalcified and new carious surfaces at 6 months (Table 5).

When children with 1 $\log_{10}$ or more reduction in *S. mutans* ("change" group) were compared with children with less than 1 $\log_{10}$ reduction ("no change" group), they differed significantly for the time that mother had been in North America. The difference in distribution of children that were Chinese in "change" and "no change" group also approached significance. Other variables were not significantly different between the two groups (Table 10).

6.2. Limitations of the study

A project conducted in a private practice provides a unique opportunity for a clinical trial because, for example, a dental practice provides access to a large number of qualified subjects to recruit for a study. However, a busy dental practice introduces difficulties that may not exist in more controlled clinical settings. For instance, in a

more controlled setting it may be possible to allocate a specific time of day to schedule study participants but in a private clinic any interference in routine may prove inconvenient and will not be practical.

6.2.1. Sample size

Although the present study was a "pilot study" to test the effectiveness of betadine on decreasing levels of *S. mutans*, a larger sample size would have provided more confidence in our findings. Transporting the plaque samples to the University of Washington, Seattle, was a key factor that limited our sample size. The reason that the samples were transported to Seattle was to allow DNA probe analysis of specimens to specifically detect and identify the *S. mutans* and to determine if betadine had qualitatively affected the *S. mutans*. However, transporting the samples to Seattle introduced several complications such as:

1) Harvesting of samples was possible on only one of the three GA days, because samples needed to be processed on a weekday.
2) Curtailment of sampling by 2:00pm because of courier schedule.
3) Added expenditure of time and money, arranging and paying couriers and ensuring safe passage across the U.S. border.

Unfortunately, after all samples were finally collected and quantified, budget restrictions at the University of Washington meant that the DNA probe analysis was never done.

The logistics of the dental office itself presented difficulties in increasing our sample size ranging from delay of study start because of the office renovations, extended closure time of the GA service for personal issues of the professional staff and simple patient "no-shows".

6.2.2. Equivalence between experimental and control groups:

Any sources of selection bias were eliminated by randomly dividing the subjects into experimental and control groups. The groups were well matched for almost all initial demographics, dental and microbiology data except for a significant difference in the gender distribution between the groups (P=0.03). However, when comparisons were made between the "change" and "no change" group from our study, gender was not significantly associated with reduction of *S. mutans* levels (Table 10). In addition, the relationship between gender and detection of *S. mutans* or dental caries has been explored in infants and preschool children by other investigators and no significant association has been reported (Al-Hosani and Rugg-Gunn 1998, Habibian *et al.* 2002).

6.2.3. Blindness of the study

The study was single blind rather than double blind. The subjects were aware to which group they belonged. However, the examiners did not know the group of each child in the final examination. The use of a coding system for the plaque samples ensured that the source of the samples was unknown when the laboratory assays were performed. Including a placebo treatment for the control group would have been required to make the study double blind. However, because the major focus of the study was on children in the experimental group to allow exploration of the possible effects of betadine, making all participants in both the experimental and control groups return every two months would likely have increased the number of dropouts. As it was, some of the subjects had their final 6 month recall at a time longer than 6 months (Table 9) and two of the subjects in the control group who were unable to come back to the office had their 6 month dental examination performed in their home. Therefore, the study design was compromised to "no-treatment control" in

stead of "placebo control" and the control group returned only once at the 6 month surgical follow up visit.

6.2.2. Recall bias

Children in the experimental group were seen in the dental office every two months for betadine application and their parents received an honorarium at each visit. The children in the control group were examined only at the 6 month follow up. Frequent dental visits and extra honorarium for the experimental group might have encouraged the children and their parents to follow the Monarch oral hygiene and dietary instructions better than the control group. This source of bias would have been eliminated if the study had included a placebo treatment for the control group.

6.2.3. Difficulties in communication with non-English speaking parents

About 80% (20 out of 25) of the study group did not have English as their first language, and 28% (7 out of 25) had only a limited understanding of English. Because the questionnaires and consents had not been translated into other languages, multilingual staff members from Monarch helped with interpretation. This approach was adequate for the demographics, child feeding and dental health data but the Evens and RAPIDD instruments were quite complicated and subjective. Therefore, due to lack of a reasonable confidence about the reliability of the responses to these two instruments plus the small number of subjects, the results from these instruments were not analyzed.

6.2.4. Inter-examiner reliability

To improve reliability, all participating Monarch staffs were calibrated to a standardized technique for the dental exam, the mouth swabs and the application of betadine. However, to fit in conveniently with office routines, procedures were

performed by a variety of calibrated staff members. Unlike laboratory measurements, no absolute standards exist to determine examiner agreement and consistency. To ensure a consistent technique, the project manager (MA) observed all procedures as they were done.

6.2.5. Longer follow-up for a few subjects

Although the second visit to collect final data had been scheduled at 6 months after the first visit for all children, 4 subjects (1 in experimental group and 3 in control group) had longer follow up than 6 months because of parent's inability to attend at the scheduled appointment (Table 9). Besides the complications that the delay caused for the project, it was interesting to detect no *S. mutans* for the "delayed" child in the experimental group, while all three "delayed" children in the control group had *S. mutans* in their final mouth swab.

In a study involving children of similar age, extensive dental treatment under GA has been reported to reduce the caries associated microbial populations at 1 month and 6 month post-treatment; however, a slight re-colonization was reported at the later follow up (Twetman *et al.* 1999). Therefore, any anti-bacterial effect of betadine on *S. mutans* may have been masked by the influence of the extensive "one-time" dental treatment under GA. The effect of betadine may be more apparent at a longer follow up when the cariogenic bacteria start to re-colonize. This effect may be more pronounced after 6 months.

6.3. Rationale for recruiting children scheduled for general anesthesia

The extensive restorative treatment often required for children with ECC is one of the major determining factors in using general anesthesia (Berkowitz *et al.* 1997). Children with more extensive carious lesions also have significantly higher

levels of *S. mutans* (Okada et al. 2002). Several investigators have reported the susceptibility of children with ECC to future caries development following comprehensive treatment under general anesthesia. (Almedia *et al.* 2000, Peretz *et al.* 2000). Therefore, more aggressive antimicrobial therapies may be required for this group of children to prevent the future development of carious lesions. All these factors together suggested that children needing extensive treatment under GA would be ideal candidates for this pilot study. In addition, having a scheduled GA appointment will ensure that all restorative and surgical treatments will be completed in a timely manner before the intervention and no subject will have untreated caries at baseline.

6.4. Methods of collecting *S. mutans*

To determine the *S. mutans* level of a child, saliva or plaque samples reflecting the number of colonized tooth surfaces are routinely used. Methods for collection of both plaque and saliva samples vary among studies. Saliva samples have been obtained before or after stimulating the salivary flow, and collected by spitting, swabbing, as a smear on a wooden spatula or a tongue loop or with a plastic strip (Kohler and Bratthall 1979, Beighton 1986, Jensen and Bratthall 1989). Pooled plaque samples can be obtained from individual teeth or sites on the teeth. However, a higher predictive value has been found for pooled plaque samples, obtained from individual teeth or sites on the teeth, rather than saliva samples (Sanchez-Perez 2001). Because of the higher odds ratio between caries and *S. mutans* in plaque samples compared to that in saliva, it has been suggested that plaque sampling may be the most accurate method to use, and also eliminates the inconvenience associated with stimulation of the salivary flow (Dasanayake 1995, Sanchez-Perez 2001). In addition, plaque

sampling is more practical and convenient than saliva sampling for young children who may have a dry mouth because of "fasting prior to GA".

6.5. Effect of betadine on outcome measures

The present study had two outcome measures. The main outcome measure was the difference in the number of *S. mutans* between experimental and control groups at 6 months, and the second measure was the incidence of new carious lesions for both groups at the same end-point. Although two earlier studies have indicated a significant reduction in plaque or saliva levels of *S. mutans* after a topical application of an iodine solution (Gibbons *et al.* 1974, Caufield and Gibbons 1979), support for this in the literature is relatively sparse. In addition, no oral microbiological analysis related to application of an iodine solution has been conducted for preschool children.

Similar to previous studies, the current study has demonstrated a greater reduction of *S. mutans* levels for the experimental group compared with the control group, although the difference in our study was not statistically significant (P=0.58). In addition, the proportion of children in the experimental group who had more than 1 $\log_{10}$ reduction in the number of *S. mutans* at 6 months (10 out of 13) was higher than that in the control group (7 out of 12) (Table 10). This finding also suggests that betadine may decrease the levels of *S. mutans*.

Regarding the second outcome measure, the incidence of new carious lesions for the experimental and control groups at 6 months, the "f" portion of the dmfs for all children in both experimental and control groups was actually increased at 6 months. This increase was because of the stainless steel or composite crowns used for a large number of the teeth. However, in agreement with previous studies (Lopez *et al.* 1999, Lopez *et al.* 2002), the number of subjects who experienced new caries or decalcified

surfaces in the control group (3 out of 12) was higher than that in the experimental group (1 out of 13). The difference was not significant (P=0.27).

Some of the reasons why betadine did not demonstrate as significant an effect on *S. mutans* and caries as we had hoped were:

Small sample size: The small sample may mean that no difference was detected even when a real difference may have existed. Therefore, caution should be employed when reporting a "no treatment effect". Our findings show similar trends as previous studies, even though the differences we observed were not significant. We simply did not have enough subjects to draw a definitive conclusion.

Low initial level of *S. mutans*: Levels of *S. mutans* at baseline for about 50% (12 out of 25) of children, 5 out of 13 for experimental and 7 out of 12 for control group, were surprisingly low (less than 10^4) (Table 6, 7). However, the dmfs of these children was quite high (21.3±7), despite their low levels of *S. mutans*. Therefore, in spite of the caries experience of our sample, levels of *S. mutans* were not as high as expected. The effect of betadine may have been more pronounced if the baseline levels of the *S. mutans* were higher.

Effect of betadine masked by dental treatment under GA: Extensive dental treatment under general anesthesia including extraction of unrestorable or abscessed teeth and restoration of caries and retentive sites has demonstrated a significant and immediate reduction in the levels of microorganisms associated with caries. This effect lasted about 6 months (Nickman and Conry 1998, Twetman *et al.* 1999). In addition, during the time immediately after surgery, families may be more receptive to preventive "messages", because the child has been relieved of pain and discomfort. Therefore, the effect of an additional chemotherapeutic intervention for

our group of children may have been undetectable. The combined effect of a significantly diminished bacterial load and decreased cariogenic challenge at 6 months for children in both groups meant betadine did not have a measurable influence on the experimental group.

Short follow-up: Based on previous findings demonstrating a gradual re-colonization of *S. mutans* and, therefore, an increase in the number of *S. mutans* after 6 month post-treatment (Twetman *et al.* 1999), it is reasonable to assume that the difference between the experimental and control groups will be more significant at a longer follow up period. Our findings for 4 subjects with longer follow up (Table 9) support this hypothesis.

6.6. Overall reduction of *S. mutans* for all children

When the mean $\log_{10}$ *S. mutans* at 6 months was compared with the mean $\log_{10}$ *S. mutans* at baseline, a significant reduction was found for all children in the study (Table 8). Some of the contributing factors to the overall decline in *S. mutans* levels are the following:

6.6.1. Effect of restorative treatment

The presence of carious lesions and other retentive sites in the mouth are expected to increase microbial colonization (Linquist and Emilson 1990). Therefore, it may be assumed that restoration of open carious lesions removes retentive sites harbouring *S. mutans* from the dentition and diminishes the number of cariogenic microorganisms. However, only a few studies have established the change in oral bacteria before and after restorative treatment. While one group of investigators suggested that successful routine restorative treatment does not alter mutans

streptococci numbers in 4-10 year old children (Gregory *et al.* 1998), other investigators demonstrated that in preschool children extensive restorative dental treatment, at one appointment under general anesthesia, effectively reduces the level of caries-associated microorganisms for a period of at least 6 months (Nickman and Conry 1998, Twetman *et al.* 1999).

The salivary levels of *S. mutans* associated with restored teeth in 6 to 7 year old school children has been compared to the levels associated with both decayed and sound teeth (Petti *et al.* 1997). Levels of *S. mutans* associated with filled teeth were significantly lower than those of decayed teeth. No significant differences were found for *S. mutans* levels between caries-free subjects and subjects with fillings and no decay. It follows that treatment of carious surfaces will lead to a lowering of *S. mutans* concentration. Therefore, the reduction of *S. mutans* in most of the children in our study may be closely related to the effect of restoring carious lesions.

6.6.2. Effect of stainless steel and composite crowns

Another contributing factor to the overall decline in the *S. mutans* levels may have been the type of restoration. Almost all of the children had a significant number of stainless steel or composite crowns (6.4±3 per child) which may affect the adherence of microrganisms. The initial adherence of *S. mutans* to enamel and a variety of dental filling materials has been explored by previous investigators, but results demonstrated no significant differences among the materials and the control (enamel). However, accumulation of *S. mutans* on different composite materials was more than that on amalgam (Shahal *et al.* 1998, Zalkind *et al.* 1998). An earlier study examined the adherence of *S. mutans* to stainless steel crowns *in vitro* (Lubick *et al.* 1981). *S. mutans* bacterial plaque was found to accumulate on stainless steel crowns, particularly at the margins, as well as on the surfaces of control teeth. Furthermore,

the amount of clinically detectable plaque associated with the teeth restored with stainless steel crowns was not significantly different from that of unrestored contralateral control teeth (Durr *et al.* 1982). Similarly, a comparison performed between "change" and "no change" group for the total number of crowns in the present study demonstrated no significant difference between the two groups (P=0.43) (Table 10). In conclusion, it is unlikely that the type of restorative material had a significant effect on the concentration of *S. mutans*.

6.6.3. Effect of fluoride therapy

The overall reduction of *S. mutans* count may be related to the antimicrobial effects of fluoride varnish applied to all subjects after routine restorative treatments. While the results of some recent studies have demonstrated that fluoride can affect bacterial metabolism through a variety of mechanisms, including inhibition of glycolytic enzymes (Marquis 1995), another group of investigators demonstrated no significant reduction in the colonization of mutans streptococci after treatments with fluoride varnish (Ekenback *et al.* 2000). Therefore, the observed overall reduction in *S. mutans* in our study most likely was not related to the fluoride therapy received by all children.

6.6.4. Effect of overnight fasting and toothbrushing

The baseline plaque sample was taken at the GA appointment after the children had been fasting and most likely had not brushed their teeth that morning. The 6 month plaque sample was taken at a routine recall appointment, and the children not only were not fasting but also might have brushed their teeth before the appointment.

Overnight fasting and oral hygiene practices before taking a mouth swab may have some effect on the level of *S. mutans*.

The effect of fasting has been explored by previous investigators who demonstrated that fasting caused a significant decrease in saliva secretion. The rate of plaque formation increased during fasting, however, short-term overnight fasting did not change oral microflora (Birkhed *et al.* 1984, Johansson *et al.* 1984).

The effect of toothbrushing on plaque formation has been investigated. It was demonstrated that toothbrushing significantly reduced plaque levels (Cronin *et al.*2001, Warren *et al.* 2002). Unfortunately, no microbiological analysis was done to compare the number of *S. mutans* before and after toothbrushing. Since children in our study group were not asked to refrain from toothbrushing at their 6 month recall, the overall reduction of *S. mutans* observed for all children may have been related to a thorough toothbrushing before the recall dental appointment.

In summary, the factors that may have contributed to the overall reduction of *S. mutans* levels for our entire study group were:

1) "One-time" restorative treatment performed for all subjects under GA, which significantly decreased retentive sites for bacteria.
2) Improved home care and dental health behaviours during the 6 month period after the surgery.
3) Altered plaque indices at baseline and 6 month visit related to overnight fasting and differences in toothbrushing behaviour.

6.7. Comparisons between "change" group and "no change" group

6.7.1. Ethnicity

Sixty percent of the children in our study were of Chinese origin. When comparisons were performed between "change" and "no change" group, a noticeable

out of 8) in "no change" group who had less than 1 $\log_{10}$ reduction in the *S. mutans* levels, were Chinese. The possible reasons for that small change in Chinese origin children may be because of their traditional beliefs and behaviours related to oral health.

This assumption is supported by the findings of a study that explored oral health beliefs, knowledge and behaviours among a group of Chinese people (with similar number of teenagers, younger adults and older people) resident in the North East of England (Kwan and Williams 1999). Regardless of gender and age, low level of dental awareness was reported for that community. The etiology of dental caries, periodontal disease and tooth loss was poorly understood. While 94% claimed to brush their teeth as part of routine dental care, dental visiting and dietary restriction of sugar intake were reported only in 61% and 30% of the sample respectively.

Therefore, in our study; cultural norms, dental knowledge, oral hygiene and feeding practices of parents of Chinese origin may have increased their children's risk to poor dental health. However, all these families were also low income, another confounding factor (Ramos-Gomez *et al.* 1999).

6.7.2. Length of time mother in North America

The mean length of time that mothers had been in North America for the "change group" was twice as much as that for the "no change" group, and the difference was statistically significant (P=0.03) (Table 10). Dental knowledge, oral hygiene and feeding practices of the mother, the main reservoir of cariogenic bacteria as well as the main caregiver of the child, has been identified as an important behavioural factor in early childhood caries (Reisine and Douglas 1998). The knowledge and skills of the mother about her own dental health, self-care and proper care of her child are the products of her culture and the family structure. Oral health

education and community-based interventions, plus more availability of dental services in North America, may explain the significant difference between the "change" and "no change" groups. Furthermore, the increased stress experienced in families because of the financial and social instability associated with immigration may have been a risk factor for less than optimum oral health practices.

However, in our Chinese families, when the mean length of time that mothers had been in North America was compared between the "change" and "no change" groups, the difference was not significant (P=0.79). Perhaps, Chinese parents tend to keep their traditional health behaviours long after immigration and are less affected by available oral health promotion programs. Therefore, to facilitate effective oral health promotion and treatment services for Chinese families, their traditional oral health beliefs and behaviours must be recognized. While the numbers in our study were small, nevertheless, they support the need for community-based, culturally-sensitive dental health promotion programs.

Chapter 7

Conclusions

Investigators have demonstrated positive outcomes for betadine in controlling the incidence of new carious lesions for children with early childhood caries. However, no microbiological analysis was employed in these studies to explore the role of betadine in decreasing *S. mutans* levels. Our study was undertaken to determine the effect of betadine on *S. mutans* levels in children with caries that had been treated under general anesthesia. Changes in the total aerobic bacteria and *S. mutans* levels were investigated over time. The *S. mutans* counts decreased significantly at 6 months for all children in the study. Although the overall reduction of *S. mutans* counts was greater for the experimental children than for the control children, the difference was not significant. This study also demonstrated a greater experience of new caries in the control group compared to the experimental group. Again, the difference was not significant. For this group of children, the effect of rehabilitation under GA may have made a major contribution to decreasing plaque *S. mutans* at 6 month post-treatment that overwhelmed the effect of betadine. Overall, parents and children had a "favorable reception" to application of betadine and no adverse effect was reported.

When comparisons were made between children with 1 $\log_{10}$ reduction or more in *S. mutans* counts and those whose change was less than 1 $\log_{10}$, a significant difference was found between groups for the time that mother had been in North America. A difference approaching significance was also found in the distribution of children of Chinese origin between the two groups.

In conclusion, although the findings of the present study were not compelling enough to recommend betadine (10% povidone-iodine) therapy as a preventive method for ECC, there is a suggestion that this iodine containing solution may have an effect on *S. mutans*. Additional research with more subjects and a longer time period after application are indicated. There is still much to be investigated about the effect of betadine on oral bacteria and on the caries process. Little is known about whether betadine has a qualitative effect on bacteria. Perhaps, betadine may still prove to be a useful adjunct to enhance the currently used behavioural approaches to prevention of early childhood caries.

Chapter 8

Bibliography

Achong RA, Briskie DM, Hilderbrandt GH, Feigal RJ, Loesche WJ (1999). Effect of chlorhexidine varnish mouthguards on the level of selected oral microorganisms in pediatric patients. *Pediater Dent* 21:169-75.

Adamietz IA, Rahn R, Bottcher HD, Schafer V, Reimer K, Fleischer W (1998). Prophylaxis with povidone-iodine against induction of oral mucositis by radiochemotherapy. *Supprt Care Cancer* 6:373-7.

Addy M, Moran J, Griffiths A, Wills-wood JI (1985). Extrinsic tooth discolouration by metals and chlorhexidine. 1: surface protein degeneration or dietary precipitation? *Br Dent J* 159:331-4.

Alaluusua S, Mamivirta R (1994). Early plaque accumulation – a sign for caries in young children. *Community Dent Oral Epidemiol* 22:273-6.

Al-Hosani E, Rugg-Gunn A (1998). Combination of low parental educational attainment and high parental income related to high caries experience in pre-school children in Abu-Dhabi. *Community Dent Oral Epidemiol* 26:31-6.

Al Ghanim NA, Adenubi JO, Wyne AA, Khan NB (1998). Caries prediction model in preschool children in Riyadh, Saudi Arabia. *International J Pediatric Dent* 8:115- 22.

Al Mahammadi SM, Rugg-Gunn AJ, Butler TJ (1997). Caries prevalence in boys aged 2, 4 and 6 years according to socioeconomic status in Riyadh, Saudi Arabia. *Community Dent Oral Epidemiol* 25: 184-6.

Almedia AG, Roseman MM, Sheff M, Huntington N,Hughes CV (2000). Future caries susceptibility in children with early childhood caries following treatment under general anesthesia. *Pediater Dent* 22:302-6.

American Academy of Pediatric Dentistry (1996). Infant oral health care. *Pediater Dent* 18:25.

Bassett S, MacDonald H, Woods S (1999). Assessing risk for early childhood caries in infants. Project in British Columbia as of July 1999. Ministry of Health, Victoria B.C.

Beighton D (1986). A simplified procedure for estimating the level of *S. mutans* in the moutn. *Br Dent J* 160:329-330.

Berkowitz RJ, Standjord S, Jones P, Hughes C, Barsetti J, Grdon EM, *et al.* (1987). Stomatologic complications of bone marrow transplantation in a pediatric population. *Pediatr Dent* 9:105-10.

Berkowitz RJ, Moss M, Billings RJ, Weinstein P (1997). Clinical outcomes for nursing caries treated using general anesthesia. *J Dent Child* 64:210-1.

Billings RJ (1996). Symposium: needed- A research agenda for nursing caries. *J Public Health Dent* 56:37-8.

Birkhed D, Heintze U, Edwardsson S, Aly KO (1984). Short-term fasting and lacto-vegetarian diet does not affect human saliva. *Scand J Dent Res* 92:408-11.

Bogash RC (1956). A three year observation of a new topical germicide. *Bull Am J Hosp Pharm* 13:226-9.

Boue D, Armau E, Turby G, (1987). A bacteriological study of rampant caries in children. *J Dent Res* 66:23-8.

Bowen WH, Schilling K, Giertsen E, Pearson S, Lee SF, Bleiwweis A, *et al.* (1991). Role of a cell surface associated protein in adherence and dental caries. *Infect Immun* 59:46060-11.

Bowen WH, Pearson SK (1993). Effect of milk on cariogenesis. *Caries Res* 27:461-6.

Bowen WH, Pearson SK, Rosalen PL, Miguel JC, Shih Ay (1997). Assessing the cariogenic potential of some infant formulas, milk and sugar solutions. *J Am Dent Assoc* 128:865-71.

Bratthall D (1972). Demonstration of *S. mutans* strains in some selected area in the world. *Odont Revy* 23:401-10.

Bratthall D (1991). The global epidemiology of mutans streptococci. In: Johnson NW, editor. Risk markers for oral diseases. Vol 1. Dental caries. Cambridge: Cambridge Univ. Pr. p. 287-312.

Brecx M, MacDonald LL, Legary K *et al.* (1993). Long-term effects of Meridol and chlorhexidine mouthrinses on plaque, gingivitis, staining, and bacterial vitality. *J Dent Res* 72:1194-97.

Brown JP, Junner C, Liew V (1985). A study of *S. mutans* levels in both infants with bottle caries and their mothers. *Aust Dent J* 30:96-8.

Burks RJ (1998). Povidone-iodine solution in wound treatment. *Phys Ther* 78:212-18.

Caufield PW, Gibbons RJ (1979). Suppression of *S. mutans* in mouths of humans by a dental prophylaxis and topically-applied iodine. *J Dent Res* 58:1317-26.

Carlos JP, Gittlesohn AM (1965). Longitudinal studies of natural history of caries II. *Arch Oral Biol* 10:739-51.

Carlsson J (1989). Microbial aspect of frequent intake of products with high sugar concentrations. *Scand J Dent Res* 97:110-114.

Centers for Diseases Control and Prevention (CDCP) (1994), Conference, Atlanta, GA.

Chen M-S (1995). Oral health of disadvantaged populations. In Cohen LK, Gift HC (eds). *Disease prevention and oral health promotion.* Copenhage, Munksgaard pp:153-212.

Chow IC (1990). Tooth-bound fluoride and dental caries. *J Dent Res* 69(Spec Iss): 595-600.

Clarke JK (1924). On the bacterial factor in the etiology of dental caries. *Br J Exp Pathol* 5:141-6.

Cleaton-Jones PE, Hargreaves JA, Roberts G, Williams SDL, Leidal TI (1989). The dmfs and dmft of young South African children. *Community Dent Oral Epidemiol* 17:38-40.

Cronin MJ , Dembling WZ, Jacobs DM, Law MA, Warren PR (2001). A comparative single-use clinical study of the efficacy of two manual toothbrushes with angle bristles. *Am J Dent* 14:263-6.

Curzon MEJ, Drumond BK (1987). Case report – Rampant caries in an infant related to prolonged on-demand breast feeding and lacto-vegetarian diet. *J Pediatr Dent* 3:25-8.

Dajani AS, Taubert KA, Wilson W, *et al.* (1997). Prevention of bacterial endocarditis: Recommendations by the American Heart Association. *J Am Dental Assoc* 128:1142-51.

Dasanayake AP, Caufield PW, Cutter GR, Roseman JM, Kohler B (1995). Differences in the detection and enumeration of mutans streptococci due to differences in methods. *Arch Oral Biol* 40:345-51.

Davis BA, Raubertas RF, Pearson SK, Bowen WH (2001). The effects of benzoate and fluoride on dental caries in intact and desalivated rats. *Caries Res* 35:331-337.

Derkson GD, Ponti P (1982). Nursing bottle syndrome; prevalence and etiology in a non-fluoridated city. *J Can Dent Assoc* 48:389-93.

Dilley GJ, Dilley DH, Machen JB (1980). Prolonged nursing habit: a profile of patients and families. *J Dent Child* 47:102-8.

Downer MC (1994). The 1993 national survey of children's dental health: a commentary on the preliminary report. *Br Dent J* 176:209-14.

Dungeman H, Rakoski J (1978). Iodine allergy, facts and phontoms. Proceeding of the World Congress on Antisepsis. Lund, Germany: Mondipharma Gmbh Limburd, 21-3.

Durr DP, Ashrafi MH, Duncan WK (1982). A study of plaque accumulation and gingival health surrounding stainless steel crowns. *J Dent Child* 49:343-6.

Ekenback SB, Linder LE, Lonnies H (2000). Effect of four dental varnishes on the colonization of cariogenic bacteria on exposed sound root surfaces. *Caries Res* 34:70-74.

Emilson C-G (1994). Potential efficacy of chlorhexidine against mutans streptococci and human caries. *J Dent Res* 73: 682-91.

Ernst CP, Prockl K, Willershausen B (1998). The effectiveness and side effects of 0.1% and 0.2% chlorhexidine mouthrinses: a clinical study. *Quintessence Int* 29: 443-8.

Evans DJ, Rugg-Gunn AJ, Tabari ED, Butler T (1996). The effect of fluoridation and social class on caries experience n 5-year-old Newcastle children in 1994 compared with results over the obvious 18 years. *Community Dent Oral Epidemiol* 13:5-10.

Evens CC (1997). Snacking patterns as a risk factor for early childhood caries. Doctoral dissertation, University of Washigton, Department of Epidemiology. Dissertation Abstracts International.

Fardal O, Turnbull RS (1986). A review of the literature on use of chlorhexidine in dentistry. *J AM Dent Assoc* 112:863-69.

Febres C, Echeverri EA, Keene HJ (1997). Parental awareness, habits, and social factors and their relationship to bottle tooth decay. *Pediatr Dent* 19:22-7.

Federation Dentaire Internationale (1994). Second international conference on declining caries. *International Dental Journal* 44 No. 4 (supplement1).

Fejerskov O, Clarkson BH (1966). Dynamics of caries lesion formation. In: Fejerskov O, Ekstrand K, Burt BA, editors. Fluoride in dentistry. Copenhagen, Munksgaard, pp:215-29.

Fine PD (1985). A clinical trial to compare the effect of two antiseptic mouthwashes on gingival inflammation. *Journal of Hospital Infection* 6(Suppl):189-93.

Firestone AR (1982). Effect of increasing contact time of sucrose solution of powdered sucrose on plaque pH *in vivo*. *J Dent Res* 61:1243-4.

Firestone AR, Schmid R, Muhlemann HR (1984). Effect on the length and number of intervals between meals on caries in rats. *Caries Res* 18:128-33.

Fleischer W, Reimer K (1997). Povidone iodine antisepsis. State of the art. *Dermatol* 195(suppl 2): 3-9.

Florta L, Gjermo P, Rolla G, Waerhaug J (1971). Side effects of chlorhexidine mouthwashes. *Scand J Dent Res* 79:119-25.

Fukui Y, Fukui K, Moriyama T (1973). Inhibition of enzymes by human salivary immunoglobulin A. *Infect Immun* 8:335-340.

Funakoshi S, Doi T, Nakajima T, Suyama T, Tokuda M (1982). Antimicrobial effect of human serum IgA. *Microbiol Immunol* 26:227-239.

Gennaro A (1990). Ed. Povidone iodine. Remington's pharmaceutical Sciences. Easton, PA: Mack publishing Company, p: 1169.

Gregory RL, El-Rahman AMA, Avery DR (1998). Effect of restorative treatment on mutans streptococci and IgA antibodies. *Pediatr Dent* 20:273-77.

Gibbons RJ, Depaola PF, Spinell DM, Skobe Z (1974). Interdental localization of *S. mutans* as related to dental caries experience. *Infect Immun* 9:481-8.

Gibson S, Williams S (1999). Dental caries in preschool children: Association with social class, toothbrushing habit and consumption of sugars and sugar-containing foods. *Caries Res* 33:101-123.

Gisselsson H, Birkhed D, Bjorn AL (1994). Effect of a 3-year professional flossing program with chlorhexidine gel on approximal caries and cost of treatment in preschool children. *Caries Res* 28:394-9.

Gjermo P (1978). Phamkodynamische aspekte uber die plaqueehemmende wirkung von chlohexidine. *Zahnarztl Wochenschr* 87: 337-8. Article in German.

Grindefjord M, Dahlof G, Ekstorm G, Hojer B, Modeer T (1993). Caries prevalence in 2.5 year old children. *Caries Res* 27:505-10.

Grindefjord M , Dahlof G, Nilsson B, Modeer T (1996). Stepwise prediction of dental Caries in children up to 3.5 years of age. *Caries Res* 30:256-66.

Grossi G, Skrepcinsky FB, DeCaro T, *et al.* (1997). Treatment of periodontal diseases reduces glycated hemoglobin. *J Periodontol* 68:713-19.

Gustafsson BE, Quensel CE, Swenander-Lanke L, *et al.* (1954). The Vipeholm dental caries study. The effect of different level of carbohydrate intake on caries activity in 436 individuals observed for 5 years. *Acta Odont Scand* 11:232-6.

Habibian M, Lawson M, Stevenson R, Harris S (2001). Dietary habits and dental health over the first 18 month of life. *Community Dent Oral Epidemiol* 29:239-46.

Habibian M, Beighton D, Stevenson R, Lawson M, Roberts G (2002). Relationships between dietary behaviours, oral hygiene and mutans streptococci in dental plaque of a group of infants in Southern England. *Arch Oral Biol* 47:491-8.

HajishingallisG, Nikolova E, Russell MW (1992). Inhibition of *S. mutans* adherence to saliva-coated hydroxy apatite by human SIgA antibodies to the cell surface protein antigen I/II: reversal by IgA1 protease cleavage. *Infect Immun* 60:5057-5064.

Harrison R, Wong T, Ewan C, Contreras B, Phung Y (1997). Feeding practices and dental caries in an urban Canadian population of Vietnamese preschool children. *J Dent Chil* 64:112-7.

Hasenau C, Clasen BP, Roettger D (1988). Use of standardized oral hygiene in the prevention and therapy of mucositis in patients treated with radiochemotherapy of head and neck neoplasms. *Laryngol Rhinol Otol (Stuttg)* 67:576-9 (Article in German).

Hinds K, Gregory J (1995). National diet and nutrition survey: children aged 1.5 to 4.5 years. London; HMSO; Vol. 2.

Holbrook WP, deSoet JJ, de Graaff J (1993). Prediction of dental caries in preschool children. *Caries Res* 27:424-30.

Horowitz HS (1998). Research issues in early childhood caries. *Community Dent Oral Epidemiol* 26; Supplement 1: 67-82.

Hoyos DF, Murray JS, Show L (1977). The effect of chlorhexidine gel on plaque and gingivitis in children. *Br Dent J* 142: 366-69.

Imfeld T, Muhlemann HR (1978). Cariogenicity and acidogenicity of food, confectionery, and beverages. *Pharmacol Ther Dent* 3:53-68.

Ismail AL (1998). Prevention of early childhood caries. *Community Dent Oral Epidemiol* 26; Supplement 1:49-61.

Jensen B and Bratthall D (1989). A new method for the estimation of mutans streptococci in human saliva. *J Dent Res* 68:468-71.

Johanssen I, Ericson T, Steen L (1984) Studies of the effect of diet on saliva secretion and caries development: the effect of fasting on saliva composition of female subjects. *J Nutr* 114:2010-20.

Johnsen DC (1982). Characteristics and background of children with nursing caries. *Pediater Dent* :218-24.

Johnson CP, Gross SM, Hillman JD (1980). Cariogenic potential in man and *in vivo* in the rat of lactate dehydrogenase mutants of *S. mutans. Arch Oral Biol* 25:707-13.

Johnson NW (1991). Risk markers for oral disease. Vol. I. Dental caries Cambridge: Cambridge University press.

Kalsbeek H, Verrips GH (1994). Consumption of sweets and caries experience of primary school children. *Caries Res* 28:477-83.

Kanellis MJ (2000). Medicaid costs associated with hospitalization of young children for restorative dental treatment under general anesthesia. *J Public Health Dent* 60:28-32.

Kanellis MJ (2000). Caries risk assessment and prevention: Strategies for Head Start, Early Head Start, and WIC. *J Public Health Dent* 60:210 17.

Karjalainen S, Eriksson A-L, Ruokola M, Toivonen A (1994). Caries development after substitution of supervised fluoride rinses and toothbrushing by unsuppressed used of fluoride toothpaste. *Community Dent Oral Epidemiol* 22:421-4.

Karjalainen S, Soderling E, Sewon L, Lapinleimu H, Simell O (2001). A prospective study on sucrose consumption, visible plaque and caries in children from 3 to 6 years of age. *Community Dent Oral Epidemiol* 29: 136-42.

Kaste LM, Marianos D, Chang R, Phipps KR (1992). The assessment of nursing caries and its relationship to high caries in the permanent dentition. *J Public Health Dent* 52:64-8.

Kaste LM, Gift HC (1995). Inappropriate infant bottle-feeding. *Arch Pediatr Adolesc Med* 149:786-91.

Kaste LM, Selwitz RH, Oldakowski JA, Brunette JA, Winn DM, Brown LJ (1996). Coronal caries in the primary and permanent dentition of children and adolescents 1-17 years of age: United States 1988-1991. *J Dent Res (Spec Iss)* 75: 631-41.

Kenrad B (1990). Toxine effect from chlorhexidine gluconate: case report. *Tandlaegblade* 94:489-91.

Keyes PH (1960). The infection and transmissible nature of experimental dental caries. Finding and implication. *Arch Oral Biol* 1:304-20.

Kimmel L and Tinanoff N (1991). A modified mitis salivarius medium for a caries diagnostic test. *Oral Microbiol Immunol* 6:275-9.

Kohler B and Bratthall D (1979). Practical method to facilitate estimation of *S. mutans* levels in saliva. *J Clin Microbiol* 9:584-8.

Kohler B and Andreen I (1996). Early colonization of mutans streptococci influenced caries prevalence in the permanent dentition (abstr). *J Dent Res* 75(spec iss): 35.

Kovesi G (1999). The use of betadine antiseptic in the treatment of oral surgical, parodontological and oral mucosal diseases. *Fogorv Sz* 92:243-50 (Article in Hungarian).

Kowash MB, Pinfield A, Smith J, Curzon MEJ (2000). Effectiveness on oral health of a long-term health education program for mothers with young children. *British Dent J* 188:201-5.

Kwan SY and Williams SA (1999). Dental beliefs, knowledge and behaviour of Chinese people in the United Kingdom. *Community Dent Health* 16:32-9.

Lenander-Lumikari M, Loimaranta Y, Hannuksela S, Tenova J, Ekstrand J (1997). Combined inhibitory effect of fluoride and hypothiocyanite on the viability and glucose metabolism of *S. mutans*, serotype c. *Oral Microbiol Immunol* 12:231-235.

Li Y, Caufield PW (1995). The fidelity of initial acquisition of mutans streptococci by infants from their mothers. *J Dent Res* 74:681-5.

Li Y, Navia JM, Bian J-Y (1996). Caries experience in deciduous dentition of rural Chinese children 3-5 years old in relation to the presence or absence of enamel hypoplasia. *Caries Res* 30:8-15.

Lindquist B, Emilson CG (1990). Distribution and prevalence of mutans streptococci in the human dentition. *J Dent Res* 69:1160-66.

Lindquist B, Emilson CG (1991). Dental location of *S. mutans* and *S. sobrinus* in humans harbouring both species. *Caries Res* 25:136-52.

Lingstorm P, Holm J, Birkhed D, Bjorck I (1989). Effects of variously processed starch on pH of human dental plaque. *Scand J Dent Res* 97:392-400.

Loesche WJ (1982). Dental caries is a treatable infection. Illinios: Charles C Thomas Publisher.

Loesche WJ (1986). Role of *S. mutans* in human dental decay. *Microbiol Rev* 50:353-80.

Lopez L, Berkowitz R, Zlotnik H, Mass M, Weinstein P (1999). Topical antimicrobial therapy in the prevention of early childhood caries. *Pediatr Dent* 21: 9-11.

Lopez L, Berkowitz R, Spiekerman C, Weinstein P (2002). Topical antimicrobial therapy in the prevention of early childhood caries: a follow-up report. *Pediatr Dent* 24:204-206.

Lubick HA, Schaefer LD, Beierle JW, Berson RB, Landesman HM (1981). *In vitro* adherence of *S. mutans* to stainless steel crowns. *J Dent Child* 48:25-8.

Marquis R (1995). Antimicrobial actions of fluoride for oral bacteria. *Can J Microbiol* 41:955-64.

Milnes AR (1996). Description and epidemiology of nursing caries. *J Public Health Dent* 56:38-50.

Mohan A, Morse DE, O'Sullivan DM, Tinanoff N (1998). The relationship between bottle usage / content, age, and number of teeth with mutans streptococci colonization in 6-24-month-old children. *Community Dent Oral Epidemiol* 26:12-26.

Moynihan PJ, Holt RD (1996). The national diet and nutrition survey of 1.5 to 4.5 year-old children. Summary of the findings of the dental survey. *Br Dent J* 181:328-32.

Neidner R (1997). Cytotoxicity and sensitization of povidone-iodine and other frequently used anti-infective agent. *Dermatol* (Suppl. 2): 89-92.

Nickman J, Conry J (1998). Pre- and post-restorative microbial levels in nursing caries patients. *J Dent Res* 77:116 (special issue A, abstract 81).

Nobukuni K, Hayakawa N, Nanba R, *et al.* (1997). The influence of long-term treatment with povidone-iodine on thyroid function. *Dermatol* 195(Suppl 2): 69-72.

Nyvad B, Fejerskov O (1996). Development, structure, and pH of dental plaque. In: Thylstup A, Fejerskov O, editors. Textbook of Clinical Cariology. Copenhagen: Munksgaard; pp: 89-110.

Okada M, Soda Y, Hayashi F, Doi T, Suzuki J, Miura K, Kozai K (2002). PCR detection of Streptococcus mutans and S. sobrinus in dental plaque samples from Japanese pre-school children. *J Med Microbiol* 51:443-7.

Olofsson M, Bratthall D (2000): Fluoride and different vehicles to provide fluoride for prevention or control of dental caries. Internet site of Malmo University, Faculty of odontology, Department of Cariology, http://www.db.od.mah.se/car/carhome.html.

O'Mullane DM (1994). Introduction and rationale for the use of fluoride for caries prevention. *Int Dent J* 44:257-61.

O'Sullivan DM, Tinanoff N (1993). Social and biological factors contributing to caries of the maxillary anterior teeth. *Pediatr Dent* 15:41-4.

O'Sullivan DM, Douglas JM, Champany R, Eberling S, Tetrev S, Tinanoff N (1994). Dental caries prevalence and treatment among Navajo preschool children. *J Public Health Dent* 54:139-44.

O'Sullivan DM, Tinanoff N (1996). The association of early dental caries patterns in preschool children with caries incidence. *J Public Health Dent* 56:81-3.

Paunio P, Rautava P, Helenius H, Alanen P, Sillanpaa M (1993). The Finnish family competence study: the relationship between caries, dental health habits, and general health in 3-year-old Finnish children. *Caries Res* 27:154-60.

Pennington JAT (1989). Iodine toxicity. Springfield, VA: National Technical Information Service.

Pennington JAT (1990). A review of iodine toxicity reports. *J Am Diet Assoc* 90:1571-81.

Peretz B, Faibis S, Ever-Hadani P, Eidleman E (2000). Dental health behaviour of children with BBTD treated using general anesthesia or sedation, and of their parents in a recall examination. *J Dent Child* 67:50-4.

Petti S, Campu G, Lumbau A, Tarsitani G (2001). Salivary levels of mutans streptococci associated with restorations: A case-control study. *Microbiologica* 24:281-8.

Peyrone M, Matsson L, Birkhed D (1992). Progression of approximal caries in primary molars and the effect of Duraphat treatment. *Scan J Dent Res* 100:314-8.
Pienihakkinen K, Soderling E, Ostela I, *et al.* (1995). Comparison of the efficacy of 40% chlorhexidine varnish and 1% chlorhexidine fluoride gel in decreasing the level of salivary mutans streptococci. *Caries Res* 29:62-67.

Primosch RE, Balsewich CM, Thomas CW (2001). Outcomes assessment an intervention strategy, to improve parental compliance to follow-up evaluations after treatment of early childhood caries using general anesthesia in a Medicaid population. *J Dent Child* 68:102-8.

Quinonez RB, Keels MA, Vann JR, Mciver FT, Heller K, Whitt JK (2001). Early childhood caries: analysis of psychological and biological factors in a high risk population. *Caries Res* 35:376-83.

Raadal M, Elkhider ElHassan F, Ras-mossen P (1993). The prevalence of caries in groups of children aged 4-5 and 7-8 years in Khartoum, Sudan. *Int J Pediatr Dent* 3:9-15.

Rahn R, Schneider S, Diehl O, *et al.* (1995). Preventing post-treatment bacteraemia: comparing topical povidone-iodine and chlorhexidine. *J Am Dent Assoc* 126:1145-1148.

Ramos-Gomez FJ, Tomar SL, Ellison J, Artiga N, Sintes J, Vicuna G (1999). Assessment of early childhood caries and dietary habits in a population of immigrant Hispanic children in Stockton, California. *J Dent Child* 66:395-401.

Reisine S, Litt M (1993). Social and psychological theories and their use for dental practise. *Int Dent J* 43:279-87.

Reisine S, Douglas Jm (1998). Psychological and behavioral issues in early childhood caries. *Community Dent Oral Epidemiol* 26; Supplement 1: 32-44.

Reynolds EC (1987). The prevention of subsurface demineralization of bovine enamel and change in plaque composition by casein in an intraoral model. *J Dent Res* 66:1120-7.

Roberts GJ, Cleaton-jones PE, Fatti P, *et al.* (1994). Patterns of breast and bottle-feeding and their association to dental caries in 1-to 4-year-old South African children: 2. A case control study of children with nursing caries. *Community Dent Health* 11:38-41.

Rodeheaver G, Bellamy W, Kody M, *et al.* (1982). Bactericidal activity and toxicity of iodine-containing solutions. *Arch Surg* 117:181-6.

Roeters FJM, Van der Hoever JS, Burgesdijk RC, Schaeken MJM (1995). Lactobacilli, mutans streptococci and dental caries: a longitudinal study in 2-year-old children up to the age of 5 years. *Caries Res* 29:272-9.

Rugg-Gunn AJ (1996). Diet and dental caries. In: Murray JJ. Prevention of oral disease. Oxford: Oxford University press, 3-31.

Russell MW, Hajishengallis G, Childers NK, Michalek SM (1999). Secretory immunity in defence against cariogenic mutans streptococci. *Caries Res* 33:4-15.

Sanchez-Perez L, Acosta-Gio AE (2001). Caries risk assessment from dental plaque and salivary *S. mutans* counts on two culture media. *Arch Oral Biol* 46:49-55.

Scannapieco FA (1994). Saliva-bacterium interactions in oral microbial ecology. *Crit Rev Oral Biol Med* 5:203-48.

Scheneyer L, Pigman W, Hanahan L, Gilmore R (1956). Rate of human parotid, sublingual and submaxillary secretions during sleep. *J Dent Res* 33:109-14.

Schreier H, Erdos G, Reimer K, *et al.* (1997). Molecular effects of povidone-iodine on relevant microorganisms: An electron microscopic and biochemical study. *Dermatol* 195 (Suppl. 2):111-117.

Scully Cm, Russell MS, Lehner T (1980). Specificity of opsonising antibodies to antigens of *S. mutans*. *Immunology* 41:467-73.

Seow WK (1987). Bottle caries a challenge for preventing dentistry. *Dentistry Today* 3:1-9.

Seow WK (1991). Enamel hypoplasia in the primary dentition: a review. *J Dent Child* 58:441-52.

Seow WK (1998). Biological mechanisms of early childhood caries. *Community Dent Oral Epidemiol* 26; Supplement 8-21.

Serwint JR, Mungo R, Negrete VF, Duggan AK, Korsch BM (1993). Child-rearing practices and nursing caries. *Pediatr Dent* 92:233-7.

Shahal Y, Steinberg D, Hirschfeld Z, Bronshteyn M, Kopolovic K (1998). *In vitro* bacterial adherence onto pellicle-coated aesthetic restorative materials. *J Oral Rehabil* 25:52-8.

Sigga S (1957). The chemistry of polyvinyl-pyrrolidone iodine. *J A Pharm Assoc* 46:201-4.

Silver DH (1992). A comparison of 3-year-olds' caries experience in 1973, 1981, 1989, in a Hertfordshire town, related to family behaviour and social class. *Br Dent J* 172:191-7.

Smith DJ (1987). Baby fruit juices and tooth erosion. *Br Dent J* 162:65-7.

Smith DJ, King WF, Taubman MA (1990). Salivary IgA antibody to oral streptococcal antigens in pre dentate infants. *Oral Microbiol Immunol* 5: 57-62.

Smith DJ, Taubman MA (1991). Association of specific host immune factors with dental caries experience. In: Johnson NW, editor. Risk markers for oral disease. Vol 1. Dental caries. Cambridge: Cambridge Univ Pr. p. 341-57.

Soderling E, Isokangas P, Pienihakkinen K, Tenovuo J (2000). Influence of maternal xylitol consumption on acquisition os mutans streptococci by infants. *J Dent Res* 79:882-7.

Spatafora G, Rohrer K, Barnard D, Michlek S (1995). A *S. mutans* mutant that synthesizes elevated levels of intracellular polysaccharide in hypercariogenic *in vivo*. *Infect Immun* 63: 2556-63.

Stamm JW (1993). The rule of dentifrices and mouth rinses in caries prevention. *Int Dent J* 43:517-27.

Steiner M, Helfenstein U, Marthaler TM (1992). Dental predictors of high caries increment in children. *J Dent Res* 71:1926-33.

Svanberg M, Loesche WJ (1977). Salivary concentration of *S. mutans* and *S. sanguis* and the colonization of artificial fissures in humans by these organisms. *Arch Oral Biol* 22:441-7.

Tang JM, Altman DS, Robertson DC, O'Sullivan DM, Douglas JM, Tinanoff N (1997). Dental caries prevalence and treatment levels in Arizona preschool children. *Public Health Rep* 112:65-75.

Tanzer JM, Freedman LM, Fitzgerald RJ (1984). Virulence of *S. mutans* defective in glucosyltransferase, dextran-mediated aggregation, or dextran activity. *Molecular bases of oral microbial adhesion.* Washington, DC, p. 204-11.

Tanzer JM (1989). On changing the cariogenic chemistry of coronal plague. *J Dent Res* 68(Spec Iss): 1576-87.

Tanzer JM, Livingston J, Thompson AM (2001). The microbiology of primary dental caries in humans. *J Dent Educ* 65:1028-37.

Tenovuo J, Grahn E, Lehtonen OP, Hyypa T, Karhuvaara l, (1987). Antimicrobial factors in saliva: ontogeny and relation to oral health. *J Dent Res* 66:475-479.

Tenovuo J, Lehtonen OP, Altonen AS (1990). Caries development in children in relation to the presence of mutans streptococci in dental plaque and of serum antibodies against whole cells and protein antigen I/II of *S. mutans*. *Caries Res* 24:59-64.

Tenovuo J, Lumikari M (1991). Organic factors in human saliva in relation to dental caries. In: Johnson NW, editor. Risk markers for oral disease. Vol 1. Dental caries. Cambridge: Cambridge Univ Pr. p. 382-98.

Tenovuo J, Hakkinen P, Paunio P, *et al.* (1992). Effects of chlorhexidine-gel treatments in mothers on the establishment of mutans streptococci in primary teeth and the development of dental caries in children. *Caries Res* 26:275-80.

Tinanoff N (1995). Dental caries risk assessment and prevention. *Dent Clin North Am* 39:709-19.

Tinanoff N (1997). Early childhood caries: overview and recent findings. *Pediatric Dentistry* 19:12-16.

Tinanoff N, Palmer CA (2000). Dietary determinant of dental caries and dietary recommendation for preschool children. *J Public Health Dent* 60:197-206.

Tsubouchi J, Tsubouchi M, Mayard RJ, Domoto PK, Weinstein P (1995). A study of dental caries and risk factors among young Native American infants. *J Dent Child* 62:283-7.

Twetman S, Peterson LG, Pakhomov GN (1996). Caries incidence in relation to salivary mutans streptococci and fluoride varnish applications in preschool children from low- and optimal-fluoride areas. *Caries Res* 30:347-53.

Twetman S, Grindefjord M (1999). Mutans streptococci suppression by chlorhexidine gel in toddlers. *Am J Dent* 12:89-91.

Twetman S, Fritzon B, Jensen B, Hallberg U, Stahl B (1999). Pre- and post-treatment levels of salivary mutans streptococci and lactobacilli in pre-school children. *Inter J Pediatr Dent* 9:93-8.

Twetman S, Garcia-Gody F, Geopferd SJ (2000). Infant oral health. *Dent Clin North Am* 44:487-505.

Valaitis R, Hesch R, Passarelli C, Sheehan D, Sinton J (2000). A systematic review of the relationship between breastfeeding and early childhood caries. *Canadian J of Public Health* 91:411-17.

Van Houte J (1980). Bacterial specificity in the etiology of dental caries. *Int Dent J* 30:305-26.

Van Houte J, Gibbons J, Butera C (1982). Oral flora of children with nursing bottle caries. *J Dent Res* 61:382-5.

Van Houte J, Rasso J, Prostak KS (1989). Increased pH-lowering ability of *S. mutans* cell masses associated with extracellular glucan-rich matrix and the mechanisms involved. *J Dent Res* 68:4511-9.

Van Houte J (1994). Role of microorganisms in caries etiology. *J Dent Res* 73:672-81.

Van Louveren C (1990). The antimicrobial action of fluoride and its role in caries inhibition. *J Dent Res* 69:676-81.

Van Rijkom HM, Truin GJ, Van Hof MA (1996). A meta-analysis of clinical studies on the caries-inhibiting effect of chlorhexidine treatment. *J Dent Res* 75:790-95.

Vratsanos SM (1983). On the structure and function of polyvinyl pyrrolidone-iodine complex. In: Degenes G, ed. Proceedings of international symposium on povidone. Lexington, KY, University of Kentucky, 289-301.

Wan AKL, Seow WK, Walsh LJ, Bird P, Tudehope DI, Purdie DM (2001). Association of *S. mutans* infection and oral developmental nodules in pre-dentate infants. *J Dent Res* 80:1945-48.

Warren PR, Jacobs D, Low MA, Chater BV, King DW (2002). A clinical investigation into the effect of toothbrush wear on efficacy. *J Clin Dent* 13:119-24.

Watson MR, Horowitz AM, Garcia I, Canto MT (1999). Caries conditions among 2-5-year-old immigrant Latino children related to parents' oral health knowledge, opinions and practices. *Community Dent Oral Epidemiol* 27:8-15.

Weigen JF, Thomas SF (1958). Reactions to intravenous organic iodine compounds and their immediate treatment. *Radiology* 71:21.

Weinstein P, Riedy CA (2001). The reliability and validity of RAPIDD scale: Readiness assessment of parents concerning infant dental decay. *J Dent Child* 68:129-35.

Wendt L-K, Hallonsten A-L, Koch G, *et al.* (1994). Oral hygiene in relation to caries development and immigrant status in infants and toddlers. *Swed Dent J* 102:269-73.

Wendt L-K (1995). On oral Health in infants and toddlers. *Swed Dent J* 106(suppl): 10-62.

World Health Organization (1994). Fluorides and oral health: Report of an expert committee on oral health status and fluoride use. Technical Report Series No 849. Geneva, WHO.

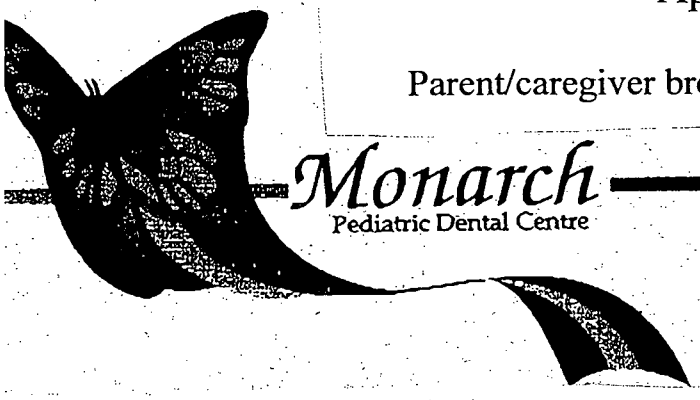
Zalkind MM, Keisar O. Ever-Hadani p, Grinberg R, Sela MN (1998). Accumulation of Streptococcus mutans on light-cured composites and amalgam: an *in vitro* study. *J Esthet Dent:* 10(4): 187-90.

Zamora JL (1986). Chemical and microbiologic characteristic and toxicity of povidone-iodine solutions. *The American Journal of Surgery* 151:400-6.

Appendices

Appendix II

Parent/caregiver brochure/information Sheet:



Monarch
Pediatric Dental Centre
SUITE #501 - 4980 KINGSWAY, BURNABY, BC V5H 4K7
TEL: (604) 430-4980 FAX: (604) 433-4981

Dear parent or caregiver:

- Monarch Dental Clinic is part of a study to try a new way to help prevent cavities in children
- We are working with the Faculties of Dentistry at UBC, and at the University of Washington on this project
- We need 30 children to participate
- Your child may be eligible to participate if he/she:
  - Will be having a general anesthetic at Monarch to get his/her teeth fixed
  - Has all the baby teeth, but so far has no adult teeth
  - Is generally a healthy child
- **And**, you are willing to bring your child back every two months for a 6 month period to have a medicine applied to your child's teeth that may decrease the number of cavity-causing germs in her/his mouth
- If you would like your child to participate, you will be asked to
  - Sign a consent form
  - Complete a survey to find out things like your child's tooth-brushing habits, favorite foods, and what you know about dental health
- Your child will be assigned, by the toss of a coin, to either
  - The group that gets the medicine every two months for 6 months: "study group"
  - The group that does not get the medicine, and just comes back for a check-up in 6 months: "control group"
- Participating families will receive some money to help cover travel costs:
  - Study group families = $60
  - Control group families = $30
- The medicine used, called Betadine (Povidone-Iodine), has been safely used in other dental studies with young children.
- The project has been carefully reviewed and approved by the Ethics Committees of UBC and the University of Washington
- Please talk to one of our staff, or your dentist if you would like to be part of this important project, or call Dr. Maryam Amin (the project investigator) at 8759519

Study procedures:
Children who participate in this study will be assigned by chance, like flipping a coin, to one of two groups. One group will have about 0.2 ml. of a 10% Povidone Iodine solution called Betadine applied to their teeth once every 2 months for 6 months. This amount is about 1/25 of a teaspoon. The other group will not have this treatment.

If I agree to participate and no matter which group my child is assigned to, I will be asked about my child's breast and bottle feeding, snacking habits, and questions about how I take care of my child's teeth. Examples of personal and sensitive questions which will be asked include: "I would not stop the bottle, even if family and friends told me to do so" or "It would be hard to give my child less sweets." I am free to not answer any questions that I do not wish to answer. Completing these questions will take about 15 - 20 minutes.

At the post-operative visit about a week to ten days after the dental treatment, my child will be assigned to one of the two groups. Group 1, the "treatment" group will have the iodine medicine applied every two months for a total of three applications. Each of these visits will take about 10 - 15 minutes The first dose to protect the teeth will be applied at the "post-operative check-up" visit. Group 2, the "no-treatment" group will return as usual in six months. At the time of the general anesthetic, a gentle scraping of the teeth will be done on all participating children to get a small amount of plaque, which is the film that forms on teeth. The plaque sample will be tested to see if it contains the germs which lead to tooth decay. At the final visit in 6 months, all participating children will have a dental examination and a gentle scraping of the teeth to collect a small amount of plaque to test. This last visit will also take about 15 minutes.

Exclusions:
If I live so far away from this dental office that returning every two months will be inconvenient, then I will be excluded from the study. In addition, because the medicine that is being used contains iodine, all children with known thyroid disease will be excluded. In addition, if my child has more than 5 baby teeth extracted at the time of surgery, he/she will not be included in the study.

Risks or Discomfort:
The only discomfort that my child will experience is that associated with a brief examination of the teeth, with having the plaque gently scraped from some of the teeth, and with having the medicine applied to his/her teeth. The study dentists from Monarch will do everything that they can to keep my child comfortable and happy during the procedure. The medicine that is being used is not a new medication, but is a 10% Povidone-Iodine solution also used in hospitals in much higher amounts to help prevent mouth infections in children who are receiving chemotherapy for cancer. The dose used in this study is a low, safe dose. Previous studies have already shown the same concentration of the iodine medicine to be effective in reducing a child's risk of developing cavities.

Very rarely use of the solution may be associated with some skin irritation; this is a rare occurrence and will not damage or scar the skin in the mouth. Also very rarely, iodine can cause an allergic reaction. If my child develops a rash or has any other adverse effects, I will call Dr. Amin at 8759519 or Dr. Harrison at 8222094; 7379091 (evenings and weekends), and take my child to my family doctor.

Benefits:
Children who get the medicine on their teeth may benefit as it may help to prevent tooth decay. All participating children will benefit from the examination at 6 months, and all parents will be told of their child's risk for getting new cavities.

Alternative treatment:
If my child is in the group that has the medicine applied and if I decide to withdraw my child from the study, or if I decide not to participate at all, my child will still be able to have regular check-ups and treatment at the Monarch Dental Centre.

Confidentiality:
All of my answers to the questionnaires will be confidential, and my identity, and that of my child, will not be revealed in any reports arising from the interviews, the dental examinations, or the plaque tests. Our names will not appear anywhere on any of the forms. All documents will be identified only by code number and will be kept in a locked filing cabinet. Security of any information kept on a computer hard drive will be maintained by password access.

The data collection forms that are used in the study may be inspected, only in the presence of Dr. Harrison or her designate, by the Health Protection Branch (HPB Canada), or by a representative of the manufacturer of the iodine solution (Purdue Frederick Company). These forms will be identified only by code number; neither my child nor I will be identified by name, initials, or date of birth.

Remuneration/compensation:
No matter which group my child is assigned to, there is no cost for any visits or procedures related to this study. I will be responsible for any of the customary dental fees for my child.

Each participating family will receive $10 at the post-operative visit. If my child is in the Betadine group, I will receive $10 at each of the two visits where Betadine is applied, and $30 at the last visit. If my child is in the other group, I will receive $20 at the last visit.

Contact:
If I have any questions or desire further information about this project, I may contact Dr. Maryam Amin, 8759519 or Dr. Rosamund Harrison at 8222094, or 7379091 after hours. If I have any concerns about my treatment or rights as a subject in this study, I may contact the Director of Research Services at the University of British Columbia, Dr. Richard Spratley at 8228598.

New Findings:
If I decide to enroll my child in this study, and, if before the study is over, a more effective treatment to prevent cavities becomes available, it will be offered to me. I will also be advised of any new information that becomes available that may affect my willingness to remain in this study.

Consent:
The participation of my child and me is voluntary and will in no way affect any services that that any of my family receives from the Monarch Pediatric Dental Group. I may withdraw my child from the study at any time without penalty. I may also refuse to answer any questions that I feel are sensitive.

Subject's statement:
The study described above has been explained to me, and I have had an opportunity to ask questions. I understand that future questions that I may have about the research or about my rights will be answered by one of the investigators listed above.

I have been given a copy of this consent form, and indicate my consent to participate in the project **"General Anesthesia Follow-up and Secondary Prevention: a Pilot"** by signing below.

In addition, I consent/ I do not consent (circle one) to let my child (named below) have the dental examinations and procedures, as described:

Date	Name of child
Date	Signature of parent or guardian
Date	Witness
Date	Investigator's Signature

Monarch Dental Clinic Project

Child Behaviour Questionnaire

Demographic Information

1. Child's date of birth: month / day / year 2. Mother's date of birth: month / day / year

3. Child's sex: ☐ boy ☐ girl

4. Since birth has the child lived anywhere else?

☐ no

☐ yes, list which city and time at each location:

5. What is the current marital status of the mother? (*check one*)

☐ married ☐ single, divorced ☐ widowed ☐ single, never married

6. What is the highest level of education that the mother has completed? (*check one*)

☐ elementary school ☐ some high school ☐ completed high school ☐ college or vocational school

7. How long has the mother been in North America? ☐ years ☐ months

8. How many people live in the child's household?

9. How many of these people are children? ☐ What are their ages?

10. How many of these people are adults? ☐ What are their ages?

11. Who spends the most hours per week in direct care of the child? *(check only one)*

☐ mother ☐ father ☐ grandparent ☐ aunt ☐ uncle ☐ sibling ☐ other (*please specify*):

If main caregiver is other than the mother, answer the questions below:

12. What is the highest level of education the main caregiver has completed? (*check one*)

☐ elementary school ☐ some high school ☐ completed high school ☐ college or vocational school

13. How long has the main caregiver lived in North America? ☐ years ☐ months

14. How many hours per week does each of the following help care for the child? (enter 0 if doesn't help)

a. ☐ no one else

b. husband or partner (hours per week) ☐

c. child's mother (hours per week) ☐

e. ☐ relative or friend (hours per week)

f. ☐ day care (hours per week)

Child Feeding Information

. Is the child using a bottle now? ☐ yes ☐ no

If no, how old was the child when the bottle feeding was stopped? _____ months old

2. If the child uses a bottle, what is **most often** contained in the bottle? *(check only one)*

☐ milk ☐ juice/pop ☐ water ☐ other _____ ☐ not applicable

3. If the child no longer uses a bottle, what was **most often** contained in the bottle when bottle feeding was stopped?

☐ milk ☐ juice/pop ☐ water ☐ other _____ *(check only one)* ☐ not applicable

4. Is the child being breast fed now? ☐ yes ☐ no

If no, how old was the child when the breast feeding was stopped? _____ months old

5. Do you (or did you) bottle feed your child as he/she falls asleep?

☐ no ☐ yes, at nap time only ☐ yes, at night time only ☐ yes, at nap time and night time.

6. Do you (or did you) bottle feed your child when he/she wakes up at night?

☐ no ☐ yes

7. On average, how often does (or did) the child walk around with a bottle to sip from during the day?

☐ never ☐ sometimes ☐ often ☐ all the time

Dental History

How old was the child when his/her teeth first appeared? ☐ months

At what age did an adult first clean your child's teeth?

- ☐ before age 1
- ☐ before age 2
- ☐ before age 3
- ☐ before age 4

Has the child ever seen a dentist before this visit? (*check one*)

- ☐ yes Why? _____
  - What was the child's age when he/she first went to the dentist? ☐ months
  - How many times has the child gone to the dentist? ☐
- ☐ no

Has the child's primary caregiver had toothaches, cavities, or bleeding gums in the past six months?

- ☐ yes
- ☐ no

ID_____ Date 91

Medical History

What is the overall health of the child? (*check one*)

☐ excellent ☐ very good ☐ good ☐ fair ☐ poor

2. How many weeks was the pregnancy of this child? [ ] weeks *(40 weeks is full term)*

3. Did you have a C-Section during this birth? ☐ yes ☐ no

4. What was the child's birth weight? [ ] pounds, [ ] ounces

5. Any extra measures to help with the birth (suction, forceps, etc.)?

☐ No ☐ Yes (*please explain)* _____

6. Has the child ever had any major illnesses?

☐ yes (*please specify*) _____

☐ no

7. How many times has the child ever had antibiotics? ☐ never ☐ 1-3 times ☐ 3-5 times ☐ more than 5 times

If yes, why? _____ for how long? _____

8. Does the child take vitamins on a daily basis?

☐ yes, which brand? _____

☐ no

9. Does the child take fluoride tablets or drops on a daily basis?

☐ yes

☐ no

Dietary Information

How many "sit down" meals does your child have each day? [ ]

What snacks are most often given to reward the child or get the child to behave? (Please list three)

_____ _____ _____

ID_____ Date 92

Think about what your child has eaten in the past month.
How often has your child eaten the following items?

If your child eats the food item less than once a day, mark "0" in the box.

If your child eats the food item about once a day, mark "1" in the box.

If your child eats the food item about twice a day, mark "2" in the box.

If your child eats the food item three times a day, mark "3" in the box.

If your child eats the food item four times or more each day, mark "4" in the box.

FOR EXAMPLE:

If your child eats "carrots" 5 times each day you would mark:
[4] **carrots**

If your child eats "popcorn" once per week you would mark:
[0] **popcorn**

- [ ] peanut butter
- [ ] jam/jelly
- [ ] eggs
- [ ] nuts
- [ ] cheese
- [ ] milk
- [ ] yogurt
- [ ] fruit juice
- [ ] banana
- [ ] raisins/ fruit roll-ups
- [ ] apple
- [ ] other fresh fruits
- [ ] cereal
- [ ] crackers
- [ ] bread/ bagel
- [ ] soft drinks (pop)
- [ ] kool aid/ fruit drinks
- [ ] ice cream
- [ ] pudding
- [ ] jello
- [ ] donuts
- [ ] cookies/ cakes
- [ ] candy
- [ ] chips
- [ ] vegetables
- [ ] noodles/ pasta
- [ ] potato
- [ ] rice
- [ ] luncheon meat

ID_____ Date 93

The five statements below refer to how you have been feeling in the past two weeks. Circle one number for each item.

	All of the time	Most of the time	More than half the time	Less than half the time	Some of the time	At no time
I feel cheerful and in good spirits.	1	2	3	4	5	6
I feel calm and relaxed.	1	2	3	4	5	6
I feel active and vigorous.	1	2	3	4	5	6
I wake up feeling awake and rested.	1	2	3	4	5	6
My daily life is filled with things that interest me.	1	2	3	4	5	6

ID_____ Date 9/4

INSTRUCTIONS: If your child is currently feeding from the bottle or breast please fill out questions 1-31 below. If your child is **NOT** currently feeding from the bottle or breast, please **SKIP** to questions 32-62 (page 8).

Please check the box that matches your level of agreement with each sentence. Check only one box for each sentence.

Questions	Strongly agree	agree	Not sure	disagree	Strongly disagree
1. It is very convenient to feed the child with a bottle.					
2. I would take the child off the bottle if the dental clinic staff told me to do so.					
3. Keeping my child's teeth healthy is important to me.					
4. It makes me feel good when I give my child something sweet to eat or drink.					
5. Without a bottle, my child's crying would keep me and my family up at night.					
6. I would change my child's snack foods if I was told that they caused tooth decay.					
7. My child benefits a lot when I clean his/her teeth.					
My child usually decides which foods and ...nks he/she will eat or drink.					
9. My child will have difficulty when I stop giving him/her the bottle.					
10. I would follow the advice of the dental staff about cleaning my child's teeth.					
11. I like the idea of a health person putting medicine on my child's teeth to protect them from getting cavities.					
12. My child is happier when I give him something sweet in his bottle.					
13. It would be very hard to give my child less sweets.					
14. I get advice on taking care of my child from radio, TV, magazines, newspaper or books.					
15. I believe giving my child fluoride vitamins every day would help my child's teeth.					
16. I feel like a mean parent if I don't give my child sweets.					
17. My child gives me a hard time when I try to brush his/her teeth.					
I feel comfortable asking a doctor/dentist ...out ways to take care of my child.					

ID_____ Date 95

Questions	Strongly agree	agree	Not sure	disagree	Strongly disagree
19. Protecting my child's teeth from getting cavities is important to me.					
20. It is not easy to give my child a fluoride vitamin every day.					
21. It is easy for me to get answers about ways to take care of my child from the health center.					
22. It is important to me that my child does not have tooth decay.					
23.Foods and drinks that are not sweet, don't taste good to my child.					
24. It is/was easier to put my child to sleep with a bottle filled with juice or milk.					
25. I would stop, or would have stopped, giving my child a bottle before he/she was ready to stop if the experts told me it would be healthier for his/her teeth.					
26. Seeing decay in my child's teeth is upsetting to me.					
27. My child decides when to brush his/her own teeth.					
28. I am/was able to put my child to sleep without feeding him/her.					
29. It makes/made me very sad to think of not giving my child the bottle he or she wants/wanted.					
30. It is often difficult to follow the doctor's/dentist's advice and change how I take care of my child.					
31. It will be/was hard not to give the breast/bottle every time my child cries for it.					

ID_____ Date 96

Please check the box that matches your level of agreement with each sentence. Check only one box for each sentence.

Questions	Strongly agree	agree	Not sure	disagree	Strongly disagree
33. It was very convenient to feed the child with a bottle.					
34. I would have taken the child off the bottle if the dental clinic staff told me to do so.					
35. Keeping my child's teeth healthy is important to me.					
36. It makes me feel good when I give my child something sweet to eat or drink.					
37. Without a bottle, my child's crying did keep me and my family up at night.					
38. I would change my child's snack foods if I was told that they caused tooth decay.					
39. My child benefits a lot when I clean his/her teeth.					
40. My child usually decides which foods and drinks he/she will eat or drink.					
41. My child did have difficulty when I stopped giving him/her the bottle.					
42. I would follow the advice of the dental staff about cleaning my child's teeth.					
43. I like the idea of a health person putting medicine on my child's teeth to protect them from getting cavities.					
44. My child was happier, when I gave him something sweet in his bottle.					
45. It would be very hard to give my child less sweets.					
46. I get advice on taking care of my child from radio, TV, magazines, newspaper or books.					
47. I believe, giving my child fluoride vitamins every day, would help my child's teeth.					
48. I feel like a mean parent if I don't give my child sweets.					
49. My child gives me a hard time when I try to brush his/her teeth.					
50. I feel comfortable asking a doctor/dentist about ways to take care of my child.					

ID_____ Date 97

Please check the box that matches your level of agreement with each sentence. Check only one box for each sentence.

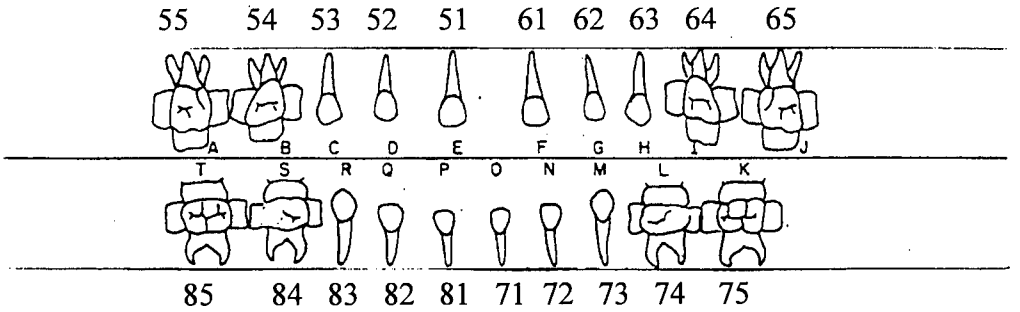
Questions	Strongly agree	agree	Not sure	disagree	Strongly disagree
33. It was very convenient to feed the child with a bottle.					
34. I would have taken the child off the bottle if the dental clinic staff told me to do so.					
35. Keeping my child's teeth healthy is important to me.					
36. It makes me feel good when I give my child something sweet to eat or drink.					
37. Without a bottle, my child's crying did keep me and my family up at night.					
38. I would change my child's snack foods if I was told that they caused tooth decay.					
39. My child benefits a lot when I clean his/her teeth.					
40. My child usually decides which foods and drinks he/she will eat or drink.					
41. My child did have difficulty when I stopped giving him/her the bottle.					
42. I would follow the advice of the dental staff about cleaning my child's teeth.					
43. I like the idea of a health person putting medicine on my child's teeth to protect them from getting cavities.					
44. My child was happier, when I gave him something sweet in his bottle.					
45. It would be very hard to give my child less sweets.					
46. I get advice on taking care of my child from radio, TV, magazines, newspaper or books.					
47. I believe, giving my child fluoride vitamins every day, would help my child's teeth.					
48. I feel like a mean parent if I don't give my child sweets.					
49. My child gives me a hard time when I try to brush his/her teeth.					
50. I feel comfortable asking a doctor/dentist about ways to take care of my child.					

Appendix V

CCOHR Study of Early Childhood Caries

ORAL HEALTH ASSESSMENT FORM

ID# _____ **DATE:** _____



CARIES BY SURFACE

0= SOUND
1= DECAYED
2= FILLED
3= DECALCIFIED (WHITE SPOTS)
4= MISSING
-- = UNERUPTED

	M	O	D	B	L
55/a					
54/b					
53/c					
52/d					
51/e					

	M	O	D	B	L
61/f					
62/g					
63/h					
64/i					
65/j					

	M	O	D	B	L
75/k					
74/l					
73/m					
72/n					
71/o					

	M	O	D	B	L
81/p					
82/q					
83/r					
84/s					
85/t					