Milk fluoridation for the prevention of dental caries - 2009

WHO

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World Health Organization

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Editors

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World Health Organization

Geneva
2009

WHO Library Cataloguing-in-Publication Data

J Bánóczy, PE Petersen, AJ Rugg-Gunn (Editors). Milk fluoridation for the prevention of dental caries. World Health Organization, 2009.

1. Milk. 2. Fluoridation. 3. Oral health promotion. 4. Dental caries prevention

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ISBN 978 92 4 154775 8 (NLM classification: QV 50)

Printed in Switzerland

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Preface

The burden of non-communicable diseases (NCD) is rapidly increasing; in response to the growing NCD problem, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) in April 2003 released the report *Diet, Nutrition and the Prevention of Chronic Diseases* (WHO/FAO, 2003). This Report contains the best currently available scientific evidence on the relationship of diet, nutrition and physical activity to chronic diseases, including oral disease. Subsequently, in 2004, WHO initiated a Global Strategy on Diet, Physical Activity and Health with the overall goal of guiding the development of sustainable actions at individual, community, national and global levels, which will lead to reduced disease (WHO, 2005a).

Oral diseases are most prevalent chronic diseases worldwide and are a significant burden to all countries. In reviews of global oral health published by the WHO it is emphasised that despite great improvements in the oral health of populations across the world, problems still persist particularly among the under-privileged groups (WHO, 2003a; Petersen, 2003; Petersen *et al.*, 2005). WHO sees oral health as an integral part of general health, and oral diseases and conditions may have wider impacts on health and wellbeing of people. In addition, oral health and general health share common risk factors, such as poor diet and nutrition, and therefore disease prevention programmes must incorporate oral disease.

Dental caries remains a major public health problem in most high income countries, affecting 60–90% of school children and the vast majority of adults (Petersen, 2003; Petersen *et al.*, 2005). It is also the most prevalent oral disease in several Asian and Latin American countries. Although for the moment it appears to be less common and less severe in several low-income countries, the WHO reports anticipate that, in light of changing living conditions and dietary habits, the

incidence of dental caries will increase, particularly as a result of growing consumption of sugars and inadequate exposure to fluorides.

It must be acknowledged that a lack of fluoride does not cause dental caries. The WHO World Oral Health Report (WHO, 2003a) is quite clear that the post-eruptive effect of sugar consumption is one of the main aetiological factors for dental caries both in terms of the amount and the frequency of sugars consumed. The recent systematic analysis (WHO/FAO, 2003) of the evidence on the role of diet in chronic disease recommends that free (added) sugars should remain below 10% of energy intake and the consumption of foods/drinks containing free sugars should be limited a maximum of four times per day. For countries with high consumption levels it is recommended that national health authorities and decision makers formulate country specific and community specific goals for reduction of consumption of free sugars. However, WHO also notes that many countries currently undergoing nutrition transition do not have adequate fluoride exposure (WHO/FAO, 2003; Petersen & Lennon, 2004).

It is the responsibility of national heath authorities to ensure implementation of feasible fluoride programmes for their country (WHO/FAO, 2003). In a WHO Expert Committee report on fluoride and oral health (WHO, 1994) and in more recent updates (WHO/FAO, 2003; Petersen & Lennon, 2004), WHO has emphasized the importance of effective prevention of dental caries through diet and administration of fluoride as part of public health programmes. In the vast majority of countries, dental caries is highly linked to socio-economic status and prevention by automatic administration of fluoride through water, salt, or milk is documented to be most equitable. The WHO position on use of fluoride is further detailed in a series of World Health Assembly Resolutions (WHA22.30, WHA28.60, WHA31.50), and in 2007 by WHA60.17 Oral Health - Action Plan for Promotion and Integrated Disease Prevention.

The World Oral Health Report (WHO, 2003a) noted that dental caries can be controlled by the joint action of communities, professionals and individuals aimed at reducing the impact of sugar consumption and emphasizing the beneficial impact of fluorides. In many low-income countries however, access to oral health services is limited; likewise, in these countries significant numbers of population groups are underserved. For these reasons, professionally applied fluoride is less relevant to public health.

Research on the oral health effects of fluoride started around 100 years ago. For the first 50 years or so it focused on the link between water and fluorides - both natural and artificial - and dental caries and fluorosis. In the second half of the 20th century this focus shifted to the development and evaluation of fluoride toothpastes and rinses, and alternatives to water fluoridation such as milk fluoridation, in countries which do not have the facilities necessary for water fluoridation.

Milk fluoridation, as an alternative vehicle for automatic population-directed administration of fluoride, began in Switzerland some fifty years ago. In 1988 the first community based scheme was introduced in Bulgaria and reached some 15,000 children. By 2000 this figure had increased to 114,000 children as programmes were introduced in four other countries. More recently there has been further expansion particularly in Thailand and Chile and there are now 800,000 children in five countries participating in the international programme. As milk fluoridation mostly targets the child population, milk fluoridation schemes have been established within the context of school health programmes (WHO, 2003b), and programmes for healthy diet and nutrition.

The evidence of milk fluoridation as a public health measure has been shown in a wealth of studies. Substantial basic research and public health research have been performed in the last decade in order to consolidate the sound scientific basis for the use of fluoridated milk. The Borrow Dental Milk Foundation encouraged these studies in the form of individual grants, by supporting PhD studies and long-term grants in collaboration with WHO; these studies brought good results, and were published in international peer-reviewed journals.

In order to perform and evaluate these studies according to common guidelines, discussions between the World Health Organization (WHO) and the Borrow Dental Milk Foundation (BDMF) were initiated during the 1980s, and effective collaboration on country programmes started in 1988. Since 2002, the WHO Global Programme for Milk Fluoridation has been implemented under the leadership of Dr *Poul Erik Petersen*. With experiences gained from national milk fluoridation programmes established within all regions of the world, the constructive collaboration between WHO and by the renamed Borrow Foundation (BF) emerged into effective work for promotion of oral health and integrated disease prevention, linking dental caries

prevention through use of fluoride with programmes for diet and nutrition and school health programmes.

The first edition of this book the reader held in their hands, was edited by *K.W. Stephen, J. Bánóczy and G.N. Pakhomov* with the title: "Milk fluoridation for the prevention of dental caries", and published by the World Health Organization and the Borrow Dental Milk Foundation in Geneva, 1996.

The aim of the new edition of this publication is to offer help to public health planners and administrators at community or national levels in establishing a sound basis, supported by scientific evidence, for the planning, implementation and extension of milk fluoridation projects for the prevention of dental caries. Finally, the edition provides basic guidelines for evaluation of milk fluoridation schemes.

Geneva, 2009

Poul Erik Petersen

1 Milk, nutrition and human health

A. J. Rugg-Gunn and P. E. Petersen

1.1 Introduction

"A land flowing with milk and honey" has been considered a bountiful land and a desirable place to live. Milk has long been regarded as a nutritious food since our mother's milk sustained us at the beginning of our life. Our experience of milk from other animals began during man's transition from a hunter-gatherer to a pastoralist – probably in Mesopotamia some 8000 years ago – enabling us to benefit from animals in many ways, including the collection of milk (Southgate, 2000). Now, the dairy industry is an essential part of agricultural policy in most countries, and these policies have resulted in the breeding of high-producing stock and the development of effective and safe milk collection and delivery systems. Milk is also used to make cream, cheese and butter, but these are outside the scope of this review.

These developments are not surprising as human milk is the only source of nutrition for newly born infants and provides sufficient energy and nutrients for rapid growth and development until at least four months. Non-human milks differ in composition from human milk but make an important contribution to the nutrition of children and, indeed, adults. Milk consumption is considered so important that provision of milk in schools is public health policy in many countries.

1.2 Types of milk, their treatment and nutritional value

In Europe, America and Australasia, cow's milk is the most important type of milk. Reindeer milk is important in Lapland, and the mare and ass are important providers in many parts of Eurasia. In the Middle East, camel and goat's milk are traditional drinks, although now being replaced by cow's milk, while in southern and south-east Asia, buffalo milk is popular. In Africa, cows provide most of the milk, although the breeds of cow differ from those farmed in Europe. The compositions

of some of these are given in Table 1.1, and the wide variation in the concentration of several nutrients can be seen.

Table 1.1
Composition of milk from different animals (per 100ml)

	Human [1] (Mature)	Cow [1]	Goat [1]	Sheep [1]	Camel [2]	Buffalo [2]
Water (g)	87	88	89	83	89	83
Energy (kJ)	289	274	260	388	264	385
Protein (g)	1.3	3.3	3.1	5.4	2.0	4.1
Fat (g)	4.1	3.9	3.7	5.8	4.1	5.9
Lactose (g)	7.2	4.5	4.4	5.1	4.7	5.9
Calcium (mg)	34	118	100	170	94	175

Source : [1] Food Standards Agency (2002). [2] Southgate (2000).

While cow's milk remains the most widely consumed milk, it deteriorates rapidly and is usually treated to prevent, or at least slow down, this process. The least severe treatment is pasteurisation, where milk is heated to at least 72°C for 15-25 seconds which will kill all non-sporing pathogens and non-thermoduric organisms. However, pasteurised milk should be kept in a refrigerator to prevent the growth of sporing organisms. Ultra-heat-treated (UHT) milk can be stored at room temperature for a long time when packed aseptically in packs which exclude oxygen. The process involves heating milk to 130°C for one second. Full sterilisation of milk involves heating milk to 117 to 123°C for 10 to 12 minutes (Varnam & Sutherland, 1994). Both UHT and sterilised milks taste slightly different to pasteurised milk. Controlled fermentation of milks has been practised for millennia, mainly as a way of prolonging its drinkable life. Lactobacilli are used to convert the lactose to lactic acid so that the fall in pH inhibits growth of many pathogens. A further advantage is the establishment of desirable intestinal flora. To make transport and storage of milk easier and to increase shelf-life, whole milk can be reduced to a powder which can be reconstituted as required, in the home or local premises. The powder is now widely produced by spray drying, which is less destructive of vitamins and proteins than the previous heated-roller system of drying.

In recent publications, WHO and UNICEF (2003, 2007) strongly recommend that infants should be exclusively breastfed for the first six months of life to achieve optimum growth, development and health. They emphasise that breastfeeding is an unequalled way of providing

ideal food for the healthy growth and development of infants. After the age of six months, to meet the child's evolving nutritional requirements, infants should receive nutritionally adequate and safe complementary food while breastfeeding continues up to two years of age or beyond. There are few medical reasons why infants should not be breastfed; for example, it is now recommended that HIV-infected mothers breast-feed their infants for the first six months and abrupt cessation at six months of age is also not recommended (WHO, 2007).

Complementary foods, given after six months of age, can be low-cost complementary foods or industrially processed complementary foods (WHO and UNICEF, 2003): they should be nutritionally adequate and safe, and additional micronutrient supplementation may be necessary (WHO and UNICEF, 2007). Cow's milk is unsuitable for infants under six months and 'formula milk', based on cow's milk, is now widely manufactured and marketed in many countries. The composition of these formula milks is based on knowledge of the nutritional requirements of infants at various ages. They must be marketed and distributed following the International Coding of Marketing of Breast-milk Substitutes, and prepared in accordance with Codex Alimentarius standards (WHO and UNICEF, 2003). Formula 'milk' is also made from the soybean and is used by those who wish to avoid milk from animals.

Milk provides energy and essential nutrients – proteins, fats, carbohydrate, vitamins and minerals. The major protein in milk is casein which, in cow's milk, is about 80% of total protein. Other major proteins are lactalbumin and immunoglobulins. Casein – which is actually present in milk as caseinogen, being converted into casein by gastric enzymes - is a major factor in retaining calcium and phosphate in solution in milk (Southgate, 2000). The profile of amino acids in milk complement those in grains and cereals, which is of considerable benefit in communities where grains and cereals predominate. Almost all fat is 'saturated' and milk contains very little unsaturated fat.

Because consumption of fats in general, and saturated fats in particular, is too high in many developed countries, children and adults are now urged to choose milks with lower fat contents. These are either semi-skimmed or skimmed milk (Table 1.2). Other than a decrease in energy and fat content, there is little change in nutritional composition except a reduction in fat-soluble vitamins. It is important that young children are given concentrated sources of energy. Thus, whole milk, not reduced-fat milk, should be given to children up to two years of

age. Recommendations regarding the appropriate fat content of milk for children over two but under five years vary. WHO (2005b) accept skimmed milk as an option for children from two years of age onwards. In the UK, the current recommendation (Food Standards Agency, 2008) is that children over two but under five years of age can be given semi-skimmed, but not skimmed milk provided they have a good appetite and are eating a varied diet. Countries may vary in the ages at which they recommend children change from full fat to semi-skimmed milk and from semi-skimmed to skimmed milk.

Table 1.2
Composition of some cow's milk products (per 100ml)

	Whole	Semi-skimmed	Skimmed
Water (g)	88	90	91
Energy (kJ)	274	195	136
Protein (g)	3.3	3.4	3.4
Fat (g)	3.9	1.7	0.2
Lactose (g)	4.5	4.7	4.4
Calcium (mg)	118	120	122

Source : Food Standards Agency (2002)

Almost all the carbohydrate in milk is lactose, which is a disaccharide and, as a sugar, will be discussed in relation to dental health later in this chapter. The inorganic (often known as 'mineral') content of milk is essential for infant development. As can be seen for calcium in Table 1.1, the concentrations of these are generally higher in cow's milk than in human milk. Milk is a very important source of calcium, supplying well over half the daily requirement and intake for children in many countries. The next most important inorganic components are iodine and phosphorus, followed by potassium and magnesium, zinc and selenium. Milk is not a significant source of iron or copper. There is some variation in the concentration of nutrients in milk between seasons and countries, and national food tables should be consulted for more precise information. Unlike many other foods, the availability of these inorganic nutrients (that is, their absorption from the gut into the blood stream) is high from milk. Milk contains both fat-soluble vitamins (e.g. A, D and E) and water-soluble vitamins (mainly B). Concentrations of fat-soluble vitamins will be much lower in reduced-fat, compared with whole milk: for example, for vitamin A (retinol equivalent), concentrations in whole, semi-skimmed and skimmed milks are about 37µg, 21µg and 1µg per 100g, respectively (Holland

et al., 1989). Fortification with vitamins may occur in some proprietary brands where skimmed milk is used. The concentrations of some B vitamins are reduced by heat treatments and storage, especially in UHT milks. Between 0 and 10 per cent of the thiamin, riboflavin, vitamin B_{12} and folate, and about 10 per cent of vitamin C are destroyed but, in a varied diet, milk is not an important source of these nutrients. Up to 50 per cent of vitamin C and folate is lost during prolonged storage of UHT milk (Food Standards Agency, 2008). However, it should be remembered that heating and storage reduces the nutrient content of many foods, not just milk, and considerable nutrient value remains.

1.3 Milk consumption around the World

Per capita milk consumption varies considerably between regions of the world. In 1997-99, it was estimated that the worldwide average consumption was 78kg of milk per person per year (Table 1.3). Consumption was highest in industrialised countries, at 212kg/person/year: this is four times the estimated consumption in developing countries (45kg/person/year), although consumption was higher in Latin American countries (110kg/person/year) than in many other regions in this group of developing countries.

Table 1.3
World trends in milk consumption (kg/person/year)

	1964-66	1997-99	2030
World	74	78	90
Developing Countries	28	45	66
Near East & North Africa	*69*	*72*	*90*
Sub-Saharan Africa	*29*	*29*	*34*
Latin America & Caribbean	*80*	*110*	*140*
East Asia	*4*	*10*	*18*
South Asia	*37*	*68*	*107*
Transition Countries	157	159	179
Industrialised Countries	186	212	221

Source : WHO, 2003a

Worldwide, there has been a modest increase in the *per capita* consumption of milk since 1964-66 from 74 to 78kg/person/year (Table 1.3). This is predicted to rise to 90kg/person/year by the year 2030, with rises expected in all three categories of country (industrialised, transitional and developing). In terms of global importance, the increases (1965 to 1998) and the predicted increases (1998 to 2030) in

East Asia and South Asia are the most significant, because of their large populations. The predicted increase in Latin America and the Caribbean is also substantial.

Projected annual growth rates over a seven year time period are given in Table 1.4 for a number of countries for liquid and powdered milks. Data were unavailable for some countries (including the Indian sub-continent, Australasia and North America) and some categories of milk. A growth in production of liquid and powdered milk is predicted in all countries listed, but is considerably larger in China. Most countries are self-sufficient in milk: while Australia and New Zealand, and Europe produce more milk than they consume, SE Asia, the Middle East and Central America are net importers of milk (Goldberg & Herman, 2006).

Table 1.4
Projected percentage annual growth rates for milk, 2003 to 2010, for some regions of the world

	Liquid Milk	Whole Milk Powder	Skimmed Milk Powder
China	20	7	17
SE Asia	6	4	-
EU	4	4	-
CEEC (Non-EU)	3	10	10
Russia, Ukraine, Belarus	3	4	9
Latin America	3	-	-
Middle East, North Africa	3	-	12

- = data unavailable
Source : Goldberg & Herman, 2006

Changes in milk consumption in China, with its population of 1.3 billion people, will have a major impact on world milk production and consumption. Traditionally, milk has not been popular in China due to taste and dietary preferences. Difficulties in transporting fresh milk and the relative high cost of milk are other reasons for low consumption by Chinese people in the past. In 2003, *per capita* milk consumption was just 5.6kg/year (Fuller & Beghin, 2004), but this should be compared with the figure of below 1kg/year 20-30 years previously. Almost all the growth in consumption in China has occurred in urban areas, where consumption reached nearly 16kg/person/year in 2002, compared with less than 1kg/person/year in rural areas

(Fuller & Beghin, 2004). Consumption is twice as high in Beijing and Shanghai as in the southern city of Guangzhou. An important factor is that 87% of urban homes now have a refrigerator, since UHT milk is ten times the price of fresh bagged milk. Although China imports milk, home production doubled between 1997 and 2002 (Fuller & Beghin, 2004). These changes in China are due to the Chinese government's policy of promoting the dairy industry to improve the health of the population, and the rapidly changing dietary habits in the cities (Zhou *et al*., 2002). Very recently, there has been a major safety issue regarding milk production in China which may well affect consumption now and during the next few years. In September 2008, it became clear that melamine had been added illegally to infant formula and related dairy products (WHO 2008a; FAO, 2009). By December 2008, over 50,000 children in China had been hospitalized (WHO, 2008b).

1.4 The effect of the rise in non-alcoholic beverage consumption on milk consumption

Although information given in Tables 1.3 and 1.4 for industrialised and EU countries respectively, indicates an increase in milk consumption, in some European countries consumption has declined in recent years. The European countries which have recorded a decline in milk availability are mainly in the north and west of Europe (Trichopoulou *et al*., 2002). According to these authors, in Norway for example, total milk availability fell by 28% between 1986-88 and 1996-98, with 72% of the milk being low-fat milk in 1996-98. These authors also reported a rise in non-alcoholic beverage consumption over the same period; non-alcoholic beverage consumption being highest in those countries with the greatest decline in milk consumption.

An increase in non-alcoholic beverage (soft drink) consumption and a parallel decrease in milk consumption can be seen in Table 1.5, using data for English adolescents (Zohouri *et al*., 2004). Similar time trends for non-alcoholic beverage and milk consumption by children and adolescents have been reported by Heller *et al*. (1999) in the USA and by Sichert-Hellert *et al*. (2001) in Germany. The parallel rise in childhood obesity and non-alcoholic beverage consumption has been noted by health authorities. To quote WHO/FAO (2003): "The high and increasing consumption of sugars-sweetened drinks by children in many countries is of serious concern." The WHO classified the level of evidence linking sugars-sweetened soft drinks to obesity as 'probable',

although it should be recognised that the aetiology of obesity is multi-factorial. In addition, these drinks are a major source of free sugars in the diets of young people and the WHO/FAO (2003) classified the level of evidence linking free sugars to dental caries as 'convincing'. Thus, sugared non-alcoholic beverages are a risk for both general and oral health.

Table 1.5
Reciprocal changes over 20 years (1980-2000) in consumption (g/day) of milk and soft drinks (non-alcoholic beverages) in English adolescent children

	Males			Females		
	1980 (n=193)	**2000 (n=196)**	**Change (%)**	**1980 (n=212)**	**2000 (n=228)**	**Change (%)**
Milk	251	148	41 ↓	190	109	43 ↓
Soft Drinks	111	321	189 ↑	107	267	150 ↑

Source : Zohouri et al., 2004

1.5 Public health milk programmes and health implications

Although milk has been viewed as a nutritious food for millennia, the identification of 'protective factors' in foods dates from around 1918 (Southgate, 2000). This was the age of vitamin discovery: those of relevance to milk are vitamin A in 1913, vitamin D in 1922 and vitamin B_2 (lactoflavin) in 1916. By 1920, in the UK, 'school milk clubs' were promoted and paid for by local authorities and charities. The UK government, though, was reluctant to pay for the milk and it was not until 1940 that cheap milk was made available through the Maternal and Child Welfare Scheme and, eventually in 1944, free school milk (189ml each school day) was available to all schoolchildren in the UK. This ended, in the UK, in 1971 and, from then, the cost has been borne by local authority and parent. In 1977, the European Union (EU) introduced the School Milk Scheme, to encourage milk consumption by children, subsidising the cost of whole and semi-skimmed school milk.

More recently, the World Food and Agricultural Organisation (FAO) has sought to co-ordinate information on school milk programmes (Griffin, 2005), and it is intended that an International School Milk Information Centre be established based at FAO in Rome. Among the Centre's activities is the move to develop and strengthen school milk programmes internationally. Replies to a questionnaire to countries

about their programmes showed diversity in programme development, funding and control, and effect on national milk consumption. In Thailand, for example, school milk accounts for 25% of national milk consumption, compared with 4% in Sweden. State funding varies from total subsidy downwards. At the highest level, in Finland and Sweden, free milk is provided for children until they complete secondary education. The financial cost of such programmes can be crucial but should be weighed against short and long-term health benefits. In Kenya, the government-funded school milk programme provided 44 million litres in 1989, but only 3 million litres in 1997, before being abandoned (Griffin, 2005). The FAO has recently introduced a World School Milk Day, which is celebrated on the last Wednesday in September: over 30 countries participated in 2004 (Griffin, 2005). There is, therefore, support for public health milk, including school milk, programmes from both health and agricultural authorities.

The WHO/FAO report on diet, nutrition and the prevention of chronic disease (WHO/FAO, 2003) emphasised the importance of good nutrition in children. The ministries of health in countries have been urged to ensure that the mechanisms for intersectional collaboration are carefully considered. Strategies include taxation and pricing, food labelling, school lunch policies and support to nutrition programmes. An example which is relevant to the provision of milk can be found in Chile where, for over 50 years, the National Complementary Feeding Programme (PNAC) has provided powdered milk during pregnancy and lactation, and for the child powdered milk up to 2 years and a milk derivative until 6 years of age. One of the three main objectives of PNAC is to promote breast-feeding by providing supplements to mothers during pregnancy and lactation (Uauy and Kain, 2002). The national coverage is about 90% (Mariño *et al*., 2001).

Approximately one fifth of the world's children live in China. The central government has recognised the need to improve the nutrition of children, and a national school milk programme was implemented in 2000, covering 20 provinces, municipalities and autonomous regions (China Daily, 2001). This followed the approval by the State Council of China in 1997 of a National Action Plan for Nutrition and a successful trial, involving 2000 schools in five cities, which began in 1999 (Jun *et al*., 2004). In the words of Zhang Baowen, Vice-Minister of the State Ministry of Agriculture, "China has more than 200 million school students, and comprehensive implementation of its school milk programme is of far-reaching importance." (China Daily, 2001).

In many countries, there has been a move away from state subsidy of school milk. In New Zealand, for example, state support for the school milk scheme was discontinued in 1967. Since then milk, as a drink, has faced ruthless competition from soft drinks, such that milk consumption has declined in New Zealand by 30% during the past 20 years (Wham & Worsley, 2003). On the other hand, in recognition of the health risks posed by sugared non-alcoholic beverages, it is government policy in the UK, to provide only water, milk and fruit juice in vending machines on school premises (HM Government, 2007); this had been urged by WHO as part of school health promotion (WHO, 2003b; Kwan et al., 2005). In many countries, concern that intake of saturated fats from dairy foods needs to be reduced has resulted in semi-skimmed milk, containing 1.7% fat, being recommended in place of whole milk (Department of Health, 1994; Health Education Authority, 1995). Recent trials have shown that reducing the fat content still further to 1.0% has little effect on taste (Dairy Council, personal communication).

Milk is a valuable source of many nutrients. Considering growth, Baker *et al.* (1980) showed, in a randomised, controlled trial in the UK, that provision of free milk to children in school increased gain in height, compared with no milk provision. Although statistically significant, the 3% gain in height was considered modest. More attention is given to the role of milk as a provider of calcium and other micronutrients.

Calcium is essential for the development of bone and, to a lesser extent, teeth, and in many countries a high proportion of children consume less than the recommended amount of calcium. For nearly all children, milk is the major source of calcium. Over 30% of adult bone mineral is deposited in the skeleton during adolescence (Flynn, 2003). Recently, a considerable amount of research has been undertaken aimed at establishing whether calcium dietary supplementation (by milk is one option) leads to short term and long term health benefits (Flynn, 2003; WHO/FAO, 2003; Ginty & Prentice, 2004; Prentice, 2004; Lanou *et al.*, 2005). By short term is meant – optimising the peak bone mass, which occurs in early adulthood; by long term is meant — decreasing the risk of osteoporosis and osteoporotic fractures in older age. These two are linked since the WHO (WHO/FAO, 2003) considered peak bone mass to be a major determinant of risk of osteoporosis in later life.

Despite this research, many scientific uncertainties remain and further information is required to clarify the role of dietary milk in improving and maintaining bone health (Lanou *et al.*, 2005): it is an area of considerable public health significance (Ginty & Prentice, 2004) and it is clear that each country has to determine the need for milk supplementation in children, adolescence and adulthood, since there are many reasons for doing so and the strength of each of these varies between populations. In this respect, it should be remembered that milk is a 'cocktail of nutrients' (Gurr, 1994) and not just a provider of calcium.

1.6 Milk intolerance

Many foods can cause unwanted effects and milk is no exception. There are two broad categories of unwanted effect – food intolerance (or non-allergic food hypersensitivity) and food allergy (WHO, 2006). Allergy is characterised by an abnormal immunological reaction, while food intolerance is not. As far as milk is concerned, an IgE-mediated allergic reaction is caused by one or more of the milk proteins, and milk intolerance is due to an inability to digest lactose (lactose intolerance). It is difficult to determine prevalence of food allergies, but it is considered to affect about 1 to 4% of individuals (Mills *et al.*, 2007): milk is one of the more common food allergens in children. Milk protein allergy is much less common that lactose intolerance.

Lactase is one of several disaccharidases produced in the small intestine: it digests lactose in preparation for absorption. Lactase activity is highest in infancy (when it is most needed) and then falls rapidly. However, it is rarely lost completely and nearly all children and adults retain the ability to digest lactose. This ability varies somewhat between races, being highest in Aryan and some African racial groups. 200ml of cow's milk contain about 9g lactose, which is well tolerated by most people. Physical damage to the small intestine, due to gastroenteritis for example, often causes secondary lactase deficiency. Lactose undigested in the small intestine is fermented in the lower intestine causing flatulence, pain and diarrhoea. In people with very little lactase activity, digestion improves when milk is taken with food as this prolongs transit time. Yoghurt and yoghurt drinks contain much less lactose than fresh milk and are therefore better tolerated by those with little lactase activity (Cummings, 2000).

There is considerable antigenic similarity between proteins in cow's milk and proteins in milk of goats and sheep, and even soya milk (but not human milk). Because of the myriad of antigens in food, diagnosis of milk protein hypersensitivity, and indeed diagnosis of lactose intolerance (because it may be secondary to many diseases), need to be made by competent professionals. Moderate intake of cow's milk is well tolerated by the vast majority of children and adults.

1.7 Milk and dental health

1.7.1 *Introduction*

Some of the earliest investigations regarding milk and dental health were carried out by Sprawson (1932a,b,c, 1934, 1947), who concluded that milk improved oral health. Since then, much research, both clinical and non-clinical, has been published and, almost uniformly, milk is not seen as a cause of dental caries. The UK Department of Health (1989) COMA report on dietary sugars and human disease concluded that: 'Although lactose alone is moderately cariogenic, milk also contains factors which protect against dental caries, so that milk without added sugars may be considered to be virtually non-cariogenic'. In a review of diet, nutrition and the prevention of chronic diseases, WHO (WHO/FAO, 2003) classified evidence linking diet to several diseases including dental caries: the strength of the evidence for a 'decreased risk' of dental caries from milk was classified as 'possible'. Human milk will not be considered in this review: for further information see Rugg-Gunn (1993).

1.7.2 *Review of evidence – milk and dental caries*

Eighty per cent of carbohydrate in milk is lactose; various other components of milk have been considered to be protective against dental caries, namely the minerals, casein and other proteins and lipids. Cow's milk contains about 4.5g lactose per 100g milk (Table 1.1). This amount could be sufficient to classify milk as cariogenic, but there is much evidence that lactose is the least cariogenic of the common dietary sugars (Rugg-Gunn, 1993). In addition, the high concentrations of calcium and phosphorus in milk will help to prevent dissolution of enamel (which is largely calcium and phosphate) and other factors may be protective as well. Thus, it is possible that milk could be caries-promoting (due to the lactose content), caries-preventing, or somewhere between these two.

The epidemiological evidence from older studies relating consumption of cow's milk to dental caries experience is equivocal. Lacto-vegetarian children were reported by Gillman & Lennon (1958) to have lower caries experience than other children, although Linkosalo & Markkanen (1985) reported no difference. While Zita *et al.* (1959) and Rugg-Gunn *et al.* (1984) recorded positive relations between consumption of cow's milk and dental caries experience in their epidemiological surveys, in more recent studies, Petridou *et al.* (1996), Petti *et al*. (1997) and Levy *et al*. (2003) reported that milk consumption in children was associated with lower caries experience. The study of Petti *et al*., in Italian children who, on average, drank about 209ml of milk per day, showed that the inverse relation between milk and caries was strong in children with the highest frequency of sucrose consumption. In another publication, Marshall *et al*. (2003) reported that 'milk consumption had a neutral association with caries' experience in young American children, and Mattos-Graner *et al*. (1998) reported that, in young Brazilian children, milk in bottles was not associated with dental caries unless sugar, or sugar and cereal, were added to the milk. Recently, Sohn *et al.* (2006), using the US NHANES data, reported that children drinking milk had less caries than those drinking carbonated soft drinks, but a similar caries level to those consuming plain water or juice. Only the studies of Petti *et al.* (1997), Levy *et al.* (2003), Marshall *et al.* (2003) and Sohn *et al.* (2006) used multivariate analyses to control for possible confounding factors, and the results of other studies should be interpreted cautiously.

Evidence from animal experiments not only indicates that cow's milk is non-cariogenic, but also strongly suggests an anti-cariogenic effect. The extensive studies of Stephan (1966) labelled milk as non-cariogenic. Earlier indications that milk was anti-cariogenic (Sperling *et al.*, 1955; Shaw *et al.*, 1959) were followed up by Reynolds & Johnson (1981). They found that supplementation of a cariogenic diet with cow's milk reduced substantially dental caries incidence, and that this was not due to reduced consumption of the cariogenic diet. In a review, Bowen & Pearson (1993) came to the same conclusion. The caries-preventive effect of milk, in comparison with water, has also been reported by König (1960), Bánóczy *et al*. (1990) and Stösser *et al*. (1995a). A severe test of the cariogenic or cariostatic properties of milk was carried out by Bowen *et al.* (1991), using desalivated rats which are therefore much more caries-susceptible. The rats given milk

or lactose-reduced milk remained essentially caries-free, while those given sucrose or lactose in water developed caries. Using the same model a few years later, Bowen *et al*. (1997) found a similar result regarding the very low caries potential of milk. The authors concluded 'that [cow's] milk does not promote caries, even in the highly caries-conducive environment engendered', and 'that milk or lactose-reduced milk can be used safely by hyposalivatory patients as a saliva substitute'. More recently, the same group (Bowen and Lawrence, 2005) reported a further study which used the same animal model, stating "Our observations confirm and extend our previous results that showed very clearly that cow milk is essentially noncariogenic."

Several studies have shown that the fall in plaque pH after drinking milk is negligible (Jenkins & Ferguson, 1966; Frostell, 1970; Edgar *et al*., 1975; Mor & McDougall, 1977; Rugg-Gunn *et al.*, 1985). In the studies of Rugg-Gunn *et al.* (1985), 14 volunteers rinsed their mouths with cow's milk, human milk, lactose solution, or sucrose solution. Sucrose solution caused substantial falls in plaque pH, while the milks depressed plaque pH only slightly.

Bibby *et al.* (1980), using an artificial mouth test system (Orofax), found that the inclusion of milk solids reduced the cariogenicity of sugar-containing foods, while similar results were obtained by Thompson *et al.* (1984) using an enamel slab intra-oral device. Jensen *et al.* (2000), using a similar system which held slabs of enamel or dentine in the mouth of volunteers, showed that milk (with three levels of fat content) was not cariogenic for enamel or dentine. In addition to the plaque pH studies mentioned above, Jenkins & Ferguson (1966) conducted *in vitro* comparisons of 4 per cent lactose solutions and cow's milk. They concluded that, within the limits of their experiments, their results 'gave no grounds for suggesting that milk has a local effect on the teeth which would favour caries', and suggested that the negligible fall in plaque pH was partly due to milk's high buffering power, and the low level of dissolution of test enamel was due to the protective action of milk's high levels of calcium and phosphate. Rugg-Gunn *et al.* (1985) also reported that both cow's milk and human milk protected enamel from dissolution in *in vitro* experiments, compared with sucrose or lactose, but that human milk was less protective than cow's milk, as would be expected from their different calcium and phosphorus content. Four further *in vitro* studies investigated the caries preventive effect of milk. McDougall (1977) and Mor and Rodda (1983)

showed that (a) demineralisation of enamel in an acid buffer was reduced by intermittent exposure to milk, and (b) that milk aided the remineralisation of demineralised enamel. Arnold *et al*. (2003) showed that milk inhibited enamel demineralisation compared with saline or a remineralising solution, and Ivancakova *et al*. (2003) reported that milk reduced the rate of root caries progression.

1.7.3 *The actions of the constituents of milk*

The principal constituents of whole cow's milk, other than carbohydrate (4.5%), are fat (up to 3.9%), protein (3%), calcium (118mg/100g) and phosphorus (92mg/100g). Oral clearance is influenced by the ingredients of foods as well as by salivary flow, action of the tongue, cheeks and lips, and by other factors. One ingredient which accelerates oral clearance is fat (Bibby *et al.*, 1951; Swenander Lanke, 1957; Frostell, 1969; Brudevold *et al.*, 1990). This is probably due to a physical action of holding all the particles together. Proteins are adsorbed well onto enamel surfaces: Pearce & Bibby (1966) tested 11 proteins and found that casein and globulins were adsorbed in greatest amount and albumins the least.

Casein is a phosphoprotein and represents about 80% of all proteins present in milk: it is considered to be one of the main ingredients responsible for the caries-protective action of milk (Johansson, 2002). Decreases in the development of dental caries in rats have followed increases in the casein content of their diet (Bavetta & McClure, 1957; Holloway *et al.*, 1961; Reynolds & Black, 1987a,b*).* The reasons for the caries preventive effect of caseins have been studied by Vacca-Smith *et al*. (1994) and Vacca-Smith & Bowen (1995, 2000). Casein appears to prevent adherence of salivary components and bacteria to enamel and pellicle, and to reduce the activity of glucosyltransferase, thus reducing glucan formation and plaque adherence. In a series of rat caries experiments, Guggenheim *et al.* (1999) demonstrated the marked caries preventive effect of 'milk micellar casein' when incorporated into a cariogenic diet. Large reductions in the proportion of S. *sobrinus* were observed leading the authors to conclude that the micellar casein interfered with the adhesion of some plaque bacteria.

Harper *et al.* (1987) questioned whether casein was the most caries-protective constituent in milk, following experiments in rats which tested the caries-reducing potential of three mineral-rich milk concentrates with various levels of whey protein, calcium, and phosphorus,

but negligible levels of casein. The results suggested that considerable protection could be afforded by calcium and phosphate compounds in the absence of casein. While the caries protective effect of phosphates in the diets of rats needs to be interpreted with care (Rugg-Gunn, 1993), the results agree with other *in vitro* studies which demonstrate the favourable role of the high concentrations of calcium and phosphorus in milk (Jenkins & Ferguson, 1966; Rugg-Gunn *et al*., 1985). Support for this view came from the studies of Grenby *et al.* (2001) which showed, in *in vitro* experiments, that the 'removal of lactose, fat, casein and other proteins had little influence on the protective effect of the milk fractions. Besides calcium and phosphorus, milk contains other more powerful protective factors against demineralisation, which were identified as proteose-peptone fractions 3 and 5.'

1.7.4 *Addition of sucrose and cocoa to milk*

In some countries, it is common to flavour milk, especially milk for children, with sugar and other flavourings. The use of flavoured, fluoridated milk has been reported in Hungary (Bánóczy *et al.*, 1983, 1985; Gyurkovics *et al.*, 1992), the USA (Rusoff *et al.*, 1962; Legett *et al.*, 1987) and China (Bian *et al.*, 2003). In most countries, the sugar added is sucrose and the most common other flavouring is cocoa. It is reasonable to assume that adding sucrose to milk will increase cariogenicity, but at what concentration will the added sucrose overcome the caries-protective properties of plain milk, is an interesting question. The question is complicated by the knowledge that cocoa itself has caries-protective properties (Gustaffson *et al.*, 1954; Rugg-Gunn, 1993).

The effect of adding sucrose to cow's milk has been investigated in a variety of studies. In an uncontrolled, observational epidemiological survey, Mattos-Graner *et al.* (1998) recorded that children who had drunk milk with added sugar had higher caries experience than children who had drunk milk with no added sugar. Dunning & Hodge (1971) reported results of a two year clinical trial in American children and young adults. Caries increment was slightly higher (of borderline statistical significance) in children drinking milk with 6% sugar added, compared with children drinking plain milk. Using the rat caries model, Bowen & Pearson (1993) studied the effect of adding 10% sucrose or 10% fructose to milk. There was little difference in cariogenicity between these two sugars and both were more cariogenic than milk alone

but less cariogenic than 10% sucrose in water. A further aspect of this experiment showed that 4% lactose in water was of very low cariogenicity. In a second series of experiments, these authors found that caries development was similar in groups of animals receiving 2%, 5% or 10% sucrose in milk. This caries development was greater than that recorded for rats receiving milk alone and less than that recorded for rats receiving 10% sucrose in water. From these results they concluded "..that lactose has little capacity to promote caries.."; "It is clear that the addition of as little as 2% sucrose to milk enhances the caries activity of milk even though the milk-sucrose solutions are significantly less cariogenic than the water-sucrose solutions." and ".. the practice of adding any sugar to milk should be discouraged.". A small and statistically non-significant increase in enamel softening was recorded by Thompson *et al.* (1984) when 5% sucrose was added to cow's milk, in an intra-oral enamel slab experiment. However, softening was much greater when the enamel was exposed to 5% sucrose in water. Two plaque pH studies have indicated that the addition of 5% and 10% sucrose to cow's milk increased acidogenicity, but this increase was less than when sucrose was added to water (Mor & McDougall, 1977; Moynihan *et al.,* 1996).

There appears to be only one study comparing the effect on caries development of cocoa with any other flavouring. In the intra-oral enamel slab experiment of Thompson *et al.* (1984), mentioned above, there was a hint that milk flavoured with cocoa caused less enamel softening than milk flavoured with strawberry; both milks also containing about 5% sucrose.

Thus, it is probable that adding sugar to milk increases risk of caries development; the concentration of added sugar at which caries development might begin is uncertain but may be as low as 2%. There is too little information on the effect of cocoa flavouring to draw conclusions.

1.7.5 *Conclusion*

Cow's milk can be considered non-cariogenic. Evidence from several types of study contributes to this conclusion. While epidemiological evidence is equivocal, information from animal experiments shows clearly milk's non-cariogenicity and, indeed, the caries-protective role of milk. *In vivo* and *in vitro* demineralisation and remineralisation (enamel slab) experiments also indicated the low cariogenic potential of milk and also demonstrated its caries-protective role. These actions

would appear to be due to (a) lactose being the least cariogenic of dietary sugars, (b) the protective role of casein and possibly fats, and (c) the protective role of calcium and phosphorus. Milk's favourable dental profile is likely to be compromised by the addition of sucrose.

1.8 Summary

Milk is an essential food in our early life. Cow's milk is now the most commonly consumed milk and is a highly nutritious food. Most milk is pasteurised, although ultra-heat-treated (UHT) and sterilised milk have better shelf-life. Powdered milk is easier to transport and store and has good shelf-life. World consumption of milk is forecast to rise; dramatically so in some countries. Many health authorities subsidise the provision of milk to children, and school milk programmes exist in many countries: these programmes are supported by the WHO and the FAO. Lactose intolerance depends on the quantity of milk consumed, and virtually all children tolerate moderate amounts of milk. Lactose intolerance is often secondary to gastro-enteritis. Cow's milk is non-cariogenic. Despite containing about 4% sugar, other factors in milk ensure that it is not a threat to oral health; indeed, it is more likely to be protective. Milk's favourable dental profile is likely to be compromised by the addition of sucrose.

2 Clinical studies

J. Bánóczy and A. J. Rugg-Gunn

2.1 Introduction

Some years after the successful introduction and initial good results of water fluoridation, the possibility of another vehicle for fluoride emerged. The idea of the fluoridation of milk was mentioned - nearly at the same time – in the first half of the 1950s, in three other rather distant places: Japan, USA and Switzerland.

Imamura (1959) in Japan began his experiments with fluoridated milk in the year 1952, Rusoff *et al.* (1962) in the USA in 1955, and Ziegler (1956, 1964) and Wirz (1964) in Switzerland in 1957/58. However, as most of the publications originated from the Swiss group, this literature, being more accessible, soon became well known and disseminated. Eugen Ziegler, a Swiss paediatrician, was the first to publish his ideas on milk fluoridation in 1953 describing thoroughly his method of household and school milk fluoridation, justified by physiological and toxicological considerations.

2.2 Early studies

Imamura (1959) was, however, the first to publish results of a clinical trial with fluoridated milk. In post-war Japan experiments were conducted on prevention of dental caries by adding fluoride tablets to school meals. Imamura started in 1952 to give NaF solution to schools meals at Yokohama City primary schools, where liquid dishes like milk and soup were served for 150 to 180 days a year. The daily dosage was 2.0 to 2.5 mg NaF. The pupils of the control schools drank their meals without added fluoride.

After five years, 167 eleven year old children in the experimental school, and 141 children from the comparison school were examined. The overall caries reduction for permanent teeth was 34% for those

who began school in 1952, while for the 1953 intake there was a 29% reduction. Considering the caries reduction of first permanent molars, this ranged from 14% to 20% during the same period. No adverse effects such as fluorosis, were noted, and laboratory investigation showed an increase of fluoride content in the enamel of the second primary molars in the fluoride group compared with the control children.

The second study, published in 1962 by Rusoff *et al.* came from Baton Rouge, Lousiana, USA. A school lunch programme started in 1955 with 171 children comprising a treated and a control group. A half pint of fluoridated milk containing 1 mg fluoride as 2.2 mg NaF (=285 ml milk containing 3.5 ppm F) per school day was given to children aged 6-9 years at the beginning of the study. During the vacation periods, parents of the test group children were supplied with a NaF solution, to continue fluoride dosage of milk at home.

After 3.5 years, when 65 children in the test and 64 children in the control groups were examined, an overall caries reduction of 35% in permanent teeth was recorded. However, for subjects aged 6 years at the beginning of the experiment, there was a 70% difference between the test and control groups. Eighteen months after the cessation of fluoride ingestion, at the final examination, a carry-over effect was observed: a significant difference of approximately 50% in caries rate in favour of the treated group.

In Switzerland, Ziegler (1953) proposed fluoridation of milk for small children, based on the assumption that the introduction of fluoridated salt will not be sufficient for caries-preventive purposes, due to the low intake of salt in this age-group. Epidemiological studies performed in Winterthur by Wirz in the school years 1956/57 and 1957/58 supported the necessity of introducing a new method of fluoride prevention: milk fluoridation. In about five preparatory years' work - due to the lack of agreement with the dairy industry – Ziegler (1956, 1959) elaborated his method of milk fluoridation by adding a 0.22% NaF solution to household milk. The 2.2% NaF solution, sealed in plastic bottles, was available on prescription in the pharmacies for the parents involved in the experiment on a voluntary basis. By using a measuring device, 1cc solution was added to one litre of milk, equalling 1 ppm F. Thus, the intake for pre-school children was about 0.5 mg fluoride and for pupils participating in drinking school milk intake was about 0.7 mg fluoride.

The fluoridated household milk was distributed to 749 test children, using 553 others as controls, all of whom were aged 9-44 months at the beginning of the trial. The examinations after six years (Wirz, 1964; Ziegler, 1964) showed a caries reduction of 17% deft to 30% defs in primary teeth, and 64% DMFT to 65% DMFS in permanent molars. The percentages of caries-free dentitions of primary and permanent teeth, showed significant increases in the fluoride group, compared with the control group.

2.3 The Borrow Foundation

A major change in research into and promotion of fluoridated milk for children's caries prevention occurred in 1971 when Edgar Wilfred Borrow of Padnell Farm, Cowplain, Portsmouth, U.K., established a charitable foundation. The 'Trust Deed', drawn up with the assistance of C.F.J. Baron, B.S. Konikoff and E.R. Churcher, named the body 'The Borrow Dental Milk Foundation', expressing its objectives in 12 points. The main purpose of the Foundation was: "to promote the study of and research into the fluoridation of milk for human consumption, and to publish and to disseminate to the public the results of such study and research", and "in furtherance of the objects specified above, to help its implementation by grants, equipment, lectures, scientific papers and every possible means".

In 1993, the original 'Trust Deed' was amended widening the objectives of the Foundation to include "the protection of dental health and the promotion of education in dental care for the benefit of the general public, and in particular…..to promote research into…I./ the use of fluoride in the prevention of dental caries and in particular its use in milk and milk products: II./ the effect of nutrition on teeth and in particular the (nutritional) effect of milk and milk products." In 2002 the name of the Foundation was changed to 'The Borrow Foundation'.

Based on data from the early clinical milk fluoridation studies and as a result of the promotional activities of The Borrow Dental Milk Foundation to support clinical schemes, there seemed to be justification for further investigation of this means of providing community-based fluoridation for children.

2.4 Scotland

The results and conclusions from earlier home-based (Ziegler, 1956), or school-based (Rusoff *et al.*, 1962; Wirz, 1964; Ziegler, 1964) milk studies had been criticized due to the small number of participants and non-matching between test and control groups' caries prevalence at baseline (WHO, 1970) and it was deemed worthwhile to attempt to assess the potential caries-inhibiting benefits of daily fluoridated school milk in a study beginning in Glasgow in 1976, which employed adequate pre-trial stratification and double-blind methods (Stephen *et al.*, 1981, 1984).

2.4.1 *Materials and methods*

It was decided to offer fluoridated milk trial participation to all children in first grade classes (aged 4 y) at four state primary schools situated in a predominantly low social class area. These schools were all within 3 km of a dairy which was already involved in a daily milk delivery service for the local authority concerned. Thus all potential subjects were aged between 4 year 6 months and 5 year 6 months at the outset; the vast majority belonging to social classes IV and V, only a few being in social class III. All children, therefore, came from a social class cohort, the constituent groups of which had previously been shown to have similar caries experience and dental attitudes.

After appropriate regional, district and education authority permissions had been obtained, and the proposed protocol discussed with the head teachers in the schools concerned, all parents of eligible children were circulated with details relating to the study. As a result of a letter of explanation, parental permission was obtained for 187 subjects, 83 per cent of those eligible.

As milk was to be distributed on only 200 days per annum, it was decided to add 1.5 mg F to each 200 ml plastic test pack, identified from the placebo pack by colour alone. The coding was held by the District Dental Officer who was involved in neither the daily distribution nor the clinical examinations. The sodium fluoride concentrate was prepared in 300 ml sterile containers, the contents of one container being added to 5 gallons of milk daily, giving a final concentration of approximately 7 ppm F in each 200 ml test pack.

Milk distribution and all clinical examinations were on a double-blind basis. A distributor was responsible for the uplifting of the test and placebo packs from the dairy, and the 'milk round' was timed so that

all children received their milk at least 15 minutes before the mid-morning break, thus enabling a reasonable period for topical fluoride exposure prior to possible ingestion of other food or drink. Children sucked the test or control milk through straws. Baseline clinical and radiographic examinations were performed in the University of Glasgow Dental School's Mobile Research Unit, and radiographs read separately from the clinical data. Thereafter, subjects were graded into three groups according to baseline dmft scores of 0-4, 5-8 or 9-12, and then divided equally into 'test' and 'control' groups and into four groups by 3 month wide age spans. The initial and repeat clinical examinations, conducted blind, were carried out annually by the one examiner whose repeat caries penetration examination variations had been shown to be insignificant ($P>0.9$).

Fluoride monitoring, using an Orion specific ion electrode, was carried out daily at the dairy and on a random basis by the U.K. Department of the Environment. In addition, a weekly analysis was also performed at the University Department of Oral Medicine - 96.4% of samples tested being within the desired range.

For the laboratory-based urine studies, children were asked to supply a sample at the end of a school week towards the end of the second year of the trial. With the aid of parents, urine was obtained in the morning 1 h after the fasting overnight urine had been discarded and following a glass of water, taken before breakfast. Samples were collected soon after the arrival at school and transferred to the laboratory where they were frozen until analysed.

2.4.2 *Results*

Of children receiving parental permission to participate, 94 were placed in the test group and 93 in the control. At the baseline examination in February 1975, only 46 permanent first molars (6%) were erupted, 23 in each group. The mean modified dmft score for the test children was 4.3 and for control children, 4.5, after stratification had been completed.

The experiment began in 1976/77 with 82 test and 83 control children and ended in 1980/81 with 50 and 56 children in the two groups respectively. The attrition was due partly to the moving of children to other schools, and partly to discontinuation of drinking school milk by the age of 10 years. The mean milk consumption in both groups varied between 91 and 96% of the total milk provided each year in both groups.

Details of the primary caries status in terms of the modified dmft and dmfs indices at baseline (1976) and at the first three annual re-examinations, are given in Table 2.1. No significant differences were found between the test and control data at any of these times.

Table 2.1
Primary caries status expressed as modified mean dmft or dmfs values for the test (T) or control (C) children at the 1976 baseline and at the three subsequent re-examinations

	1976		1977		1978		1979	
	T	**C**	**T**	**C**	**T**	**C**	**T**	**C**
dmft	4.3	4.5	5.4	5.2	5.9	6.2	6.3	6.0
dmfs	12.4	12.1	17.6	16.3	19.5	21.4	22.1	22.1

Source: Stephen et al. (1984)

The DMFT and DMFS values relating to all erupted teeth, along with their respective differential data, are shown in Table 2.2. Thus, while both groups started with 1976 baseline DMFT and DMFS scores of zero, only after 4 years of milk consumption did results differ statistically significantly, when the mean DMFT of the test group was 1.65 (median 1.36) compared with the mean control value of 2.56 (median 2.75; $P<0.05$). For mean DMFS indices, a 42.0% difference was found between the mean test score of 2.94 (median 1.67) and the 5.0 mean (median 4.25) for control subjects, but this was not significant ($0.1 > P > 0.05$).

By the fifth year, the mean DMFT of the fluoridated group had risen to 2.14 (median 2.20) compared with a rise of 3.11 (median 3.17) in the non-fluoridated group; a 31.2% difference *(*$P<0.05$*).* For DMFS scores, the difference was then 43.1% *(*$P<0.001$).

When only those permanent teeth which were originally unerupted at the baseline examination were considered, the data shown in Table 2.3 resulted. Here, the fifth year mean DMFT test value of 1.94 (median 2.0) was also highly significantly different from the control value of 3.02 (median 3.0). This represented a 35.8% difference *(*$P<0.02$). The mean DMFS index for the fluoridated group was 3.29 (median 2.1) compared with the mean surface score of 6.33 (median 5.0) for the non-fluoridated group, a difference of 48.0% *(*$P<0.01$).

Table 2.2

Number of permanent teeth, mean DMFT, mean DMFS and percentage differences (Δ %) between test (T) and control (C) teeth at 1976 baseline and five subsequent examinations

	1976		1977			1978			1979			1980			1981		
	T	**C**	**T**	**C**	Δ%	**T**	**C**	Δ%	**T**	**C**	Δ%	**T**	**C**	Δ%	**T**	**C**	Δ%
n (teeth)	23	23	304	278	-	718	687	-	844	828	-	676	834	-	838	918	-
DMFT	0	0	0.33	0.35	5.0	0.79	1.15	31.3	1.54	1.90	19.0	1.65	2.56	35.5**	2.14	3.11	31.2*
DMFS	0	0	0.35	0.36	2.8	1.40	1.58	11.4	3.08	3.17	2.8	2.94	5.00	42.0	3.76	6.61	43.1***

* *P*<0.05, ** P<0.02, ****P*<0.01

Source: Stephen et al. (1984)

Table 2.3

Number of permanent teeth (excluding permanent first molars which were erupted at baseline), mean DMFT, mean DMFS and percentage differences (Δ%) between test (T) and control (C) teeth at five annual examinations

	1977			1978			1979			1980			1981		
	T	**C**	Δ%	**T**	**C**	Δ%	**T**	**C**	Δ%	**T**	**C**	Δ%	**T**	**C**	Δ%
n (teeth)	284	266	-	702	671	-	824	810	-	663	816	-	823	904	-
DMFT	0.40	0.43	7.0	0.69	1.04	33.6	1.33	1.70	21.8	1.58	2.37	33.3**	1.94	3.02	35.8**
DMFS	0.43	0.45	4.4	1.09	1.26	13.5	2.93	2.99	2.0	2.85	4.72	39.6*	3.29	6.33	48.0***

* *P*<0.05; ***P*<0.02; ****P*<0.01

Source: Stephen et al. (1984)

Finally, in relation to permanent first molar interproximal data, seven surfaces were carious in the test group compared with 31 in control children, giving a 74.6% reduction in favour of subjects receiving fluoridated milk *(P*<0.001).

2.4.3 *Discussion*

After five years, this double-blind clinical study resulted in statistically significant 31.2% DMFT and 43.1% DMFS reductions in permanent tooth caries incidence of the 50 test children remaining out of the initial 94 who began the trial in 1976. While it had been hoped that no test or control subjects would have had any erupted permanent first molars present at the baseline examination, as shown in Table 2.2, 46 of these teeth had appeared in the mouths of these 4½ - 5½ year olds when they were seen at baseline. Following the stratification procedure, there were 23 such teeth in each group and, when these were excluded from the above DMFT and DMFS calculations, the differences between mean values increased (Table 2.3). Prior to the fourth year, earlier DMFT reductions, while being in favour of fluoridated rather than non-fluoridated subjects, did not attain statistical significance.

In order to determine whether those children who left the study might have influenced the final differences between test and control data, baseline values relevant to the 44 subjects in the former group and the 38 in the latter were regenerated. As a result of these calculations, it was found that the mean ages did not differ significantly (t = 0.26). The baseline mean dmft score of the missing test children was 4.66 (±SD 3.18), while that for the controls was 3.47 (±SD 2.87), a non-significant difference. Likewise, the mean test dmfs value of 13.02 (±SD 12.0) was again higher than that of the controls (9.58±SD 10.2), but not significantly so. Thus, both primary indices indicate that the inclusion of these subjects throughout the whole study would have been unlikely to have altered the result obtained.

2.5 **Hungary**

In Hungary, the opportunity arose in 1978, to conduct a clinical milk fluoridation study in kindergarten children aged 2-5 years at outset, participants being inhabitants of the self-contained Fót children's community, located 3 km north of Budapest. Here, in a closed society, about 1,000 normal, healthy children aged 2-18 years lived, the majority of whom had been abandoned by their parents. They attended the internal

school and left the Institution only for one or two summer vacation months. Thus the homogeneity and standardized living conditions in Fót seemed ideal for establishing such an early investigative programme and, one year later, it was extended to include primary school pupils aged 6-12 years.

2.5.1 *Materials and methods*

Prior to commencement of milk fluoridation, the F concentration of the Fót drinking water was determined and found to be 0.03 ppm. The F content of milk and milk products consumed in the home was assessed as 0.02 ppm, and these F determinations of water and milk were monitored throughout the study period. Milk fluoridation was implemented in early 1979. As each child consumed 200 ml milk or cocoa-milk daily for breakfast, 0.4 mg F was added to this volume for the kindergarten children and 0.75 mg F for primary school children. The fluoride aliquots (as NaF solution) were prepared in advance by the pharmacy of the Semmelweis University of Medicine, Budapest.

Thereafter, trained personnel added each F dose into the corresponding amount of milk and stirred thoroughly for at least 10 min, after which it was consumed within 30 min.

Urinary fluoride excretion was determined prior to initiation of the study, then weekly and later monthly, in urine samples collected from randomly selected children. In 1985 and 1986, due to renovation of the Institute's kitchen, fluoridated milk could not be provided. However, in 1987, its delivery was reinstated twice weekly but, in 1990, as a result of establishment re-organization, the programme was terminated.

Dental examinations were performed prior to initiation of the study (1978), then annually in December of each year. The monitoring was carried out by four dentists who were calibrated previously. Subjects were examined in a dental chair, using artificial light, a dental mirror and sharp explorer. Decayed, filled and missing teeth were diagnosed according to WHO (1979) criteria. No radiographic investigations were performed, and dmft, dmfs, DMFT and DMFS indices were calculated. Statistical analyses employed Chi-square and two tailed t-tests.

The data were evaluated after three, five and ten years of F milk consumption (Bánóczy *et al.*, 1983, 1985; Gyurkovics *et al.*, 1992). The

data were analysed longitudinally, values for the yearly examinations being correlated with the length of fluoridated milk consumption, and compared horizontally with those of a control group who lived under similar conditions. The aims of the analyses were to assess the number and ratio of caries-free children; the caries reduction in the primary and permanent dentition, as correlated with the participants' starting ages and their duration of fluoridated milk consumption.

2.5.2 *Results*

After three years, the data for 936 children were examined, although for follow-up purposes only data from 269 were analysed. At five years, 165 subjects aged 7-12 years were contactable. Of these, 72 had been involved for five years, while 93 had participated for four years in the programme. By ten years, 162 institutionalized children aged 7-14 years were available for follow-up.

The number and ratio of children with a caries-free primary dentition at the five year evaluation showed only a small difference in the group then 7-10 years of age. However, for this same age-group, a clinically, and statistically highly significant ($P<0.001$) difference in the percentage of subjects in the test group with caries-free permanent teeth (59%) was observed, compared with the control group (17%). No differences were seen in those aged 9-12 years, who had been drinking milk for only four years.

After ten years of milk fluoridation, only permanent tooth status was evaluated. In the 12 and 14 year old controls, no caries-free children were found, whereas in the corresponding test group nearly 20% of subjects were caries-free.

With respect to the caries reductions achieved in the primary dentition, these were evaluated after five years (Group T_2), and four years (Group T_1) of milk fluoridation (Table 2.4). The comparison of the dmft and dmfs mean values between test and control groups showed statistically significant differences in the 7-10 year olds. For both indices, mean values were 40% lower in the T_2 (test) group, than in the control group. However, the differences between the T_1 (test) and control group (9-12 year old) primary teeth values showed neither statistical, nor meaningful clinical variations.

Table 2.4
Comparison of dmft and dmfs mean values in the test (T) and control (C) groups after 4 (T_1/C_1) and 5 (T_2/C_2) years of milk fluoridation. Sig = statistical significance.

Group	Age (y)		N	dmft	Sig	dmfs	Sig
	At examination	At start of milk F					
T_1	9-12	5-8	83	1.42	N.S.	2.67	N.S.
C_1	9-12	-	64	1.69		2.77	
T_2	7-10	2-5	69	2.40	P<0.001	3.79	P<0.001
C_2	7-10	-	81	4.01		6.40	

Source: Bánóczy et al. (1985)

The caries reductions in first permanent molars were assessed after three and five years. Statistical analyses after three years showed significant negative correlations between DMF means and length of fluoridated milk consumption in subjects aged 5-6 years at time of evaluation. The caries reduction after three years was 74% (P<0.001). In 7-9 year olds, the permanent first molar caries reductions were less and statistically non-significant.

Data in Table 2.5 show that, after five years (Group T_2) and four years (Group T_1) of milk fluoridation, a statistically significant difference existed between first permanent molar caries for Group T_1 compared with its control Group C_1 (P<0.001), as well as between Groups T_2 and C_2. In Group T_1, the combined DMFT mean values were 25%, and the DMFS means 36% lower than those of the C_1 Group. The T_2 Group showed a clinically greater caries reduction, the mean DMFT being 54%, and the mean DMFS 53% lower than the corresponding values of the control group (C_2). The youngest age group (7 year) had the greatest DMFT reduction of 85%.

Changes in total DMFT and DMFS mean values at the fifth year assessments are shown in Table 2.6. After five years, the T_2 and C_2 group data were highly significantly different. DMFT and DMFS values respectively were 60% and 67% lower in the T_2 group. Furthermore, in each individual age group (except 9 year olds), the differences were also statistically significant, the greatest reductions being in the younger cohorts. For the four year test (T_1) and control (C_1) groups, statistically significant differences were found for the total mean

Table 2.5

Comparison of DMFT and DMFS mean values of first permanent molars between test (T) and control (C) groups, after 4 (T_1/C_1) and 5 (T_2/C_2) years of milk fluoridation. Sig = statistical significance.

	Age in years									
Group	**9**	**10**	**11**	**12**	**Total 9-12 y**					
	DMFT	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**
T_1	2.00		2.50		2.70		2.22		2.44	
		N.S		P<0.05		P<0.01		P<0.01		P<0.001
C_1	2.43		3.44		3.67		3.43		3.25	
	DMFS	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**
T_1	2.00		2.80		2.80		3.05		2.79	
		N.S.		P<0.01		P<0.001		N.S.		P<0.001
C_1	3.38		5.37		4.50		4.00		4.34	
Group	**7**	**8**	**9**	**10**	**Total 7-10 y**					
	DMFT	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**
T_2	0.26		0.68		1.94		2.42		1.04	
		P<0.001		P<0.01		N.S.		N.S.		P<0.001
C_2	1.77		1.78		3.00		4.75		2.60	
	DMFS	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**
T_2	0.26		0.68		2.06		2.57		1.09	
		P<0.001		N.S.		N.S.		P<0.05		P<0.001
C_2	1.90		1.96		3.94		6.63		3.27	

Source: Bánóczy et al. (1985)

Table 2.6

Comparison of DMFT and DMFS mean values between test (T) and control (C) groups, after 4 (T_1/C_1) and 5 (T_2/C_2) years of milk fluoridation. Sig = statistical significance.

	Age in years									
Group	**9**		**10**		**11**		**12**		**Total 9-12 y**	
	DMFT	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**
T_1	2.14		2.73		2.89		3.39		2.86	
		N.S.		P<0.05		P<0.001		N.S.		P<0.001
C_1	3.00		4.75		5.44		4.86		4.53	
	DMFS	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**
T_1	2.14		3.08		4.15		5.80		3.99	
		N.S.		P<0.05		N.S.		N.S.		N.S.
C_1	3.94		6.63		6.44		5.50		5.66	
Group	**7**		**8**		**9**		**10**		**Total 7-10 y**	
	DMFT	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**	**DMFT**	**Sig**
T_2	0.26		0.68		1.94		2.42		1.04	
		P<0.001		P<0.01		N.S.		P<0.05		P<0.001
C_2	1.77		1.78		3.00		4.75		2.60	
	DMFS	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**	**DMFS**	**Sig**
T_2	0.26		0.68		2.06		2.57		1.09	
		P<0.001		P<0.01		N.S.		P<0.05		P<0.001
C_2	1.90		2.00		3.94		6.63		3.27	

Source: Bánóczy et al. (1985)

DMFT value of the 9-12 year group (a 37% reduction), but for the overall mean DMFS, the 30% reduction was not significant. Again, for 9 year olds, no data achieved significance.

With respect to the ten year results, only at ages 12-14 years were differences between test and control DMFT and DMFS mean values significant ($P<0.05$), the greatest being demonstrated in the 14 year olds ($P<0.001$). With both indices, it was evident that those fluoridated for the longest time benefited most, the 14 year olds DMFT and DMFS mean values being approximately three times lower, than the control group means. Finally, when the overall mean caries increment was calculated between test and control groups, there was a 37% DMFT and a 40% DMFS reduction favouring subjects in the fluoridated milk group.

2.5.3 *Discussion*

The above clinical field study investigated the caries-inhibiting effectiveness of fluoridated fresh milk, after several years' regular consumption. However, since it was thought that such milk must be consumed within 30 min after the addition of fluoride, organisational difficulties could influence the practicality of implementing such a method. None the less, the conditions offered by this closed children's community were highly favourable as the teachers proved excellent collaborators. As a result, the youngsters drank the milk regularly on approximately 300 days per year. In this Hungarian study, statistically and clinically significant differences between the test and control groups' dmf values were found for the younger age groups (who began consumption of fluoridated milk at 2-5 years), and whose primary teeth were already erupted at the beginning of the programme. The longitudinal analysis of first permanent molars, as well as total DMFT and DMFS mean values of 165 children followed-up for four and five years showed evidence of strong statistically significant differences between test and control groups. The results emphasize the importance of commencing such a programme at an early age, and its continuation thereafter.

2.6 **Israel**

In 1983, a study began in Israel, with the aim of investigating the caries preventive effect of fluoridated milk consumption by a population of schoolchildren in relation to the fluoride uptake by the surface enamel. (Zahlaka *et al.*, 1987).

A total of 273 children from Bethlehem, aged 4 to 7 years at the start, participated in the 3 year study. The drinking water in Bethlehem contained less than 0.2 ppm fluoride. The children were assigned at random to test and control groups. Baseline dental examinations (deft and DMFT) were conducted using plane dental mirrors, explorers and artificial light. Before the start of the study, the examiners were calibrated. Each child was re-examined clinically during the 3 years according to WHO (1987) criteria. At the end, about 120 children could be evaluated in each group, who were present at both the initial and the follow-up examinations.

Each school day, children in the test group received 100 ml of reconstituted powdered cow's milk supplemented with 1 mg fluoride as NaF (10 ppm F). Consumption of the milk took place at 10.00 a.m. in school, except on Sundays, holidays, and during vacations. Children waited at least 15 minutes before ingesting other food or drink to avoid a potential washout effect from consuming other food or beverage shortly thereafter. The children were not instructed to swish the milk in their mouths. Children in the control group received no beverage at school.

Concerning the results, children who consumed fluoridated milk on school days for about 3 years had lower caries increments in the primary dentition (mean deft difference, 1.3) and in first permanent molar teeth (mean DMFT difference, 0.2) compared with the incremental caries scores of the children in the control group (mean deft difference, 3.5) and for the first permanent molars (mean DMFT difference, 0.5). The differences for these comparisons were statistically significant ($p<0.01$). Thus, the estimated caries reduction was 63% in the primary, and 63% in the permanent dentition.

2.7 Louisiana, USA

The second Louisiana study started in 1982 and, with a ten month hiatus, ended in 1985. The aim of the study was to examine the effect of chocolate-flavoured, sweetened, low-fat, fluoridated milk on the caries incidence of elementary schoolchildren (Legett *et al.*, 1987).

Children in grades K-4 from five demographically similar elementary schools, in a non-fluoridated community (< 0.4 ppm F) were enrolled in the study. Because test milk could be offered on only 170 school days per year, each 236 ml carton of milk contained 1 mg fluoride, which equated to a concentration of 3.9 ppm F. Standard basic cocoa

mix (1.4%) and sugar (5.8%) were added to the fluoridated milk. The children received their beverage with their lunch. The control group did not receive milk. The test milk was available on 451 days over a 39 month period. Because of a high attrition rate and because of the ten month gap, a second cohort was added one year after the trial began. Thus, there were two trials, a two-year group with 187 children (111 test, and 76 control), and a three-year group with 157 children (88 test, and 69 control).

Concerning the results, in the two-year cohort, a significant caries reduction was noted both in DMFT (77%) and DMFS (77%) mean values. The three-year cohort showed no significant DMFT reduction, and only a 22% reduction of DMFS scores, which was statistically not significant. The reason for this might be a better compliance in the two-year cohort, due to encouragement by a reward system. In the three-year cohort, the ten month break was discouraging, and many pupils refused afterwards to collaborate.

2.8 Bulgaria

In May 1988, a study to investigate the potential of a community-based milk distribution nutritional programme to deliver fluoridated milk each weekday to kindergarten and first-grade schoolchildren, commenced in Bulgaria (Pakhomov *et al.,* 1995).

This study was not designed as a controlled clinical trial, but rather as a field demonstration to determine if fluoridation of milk is an effective and practical method of reducing the incidence of dental caries in children under real-life conditions.

2.8.1 *Materials and methods*

The project was sited in the southern Bulgarian town of Asenovgrad. A dairy in nearby Plovdiv produced fluoridated milk by adding the appropriate quantity of sodium fluoride to fresh milk prior to its packaging into plastic bags. The dairy was responsible for providing milk to all kindergartens and schools in Plovdiv, and some in Asenovgrad, where approximately one-half of the kindergartens received fluoridated milk and the other half received milk without fluoride from another dairy.

Approximately 3,000 Asenovgrad children aged 3-10 years received fluoridated milk in kindergartens and schools during the observation

period. Fluoridated milk was not available for home use. In those kindergartens and schools where fluoridated milk was delivered on a regular basis, estimated daily consumption of milk and fluoride for each child was 200 ml of milk containing 1 mg fluoride (5ppm F) for 180-200 days each year. Daily fluoride analyses were completed in the Plovdiv dairy using an Orion specific ion electrode. The average age of children at the 1988 baseline examination was 3½ years (kindergarten) and 4½ years (school) respectively.

Panaguriche, a smaller town nearby, was selected as the original reference area, but it ceased as a control after three years and another township (Karlovo) became involved. At all three sites, the natural fluoride content of the drinking water was less than 0.1 ppm F and fluoride-containing dentifrice was not available in any of these communities.

The baseline examination, as well as the three- and five-year follow-up inspections, were performed by the same four examiners who were calibrated at the start of the study and immediately prior to each follow-up, by a senior WHO epidemiologist. The calibration exercise showed an overall inter-examiner agreement of 90-95% for total dmft and DMFT scores using the WHO method described by Eklund *et al.* (1993). Dental caries was recorded according to the criteria defined by the World Health Organization (1987); radiographs were not taken. Each of the four examiners saw approximately 25% of the sample and, in Asenovgrad, examinations took place in two schools. Here, at each site, the subjects were a mix of those who did, and did not, receive fluoridated milk, examiners having no knowledge as to which group the children belonged.

In each of the study groups, approximately 100 children (equal sex distribution) were selected randomly for re-examination on each occasion, corresponding to 10-25% of the total number in each cohort. The exact number of children seen at each site is shown in Tables 2.7-2.10.

2.8.2 *Results*

Results in Table 2.7 relate to 6½ year old children after three years' participation in the project. Here, the reduction in caries experience in the primary teeth of the children in 'fluoridated' Asenovgrad was 40% ($P<0.001$) as compared with a fairly stable situation in 'non-fluoridated' Panaguriche. The corresponding values for the permanent

dentition showed a DMFT reduction in Asenovgrad of 89% (P<0.001) as compared with a 14% non-significant increase in Panaguriche.

Table 2.7
Dental caries experience (dmft and DMFT) in children aged 6½ years in Asenovgrad (F- milk) and Panaguriche (Non-F-milk) at baseline (1988) and after 3 years (1991)

		1988	1991		
		Mean (SD)	**Mean (SD)**	**Percentage difference**	***P*-value (t-test)**
	n	204	139		
Asenovgrad (F milk)	dmft	5.3(3.4)	3.2(3.1)	-40%	<0.001
	DMFT	0.9(1.2)	0.1(0.4)	-89%	<0.001
	n	100	100		
Panaguriche (Non-F milk)	dmft	5.6(3.5)	5.2(3.0)	-7%	N.S.
	DMFT	0.6(1.2)	0.7(0.7)	+14%	N.S.

Source: Pakhomov et al. (1995)

In Table 2.8, data obtained from 7½ year olds, after three years' involvement, are presented. For those Asenovgrad subjects who had been consuming fluoridated milk daily during the whole study period, there was a dmft reduction from 6.7 to 3.8 i.e. a 44% difference (P<0.001). In comparison, for those who did not receive fluoridated milk in Asenovgrad, there was a dmft increase from 6.7 to 8.4, corresponding to a non-significant increase of 20%. For fluoridated Asenovgrad children there was a DMFT reduction from 1.2 to 0.2, i.e. a difference of 83% (P<0.001), while the non-fluoridated subjects' DMFT value increased by 25%, although this change was not statistically significant.

In Table 2.9, the results obtained relating to caries in the primary dentitions of 6½ and 8½ year olds in Asenovgrad compared with Karlovo, after five years of the project, are shown. Since the average age at first entry to kindergarten was 3½ years, those aged 6½ years had been drinking fluoridated milk for only three years, in contrast to the 8½ year olds who had received fluoridated milk during the full project period of five years. In the primary dentition, the dmft difference between the new reference area (Karlovo) and the test area (Asenovgrad) was 52% (P<0.001) for 6½ year olds, and 40% P<0.001) for 8½ year

old children. The corresponding figures for the permanent dentition were 89% (P<0.001) and 79% (P<0.001) respectively (Table 2.10.)

Table 2.8
Dental caries experience (dmft and DMFT) in children aged 7½ years in Asenovgrad (F- milk) and Asenovgrad (Non-F milk) at baseline (1988) and at the end of the study (1991)

		1988	1991		
		Mean (SD)	**Mean (SD)**	**Percentage difference**	***P*-value (t-test)**
	n	47	135		
Asenovgrad (F milk)	dmft	6.7(3.7)	3.8(2.8)*	-44%	<0.001
	DMFT	1.2(1.3)	0.2(0.7)**	-83%	<0.001
	n	47	101		
Asenovgrad (Non-F milk)	dmft	6.7(3.7)	8.4(4.0)***	*+25%*	N.S.
	DMFT	1.2(1.3)	1.6(1.3)****	*+33%*	N.S.

** versus *** P<0.001; ** versus **** P<0.001*
Source: Pakhomov et al. (1995).

Table 2.9
Dental caries experience (dmft) in children aged 6½ and 8½-years after 3 and 5 years' participation in the milk fluoridation project in Asenovgrad (F- milk) as compared with Karlovo (new Non-F- milk)

	Age of children in 1993	**Years of participation**	**dmft mean (SD)**	**Percentage difference**	***P*-value (t-test)**
Asenovgrad (F-milk)	6½	3	3.2(3.1) n = 139	-52%	<0.001
Karlovo (Non-F-milk)	6½	0	6.8 (4.3) n = 114		
Asenovgrad (F-milk)	8½	5	3.6 (2.6) n = 178	-40%	<0.001
Karlovo (Non-F-Milk)	8½	0	6.0 (3.1) n = 176		

Source: Pakhomov et al. (1995).

Table 2.10

Dental caries prevalence (DMFT) in children aged 6½ and 8½-years after 3 and 5 years' participation in the milk fluoridation project in Asenovgrad (F- milk) as compared with Karlovo (new Non-F-milk)

	Age of children in 1993	Years of participation	DMFT mean (SD)	Percentage difference	*P*-value (t-test)
Asenovgrad (F-milk)	6½	3	0.1 (0.4) n = 125*	-89%	<0.001
Karlovo (Non-F-milk)	6½	0	0.9 (1.3) n = 104*		
Asenovgrad (F-milk)	8½	5	0.5 (0.9) n = 178	-79%	<0.001
Karlovo (Non-F-Milk)	8½	0	2.4 (1.8) n = 176		

** Not all the children in whom dmft scores were assessed had erupted first permanent molars present at the time of examination.*

Source: Pakhomov et al. (1995).

In a more recent publication, the long-term dental caries-reducing effect of the community based fluoridated milk programme was evaluated after a 10 year period in Asenovgrad (Atanassov *et al.*, 1999). The data of 300, 11, 12 and 13 year old children participating in the programme, when compared with the data of 279 control children, showed an increase in the percentage of caries-free children. The DMFT mean values showed differences - test vs. controls - of 2.8 to 5.3 for 11 year olds, 3.3 to 6.0 for 12 year olds, and 4.2 to 7.5 for 13 year old children. Thus, the level of dental caries for children who had drunk F-milk regularly between the age of 3 and 7 years, was substantially lower than that of children who never had the opportunity to drink F-milk.

2.8.3 *Discussion*

Results of the Bulgarian community-based milk fluoridation project would appear to confirm those of previous milk fluoridation investigations summarized earlier in this chapter.

To date, practically all studies published on the caries-reducing effects of fluoridated milk have been designed as longitudinal clinical trials performed under strictly controlled conditions. Hence, the main objective of the Bulgarian investigations was to determine whether a similar effect could be obtained in a "real life" situation, and the results

of these cross-sectional studies would appear to fall at the upper end of previously reported limits of caries-preventive effect.

2.9 China

Data from the national Chinese oral health survey in 1999, showed a high prevalence (76%) of caries in the primary dentition of 5 year old children. Therefore a 5-year programme of milk fluoridation for caries prevention among 4,000 pre-school children was carried out in Beijing, China (Bian *et al.*, 2003).

2.9.1 *Materials and Methods*

In the first phase of the programme (1994 to 1997) the results were not favourable, probably due to the fact that a high amount of sugar (about 7-10%) was added to the fluoridated milk, which the children consumed only on weekdays - less than 180 days a year. Therefore, in the second phase of the programme (1997-1999), no or just a little sugar was added to the fluoridated milk, and packaged UHT milk was given to the study children for weekend consumption at home.

This was a community-based demonstration programme, carried out in 15 kindergartens in the Haidian district of Beijing, China. Each child in the participating kindergartens consumed 200 ml of fluoridated milk at a concentration of 2.5 ppm F under teacher supervision. Thus, the estimated daily consumption of fluoride was about 0.5 mg. For the weekends, the children were given two packs (250 ml) of UHT fluoridated milk. Two large kindergartens that declined the opportunity to participate in the programme were selected as controls, receiving fresh milk without sugar and fluoride for breakfast occasionally. No other additional fluoride (toothpaste) was used or consumed.

The drinking water fluoride concentration was less than 0.3 ppm (mg/l), and that of local fresh cow's milk below 0.02 ppm (mg/l).

Baseline caries examinations were carried out by four trained dentists, and an external independent dental epidemiologist was invited to carry out the 21-month evaluation examination. Examiner calibration exercises were done prior to the examinations, which were performed in the kindergartens, using portable lights, mouth mirrors, and sickle shaped probes. Dental caries was diagnosed mainly visually at the cavitation level, when caries had progressed into dentine. New caries was recorded when a tooth diagnosed as sound at the baseline was

found to have an active caries lesion at the 21 month examination. Two kinds of reversal were also identified: arrested caries and examiner reversal. Arrested caries was recorded if a cavitated lesion in a primary teeth was found to have hard cavity walls and floor when tested with a sharp probe using a light force. The net caries increment was computed by subtracting the reversals from the number of new caries.

2.9.2 *Results*

At the start of the study there were 534 children (mean age 54± 4 months) and 305 children (mean age 53± 4 months) in the test and control groups respectively. After 21 months, in the test group 417, and in the control group 247 children remained in the study. The drop-out rates were thus 22% and 19% respectively. On average, the children had consumed fluoridated milk on 379 days in the kindergartens and, since the children had consumed the packaged fluoridated milk at home at weekends, the total number of days amounted to 547 during the 21 month trial period.

There was no statistically significant difference in the baseline mean dmft score between the two groups (Table 2.11). The difference between the mean number of new carious teeth in the test and control groups was found to be statistically significant (1.2 vs. 1.8, P<0.001). In the mean number of arrested carious teeth between groups, there was also a statistically significant difference (0.3 vs. 0.1, P<0.001). The overall net caries increment over the 21-month study period was 0.4 dmft in the test group and 1.3 dmft in the control group. This amounted to a 69% reduction, and was statistically significant (P<0.001).

Table 2.11
Baseline caries experience, new caries, reversals, and net caries increment of test and control children

	Test group (n=417)	Control group (n=247)	P value
Mean baseline dmft	3.2 ± 3.7	3.5 ± 1.4	0.312
Mean new caries (dmft)	1.2 ± 1.5	1.8 ± 1.6	<0.001
Mean arrested caries (dmft)	0.3 ± 0.9	0.1 ± 0.5	<0.001
Mean net increment (dmft)	0.4 ± 1.9	1.3 ± 1.2	<0.001

Source: Bian et al. 2003.

2.9.3 *Discussion*

This study was a demonstration trial and not a strict randomized clinical trial. However, it can be concluded that the consumption of fluoridated milk by the kindergarten children in Beijing was an effective measure in preventing caries in primary teeth. The daily consumption of fluoridated milk could even arrest active dentine caries in primary teeth. The results support the view that the topical caries-preventive mechanism is important.

2.10 Chile

In Chile, special programmes were established, based on the policy of the country, providing powdered milk and milk derivatives for young children and schoolchildren. On this basis, several milk fluoridation programmes using powdered milk were introduced.

2.10.1 *The Codegua study*

A community trial with powdered milk started in Chile in 1994, based on the 50 year old National Complementary Feeding Programme (PNAC), under which every Chilean child is entitled to receive, at no charge, 2 kg of powdered cow's milk per month, from birth until 2 years of age. From 2 to 6 years, they receive 1 kg of a milk derivative (Purita Cereal) per month. Based on 90% coverage of the PNAC, the Institute of Nutrition and Food Technology (INTA) decided to use PNAC products as a fluoride vehicle for caries prevention. The programme used disodium monofluorophosphate (MFP) instead of NaF. The aim of the programme was to test the feasibility and effectiveness of using powdered milk in a rural community, with a low fluoride concentration in drinking water, on the primary dentition of children 3-6 years of age. The results of the trial were reported after four years (Mariño *et al.*, 2001). A cost-effectiveness analysis of this programme has been published (Mariño *et al.* 2007): the authors state that "the findings suggest that there are important health and economic benefits to be gained from the use of fluoridated milk products in non-fluoridated rural communities in Chile." These costs are considered further in Section 5.2.2.

Material and Methods

Two communities from the Chilean Sixth Region were selected, following a matching procedure based on different variables. Codegua, selected as the test community, and La Punta, selected as the control community, were similar in population and oral health related environmental variables. Letters of consent were requested from the parents/guardians of the children.

Following the dietary fluoride supplementation, the average daily fluoride ingestion from fluoridated milk products was estimated at 0.25 mg F/day among children 0-23 months old, 0.5 mg F/day for children 2-3 years old, and 0.75 mg F/day for children 3-6 years old. The powdered milk delivered through PNAC was prepared using a 1:10 dilution with boiled tap water. The fluoride concentration of drinking water ranged between 0.06-0.09 ppm.

Toothpaste containing fluoride was used for twice/day toothbrushing in both communities. The ratio of F/creatinine concentration of mid-morning urine samples was used as an estimator of daily fluoride urinary excretion and daily fluoride intake.

Baseline dental clinical examinations in Codegua were carried out in October 1994, and follow-up examinations every consecutive year. However, in La Punta, the baseline examination could be performed only in 1997. After that, follow-up examinations were conducted in both communities. Inter- and intra-examiner reliabilities were assessed. Oral examinations were conducted using natural light, dental mirrors and sickle probes. Clinical data were recorded and evaluated based on WHO recommendations, and analysed statistically. Dental status was assessed by using the dmfs index for the primary dentition.

Results

The baseline examinations were performed in 1994 in Codegua on 177 children, aged 3-6 years. However, in La Punta, the baseline examination was carried out in 1997, on 189 children aged 3-6 years. Although there was some variation in the mean dmfs values between the two localities, these differences were not statistically significant between 1997 and 1999.

The age specific percentage reductions between 1994 and 1999 in Codegua (Table 2.12) showed lower mean dmfs values in 1999 than in 1994. Percentage reductions ranged from 40% (4 year olds) to 78% (5 year olds).

Table 2.12
dmfs mean values and standard deviations in 3-6 year old children in Codegua by year of data collection

Age (y)	1994	1999	Reduction	P
3	3.11 ± 5.07	1.52 ± 2.48	51%	<0.06
4	5.40 ± 8.10	3.18 ± 7.27	41%	<0.05
5	13.75 ± 16.12	3.03 ± 4.83	78%	<0.01
6	19.21 ± 12.94	5.63 ± 6.23	71%	<0.01
3-6	11.78 ± 13.69	3.35 ± 5.68	72%	<0.01

Source: Mariño et al., 2001.

The comparison of the mean dmfs values between Codegua and La Punta (Table 2.13) showed lower mean dmfs values in the test community for all age groups. Percentage reductions ranged from 25% (4 year olds) to 61% (3 year olds).

Table 2.13
dmfs mean values and standard deviations in 3-6-year-old children living in Codegua and La Punta in 1999

Age (y)	La Punta	Codegua	Reduction	P
3	3.85 ± 5.67	1.52 ± 2.48	61%	<0.01
4	4.22 ± 5.00	3.18 ± 7.27	25%	<0.01
5	5.61 ± 7.05	3.03 ± 4.83	46%	<0.05
6	8.79 ± 8.89	5.63 ± 6.23	36%	<0.05
3-6	5.65 ± 7.08	3.35 ± 5.68	41%	<0.01

Source: Mariño et al., 2001.

The proportion of caries-free children in Codegua showed a statistically significant ($P < 0.05$ to $P < 0.01$) increase between 1994 (22%) and 1999 (48%), which was not present in the control community examined in 1997 and 1999. Comparing the proportion of caries-free children in 1999 between the two study sites, the percentage values were higher in Codegua (48%) than in La Punta (30%), reaching a statistically significant difference for the ages 3 and 4 years ($P < 0.05$ to $P < 0.01$).

Discussion

Results of the study obtained after four years point to a reduction in caries experience in the primary dentition, which was greater for children born after the start of this programme, or aged around 1 year when it started. Data obtained in clinical examinations of 3-6 year-old children in Codegua showed that the decrease in the mean number of tooth surfaces affected by caries ranged from 41% to 78 %.

Studies performed three years after the cessation of the milk fluoridation programme (2002) found an increase in caries experience in all age groups in the test community. Comparing the caries status of the children in Codegua and La Punta, no statistically significant differences were found in 2002. This points to the importance of a continuing maintenance of caries preventing programmes (Mariño *et al.*, 2004).

An economic evaluation of the Chilean milk fluoridation programme has been published recently by Mariño *et al.* (2007) using data from the Codegua scheme. After four years, the improvement in dental health was achieved at a yearly cost of RCH(1999) $ 1,839 per child: (1 US$ = RCH(1999) $528). On average, this programme would result in a return to society in dental treatment costs of RCH(1999) $ 950 per child per year: this represents a favourable incremental cost-effectiveness ratio for the intervention group of RCH(1999) $ 2,695 per diseased tooth averted after four years compared with the control group.

2.10.2 *The Araucania study*

In 1999, another study started with the aim of assessing the effectiveness of a dental caries prevention programme on the permanent dentition of Chilean rural schoolchildren using fluoridated powdered milk and milk derivatives. In the Ninth Chilean Region rural areas, 35,000 children using the standard School Feeding Programme (PAE), which had been in operation already for 40 years, received fluoridated products. The daily fluoride dose was assessed as 0.65 mg for children between 6-14 years.

Cross-sectional samples of children aged 6, 9 and 12 years were examined from the study communities in Araucania, at the start of the study (1999) and 36 months afterwards. The results were compared with those of a sample from a (positive) control community, where children were participating in an ongoing APF-gel programme.

No statistically significant differences were found between dmft and DMFT indices of the study and control groups either at baseline, or 36 months later. However, significant reductions ranging from 24-27% were observed in the 9 and 12 year old groups in the study communities between baseline and the 36 months examination. DMFT indices of these 9 and 12 year old children receiving fluoridated milk were not significantly different from the data of the positive control group (Weitz *et al.*, 2007).

Considering the relative costs and technical difficulties involved in these two caries preventive programmes, it seems that milk fluoridation can be used as a good alternative for those caries preventive programmes (such as the present programme with APF-gel), in rural areas where community programmes are difficult to deliver.

2.11 United Kingdom

The milk fluoridation programme in the U.K. started in 1993 with 1,600 children attending 40 primary schools in one local authority; expanding by the year 2000 to involve over 15,000 children attending schools in four local authority districts (Woodward *et al.,* 2001). The programme has been introduced in districts with poor health and most of these are in the North West of England. There were in 2004 eleven education authorities in England offering fluoridated milk, with approximately 32,000 children drinking it (Riley *et al.,* 2005), and in June 2005 this number amounted to over 40,000 children in 510 establishments. Two evaluations of the effectiveness of these programmes have been published: one longitudinal study in Knowsley (Ketley *et al.,* 2003), and one cross-sectional study from the Wirral (Riley *et al.,* 2005). The cost of operating the UK programme is discussed in Section 5.2.2.

2.11.1 *The Knowsley study*

In the district of Knowsley, a milk fluoridation programme started in 1997 in 36 schools with 4,060 children, with the aim to assess: (a) caries increment over four years in primary molars in children initially aged 3 to 5 years, and (b) caries experience in first permanent molars and incisors.

Material and Methods

From twelve schools in the district, 478 children in the nursery and reception classes were chosen for the test group, and children with a similar range of dmft indices and similar social deprivation scores from the district Skelmersdale were selected as controls. Milk was provided for both groups from the same dairy: the test children drank fluoridated milk with 0.5 mg F in 189 ml milk (2.65 ppm), consumed through drinking straws usually at mid-morning on five days per week and ideally on 180 days per year.

All parents/guardians were contacted by letter in order to enlist their co-operation. All children whose parents consented, aged 3-5 years in the test and control schools were examined at baseline and again after four years. Dental examinations took place in the schools. A random sample of 15% of subjects was re-examined to ensure intra-examiner repeatability. For the follow-up examination, the clinical examiner was blind to the children's fluoride status. Diagnostic criteria for caries were used in accordance with guidelines of the British Association for the Study of Community Dentistry. Fibre-optic transillumination was used to detect inter-proximal caries both in the permanent and primary dentition. In the results, the data are presented at the cavitation level only. The primary outcome variables were dmft and dfs four-year increments from age 3-5 years up to age 7-9 years, and DMFT and DFS at age 7-9 years.

Results

At the baseline in 1997, 478 children in Knowsley and 396 in Skelmersdale were examined (mean ages 4.7 y and 4.8 y respectively). Four years later at the follow-up, 318 children (67%) in Knowsley, and 233 children (59%) in Skelmersdale could be re-examined: the mean age of these children at baseline was 4.88±0.58, and 4.91±0.59 respectively.

The baseline dmft (for primary molars only) and the four-year increment for the test and comparison areas are presented in Table 2.14. The increment in Knowsley (2.28) was slightly higher than in Skelmersdale (1.96), but the difference in dmft increments of 0.32 (95% C.I. -0.04 to 0.68) was not statistically significant.

Table 2.14
dmft (primary molars only) at baseline and dmft four year increment for test and comparison groups and mean differences

	n	Baseline dmft (SD)	4-year dmft increment (SD)
Knowsley (F milk)	318	1.73 ± 2.23	2.28 ± 2.06
Skelmersdale (non F milk)	233	1.29 ± 2.05	1.96 ± 2.18
Mean difference		0.44	0.32

Source: Ketley et al., 2003

The baseline dfs (for primary molars only) and the four-year dfs increment are presented in Table 2.15. The increment in the test area Knowsley (4.49) was slightly higher than in the comparison area (4.12), but the difference of 0.38 (95% C.I. -0.45 to 1.21) was not statistically significant.

Table 2.15
dfs (primary molars only) at baseline and dfs four year increment for test and comparison groups and mean differences

	n	Baseline dfs (SD)	4-year dfs increment (SD)
Knowsley (F milk)	318	2.51 ± 4.35	4.49 ± 4.91
Skelmersdale (non F milk)	233	2.15 ± 4.05	4.12 ± 4.85
Mean difference		0.35	0.38

Source: Ketley et al., 2003.

Concerning the mean DMFT values at age 7-9 years (Table 2.16), there was no difference and the 95% C.I. of the difference ranged from –0.15 to 0.14. The mean DFS in the Knowsley children was slightly lower than in the Skelmersdale children, but the difference of 0.1 DFS (95% C.I. 0.3 to 0.1) was not statistically significant.

Table 2.16
DMFT and DFS at age 7-9 years in test and comparison groups after four years and mean differences

	n	DMFT (SD)	DFS (SD)
Knowsley (F milk)	318	0.40 ± 0.85	0.45 ± 1.12
Skelmersdale (non F milk)	233	0.40 ± 0.87	0.55 ± 1.35
Mean difference		0.00	-0.10

Source:Ketley et al., 2003.

Conclusion

It can be concluded, that the fluoridated school milk scheme, as configured in Knowsley, did not reduce caries within the primary and permanent dentitions up to 8 years of age.

2.11.2 *The Wirral study*

A fluoridated milk programme was implemented in Wirral nursery and primary schools in 1995/96: 5,700 children drank fluoridated milk in 63 nursery and primary schools. The objective of this cross-sectional study was to examine children attending schools with a fluoridated milk programme (0.5 mg F in 189 ml of fresh milk) and to compare their dental health with children attending schools without a fluoridated milk programme from a different district (comparison group).

Materials and Methods

The dental caries experience in the test group (Wirral) was compared with children from the schools in Sefton, where there was no fluoridated milk programme. Schools in the test group were only included if they had been receiving fluoridated milk for a minimum of six years, and if the uptake by children to drink fluoridated milk in each school was at least 50%.

Children aged 5 years in 1997/98 became part of the test and comparison cohort in 2003. Sefton was selected as control, because most of the social and dental criteria matched those from the Wirral. Both the mean dmft and the percentage of caries-free children at baseline – according to previous examinations - did not differ significantly between the two districts. The Index of Multiple Deprivation (IMD) was also matched in the two areas.

The dental examinations took place in the test and comparison schools in 2003 by trained and calibrated examiners, in accordance with the British Association for the Study of Community Dentistry (BASCD) protocol. Tooth surface data of the four permanent molars were recorded. An independent examiner was included to re-examine 12% of children, inter-examiner reliability was assessed, as well as intra-examiner reproducibility.

Results

The total study population was 773 children in the test, and 2,052 in the comparison group. All children present on the day of examination in the 14 test and 28 control schools were seen. The numbers of children examined were 690 in the test group, and 1,835 in the comparison group. The absenteeism rate for both groups was 11%.

The IMD scores for the wards where the schools were, and age of the children examined in the test and comparison groups, were very similar, with no significant differences between the two groups. The mean age of the children examined was 10.79± 0.59 years in the Wirral, and 10.83± 0.59 in Sefton.

The dental status of the first permanent molars is shown in Table 2.17. The mean DMFT of children in the test group was 1.01, and in the comparison group 1.46. The mean DT was 0.59 in the test group and 1.02 in the comparison group. The missing and filled components of the DMFT index were very similar in test and comparison groups. The mean DFS of children in the test group was 1.20 and in the comparison group 1.89. The differences between the means for the comparison and test groups for DMFT and DT and DFS were 0.49 and 0.43 and 0.74, respectively, and all were statistically significant ($p<0.001$) indicating a higher level of caries experience for the children in the comparison schools.

Table 2.17
Dental health of the first permanent molars in children examined

	Test group (SD)	Comparison group (SD)	Mean difference	p-value
Mean DMFT	1.01±1.30	1.46±1.48	0.49	<0.001
Mean DT	0.59±0.98	1.02±1.24	0.43	<0.001
Mean DFS	1.20±1.86	1.89±2.41	0.74	<0.001
% DMFT > 0	48	61	13	<0.001
% DT > 0	35	51	16	<0.001

Source: Riley et al., 2005.

In the test and comparison groups there were 48% and 61% children, respectively, with past caries experience showing a difference of 13%. The percentage of children with current dentinal decay was 35% and 51% in test and comparison groups respectively, giving a difference of 16%. These differences were all highly statistically significant ($p<0.001$). The percentage caries reductions were 31% for DMFT, 42% for DT and 37% for DFS.

Conclusion

From the findings of this study, children in the participating schools in Wirral, drinking fluoridated milk, had better dental health than children attending schools in Shefton, not drinking fluoridated milk. In Wirral, 13% less children experienced caries and 16% less children developed active decay in their permanent dentition. This study found a difference of 31% in DMFT and a difference of 37% in DFS between the test and comparison groups of children.

2.12 **Russia**

Milk fluoridation started in Russia in 1993 as a collaboration between WHO and The Borrow Foundation. In 1994, the directors of WHO Collaborating Centres in Moscow (EM Kouzmina and AG Kolesnik) proposed to look into the possibility of implementing milk fluoridation projects. The decision was taken to make Voronezh the site of the demonstration project, and the programmes in Maykop and Smolensk as ordinary projects. The projects in these three cities started in November 1994. Results of these projects, covering about 15,000 Russian children, two and three years after initiation, were reported by Kouzmina *et al.* (1998, 1999). The dmft reduction in 6 year old children in Voronezh was 68%, in Smolensk 55%, and in Maykop 63%. Prevention of caries in the permanent dentition was also recorded. Continuously, the programme has been extended to other cities in Russia: in Volgograd, several towns in Tatarstan, and currently the programme involves approximately 50,000 children.

Two detailed evaluations on the effectiveness of these programmes have been published, one from Volgograd after three years (Maslak *et al.*, 2004), and one from Voronezh after 10 years (Pakhomov *et al.*, 2005).

2.12.1 *The Volgograd study*

Due to the high caries incidence and low fluoride level in drinking water (0.18-0.20 ppm) in Volgograd, a milk fluoridation project was established in 1998, with the aim to evaluate the efficacy of the project for kindergarten children.

Material and Methods

In a three year follow-up study, 166 children, caries-free at the age of three years, were randomly assigned into test (75) and control (91) groups. The test group children consumed regularly fluoridated milk (180-200 ml/day), while the control regularly consumed ordinary milk. All children were examined yearly by calibrated examiners who were blind to the group identity of the children. Caries was diagnosed visually by probing, the results expressed as dmft and DMFT mean values. Only dentinal caries was registered.

In a cross-sectional study, 150 to 200 six year old children in a district of Volgograd were examined between 1996 and 2002 – they had participated in the fluoridated milk project from 1998 to 2001.

Results

The caries prevalence in primary teeth (Table 2.18) increased significantly ($p<0.05$) less in the test than in the control group.

Table 2.18
Caries prevalence in (%) of test and control children at the yearly examinations

Group	Age of children					
	N	**3**	**4**	**5**	**6**	**p value**
Test	75	0.0	22.7	48.0	69.3	<0.05
Control	91	0.0	45.1	65.9	82.4	

Source: Maslak et al., 2004

In the primary dentition, mean dmft values showed a statistically significant ($p<0.05$) reduction in the test group when compared with the control group after three years (Table 2.19).

Table 2.19
Mean dmft values (±s.d.) of test and control children at the follow-up examinations

Group	Age of children					
	N	**3**	**4**	**5**	**6**	**p value**
Test	75	0.0	0.40±0.09	1.17±0.18	2.5±0.26	<0.05
Control	91	0.0	0.94±0.13	2.13±0.22	3.64±0.26	

Source: Maslak et al., 2004

After three years drinking fluoridated milk, both caries prevalence in permanent teeth and mean DMFT showed statistically significant ($p<0.05$) reductions in 6 year old test group children when compared with the comparison group (Table 2.20).

Table 2.20
Caries prevalence and DMFT mean values in 6 year olds after three years of milk fluoridation

Group	N	caries prevalence %	p	DMFT	p
Test	75	1.3	<0.05	0.04±0.03	<0.05
Control	91	11.0		0.17±0.05	

Source: Maslak et al., 2004

In addition, in the cross-sectional studies of 6 year old children examined between 1996 and 2002, statistically significant decreases in both caries prevalence ($p<0.05$), and the dmft and DMFT mean values ($p<0.01$) were reported.

Conclusion

In this study, the three year milk fluoridation programme was effective in kindergarten children: statistically significant caries reductions were observed when the test and the control groups were compared.

2.12.2 *The Voronezh study*

A cross-sectional, blind study of the efficacy of fluoridated milk, including monitoring of F excretion in urine, under conditions of wide availability of fluoride containing toothpastes, was performed in Voronezh, during 10 years (1994-2004). The fluoridated milk project expanded to cover from 10,000 to 15,000 pre-school children. The aim of the study was to assess the possible caries reduction in children aged 3 to 12 years (Pakhomov *et al.*, 2005).

The first study included 335 six year old children from 11 kindergartens, who had consumed fluoridated milk regularly during three years (test group), and 175 six year old children from six kindergartens who had not consumed milk in school (control group). Fluoridated milk, with the level of 2.5 ppm F, was consumed daily in school. The results of the examinations (Table 2.21) showed a statistically significant ($p<0.001$) decrease in dmft values of the test group, when

compared with the control. The percentage of caries-free children was 42% in the test group, and 32% in the control group.

Table 2.21
Mean dmf values of 6 year old test and control children after three years of fluoridated milk consumption

Group	N kindergartens	n children	dmft (sd)	p value	% of caries-free children
Test	11	335	1.59±1.82	<0.001	42
Control	6	175	2.58±2.67		37

Source: Pakhomov et al., 2005

The second study analysed data of 3, 6, 9 and 12 year old children, when the fluoridated milk project had been running for 10 years. There was a considerable reduction in the caries experience of the children examined in 2004, when compared with those in 1994 (Table 2.22).

Table 2.22
Caries experience of 3, 6, 9 and 12 year old children in 1994 and 2004, after 10 years of consuming fluoridated milk

Year of examination	Age of children in years			
	3 dmft	6 dmft	9 DMFT	12 DMFT
1994	4.1	4.8	2.2	3.7
2004	0.8	2.6	1.2	1.5

Source: Pakhomov et al., 2005

The reduction was considerable in all age groups in 2004, probably also due to the increasing use of fluoridated toothpastes, and very active education in dental hygiene for the population in Voronezh. Fluoride intake monitoring in children 3, 4, 5 and 6 years of age has shown that fluoridated milk (daily approximately 200 ml with the level 2.5 ppm F) use is a very effective method of preventing dental caries without excessive fluoride intake.

2.13 Other studies

Several other studies investigated the effectiveness of fluoridated milk or milk products in the prevention of dental caries. However, the duration of these studies, and/or the small number of participants did not allow to definite conclusions to be reached.

2.13.1 *The Agudos study*

As reported by Lopes *et al.* (1984), the anticaries effectiveness of fluoridated milk has been studied in two primary schools of Agudos, State of Sao Paulo, Brazil. Schoolchildren aged 6 to 12 years received about 200 ml pasteurized milk for lunch every day. The schools were randomly allocated to experimental and control groups, where 456 children in the test school drank milk containing fluoride, and 321 children of the control group drank milk without added fluoride. DMFS assessments were performed by two calibrated dentists at baseline and after 16 months.

After 16 months, the drop-out was rather high, 304 students from the test school, and 198 from the control school could be re-examined. It was observed that the children from the test school had a lower caries increment than those in the control school: however, these differences were very small. The results seemed to be confounded also by the fact that restorative dental care was completely different in the two schools.

The authors, based on the above findings, concluded, that: (1) School milk fluoridation is practical; it does not cause disruption to the school daily routine. (2) The 16 months duration was not enough to demonstrate the efficacy of the intervention (daily provision of fluoridated milk at the concentration of 1 mgF/200ml). (3) There is a need to continue at least for 3 years with a longitudinal study for the verification of the effectiveness of a milk fluoridation scheme.

2.13.2 *The soya milk trial*

A study was conducted in 2000 by Werner & Perin (2004) in an Indian reservation in the Mato Grosso in Brazil on the effect of distributing fluoridated soya milk to 5-8 year old school children, during school meals for a period of 18 months. The aim of the study was to fluoridate already existing soya milk as a way of promoting dental caries prevention. The experimental group (43 children) received fluoridated soya milk (2 ppm F), while no fluoride was added to the soya milk

consumed by the control group (68 children). However, due to local circumstances, there was a lack of access to fluoridated milk during 50% of the school days. Oral data collection followed WHO guidelines.

The results were evaluated separately for groups of 5-6 year olds and 7-8 year olds. Using Student t-test to compare baseline and final results, no statistically significant differences ($p>0.05$) were found.

The authors concluded that the 18-month period (and the small numbers of participants) of sporadic ingestion of fluoridated soya milk (2 ppm) was not sufficient to demonstrate the effectiveness of fluoridated soya milk in the prevention of dental caries of school children from a Brazilian Indian Community.

2.14 Discussion of the clinical studies to evaluate milk fluoridation

2.14.1 *Number of studies*

A list of studies published up to the end of 2005 is given in Table 2.23. Abstracts have been listed only when full reports have not yet been published. There are some 20 reports of 15 studies carried out in 10 countries. The published abstract of Werner & Perin (2004) has been excluded from this list because of the very small cell sizes (13, 26, 29 and 42 subjects) and the "sporadic ingestion of fluoridated soya milk" in a Brazilian Indian community. Eight of the studies showed a caries preventive effect in primary teeth and ten studies showed a caries preventive effect in permanent teeth. Two studies, in Agudos, Brazil and Knowsley, UK, showed no effect in either dentition. One study investigated the effect of stopping milk fluoridation on caries development: caries experience in primary teeth increased after fluoridated milk cessation. A critical appraisal of these studies now follows.

2.14.2 *Lessons learnt from the evaluation studies*

Each of the main studies provides an insight into important aspects of both the conduct of such studies and the interpretation of the results. In view of this, each of the major studies will be considered in turn.

Table 2.23
List of published reports of studies into the effectiveness of milk fluoridation

Study	*Year of study*	*Authors*		*Caries prevention in:*	
				Primary teeth	*Permanent teeth*
Baton Rouge, USA	1955 - 1959	Rusoff *et al*	1962	.	+
Winterhur, Switzerland	1958 – 1964	Wirz	1964	+	+
		Ziegler	1964		
Agudos, Brazil	1976 – 1979	Lopes *et al*	1984	.	-
Glasgow, UK	1976 – 1981	Stephen *et al*	1981	-	+
		Stephen *et al*	1984		
Fót, Hungary	1979 – 1990	Bánóczy *et al*	1983	+	+
		Bánóczy *et al*	1985		
		Gyurkovics *et al*	1992		
Louisiana, USA	1982 – 1985	Legett *et al*	1987	.	+
Bethlehem, Israel	1983 – 1986	Zahlaka *et al*	1987	+	+
Asenovgrad, Bulgaria	1988 – 1993	Pakhomov *et al*	1995	+	+
		Atanassov *et al*	1999		
Codegua, Chile	1994 – 1999	Mariño *et al*	2001	+	.
Voronezh, Russia	1994 – 2004	Pakhomov *et al*	2005	+	.
Wirral, UK	1995 – 2003	Riley *et al*	2005	.	+
Beijing, China	1997 – 1999	Bian *et al*	2003	+	.
Knowsley, UK	1997 – 2001	Ketley *et al*	2003	-	-
Volgograd, Russia	1998 – 2002	Maslak *et al*	2004*	+	+
Araucania, Chile[1]	1999 – 2002	Weitz[1] *et al*	2007	+ 1	+
Cessation of milk fluoridation Codegua, Chile	1999 – 2002	Mariño *et al*	2004	+	.

**abstract only published.* *+ = evaluated, effect*
- = evaluated, no effect
. = not evaluated
[1] =equivalent to fluoride gel programme.

The study in Scotland was important because it was a randomised controlled trial designed to discover efficacy: children were randomly allocated to test and control groups and group identity was double blind. The proportion of children dropping out of the study was fairly high and the final number of subjects in the evaluation after five years was rather low. The community had high to very high caries experience (dmft = 5.2 at 5 years of age). This may partly explain the lack of effect in the primary dentition since many primary molars were already carious at the start of milk fluoridation at age 4 to 5 years. There was, though, an increment in dmft of 1.5-2.0 teeth during the 3 years and this was not reduced by the milk fluoridation programme. These results point to the need to commence the preventive programme as early as

possible in high caries communities. A substantial caries reduction was recorded in permanent teeth although it is not clear why this became apparent only after four years. The fluoride dose (1.5mg/d) was the highest tested, although the authors justify this by indicating that the children received this only on school days. One could speculate that the substantial caries reduction was related to the high fluoride dose.

The Hungarian study was a community evaluation, taking advantage of the controlled conditions in a children's home. Importantly, this allowed provision of fluoridated milk 300 days per year, compared with 200 days or less in school-based schemes. Also, it allowed a very long evaluation period of 10 years, following children who had received milk between the ages of 2 and 12 years. Substantial caries reductions were observed in both primary and permanent teeth, with the size of effect increasing with length of time receiving the milk. The daily fluoride dose was moderate, although higher than in some later studies, and the substantial caries reductions recorded could be due to the high number of days per year the milk was provided and the fairly high caries experience in these children.

The Bulgarian study was the first community trial of fluoridated milk, under real life conditions. There was a change of control community during the study which complicated comparisons, but demonstrates a problem frequently encountered in such studies. There was, though, an attempt to blind the clinical examiner to group identity. Milk was consumed only on school days but the dose was comparatively high at 1mg/d for all ages of 3 to 10 years. The analyses were 'before and after' (historical) as well as cross-sectional between test and control. This, usefully, provided information on secular change in caries experience (in the control community). Substantial caries reductions were recorded in both primary and permanent teeth, with the size of effect increasing in permanent teeth with the number of years' exposure to the fluoridated milk. Both the Hungarian and the Bulgarian studies indicate the importance of a long exposure time as possible.

The high caries experience in primary teeth was the justification for the milk fluoridation trial in China. It was planned as a demonstration trial rather than a clinical trial involving two groups of schools not assigned at random to test and control groups. There was, though, an attempt at 'blind' evaluation by using an independent external examiner. The trial was of short duration -- only 21 months – and the drop-out rate of 20% over 21 months was high: this may be the result of

choosing a 'high social class' area of Beijing, where there is high population mobility. The daily fluoride dose was low (0.5mg/d) but, by giving two packs of milk to each child for consumption during the weekend, the number of days milk was consumed reached 313 days per year, much higher than the usual 180 to 200 days per year for most school based schemes. The children were initially aged 4.5 years in this short trial, so that only primary teeth were studied. Clinical effectiveness was demonstrated, which might have been partly due to the large number of days in a year the children received the fluoridated milk. This trial was preceded by a trial where the milk was heavily sweetened and no caries preventive effect of the fluoridated milk was observed: the assumption is that the caries inducing effect of the high sucrose content negated the caries preventing effect of fluoride in milk.

Two important factors which encouraged investigation into milk fluoridation in Chile were, first, that water fluoridation could cover only the urban population and a preventive strategy was required in the rural areas and, second, that a comprehensive programme to deliver milk and milk derivatives to pre-school children and schoolchildren has existed in Chile for over 50 years. Two community trials have been undertaken – one involving pre-school children and the other, schoolchildren. Unlike several of the previously mentioned studies, fluoride-containing toothpastes were used widely in Chile by this time. The fluoride dose for each age group was determined, aided by urinary fluoride excretion studies that provided an estimate of total fluoride intake. A further safety check was the evaluation of dental fluorosis in children involved in the pre-school trial, once their permanent incisor teeth had erupted. This is the only milk fluoridation study where this has been reported, although it is only relevant when milk fluoridation is introduced at a young age. In the pre-school study, evaluation was both historical and cross-sectional, as in the Bulgarian study. Like the Bulgarian study, these evaluations were complicated by the inability to obtain data from the control community at the most appropriate time: these examples illustrate the difficulties in choosing and maintaining control groups. In the pre-school trial, the dmfs index was used to increase sensitivity in this four year study. Another interesting feature of this study was the follow up evaluation to determine the residual effect of the programme. The second study, on older children, tested milk fluoridation against a 'positive control', fluoride gel application, which was at that time the preferred preventive programme in rural areas. Since the clinical effectiveness of gel application and milk fluoridation

seemed to be the same, relative costs and technical difficulties indicated a preference for milk fluoridation. A further unique feature of the Chilean studies has been cost-effectiveness evaluation – such information is important in informing decision-makers.

There have been two evaluations of milk fluoridation in the UK. The first was a four year longitudinal study of children initially aged 3 to 5 years, while the second was a cross-sectional study of older children who had received fluoridated milk for six years. Clinical effectiveness was not observed in the first study while it was in the second study. A discussion of the possible reasons for these findings is useful. Reasons proposed for the lack of effect in the first study are: too low fluoride dose (0.5mg/d); the number of days the children received the milk (up to 180 per year) was too low; caries experience was relatively low which made it difficult to demonstrate any effect in primary teeth in children already 3 to 5 years of age; and the low caries experience (0.4 DMFT increment) and the young age of the children (7 to 9 years at evaluation) made it difficult to demonstrate any effect in permanent teeth. An unfavourable feature was the high (11%) absentee rate. Favourable aspects in design and evaluation were: in the first study, the examiner was 'blind' to group identity and, in the second study, an estimate of examiner reproducibility was provided and multivariate analyses controlled for possible confounding factors.

The two evaluations of milk fluoridation in Russia recorded clinical effectiveness in primary and permanent teeth. The first study (Volgograd) was important as it was a randomised blind design, which controlled for confounding factors and examiner bias. In these aspects, it is similar to the Glasgow trial and shows that such desirable features can be included in evaluations. The preventive programme was appropriate as caries incidence was high and water fluoride concentration low. The second study was field evaluation consisting of a series of cross-sectional surveys. The evaluation period was long – after 3 and 10 years in children who had received fluoridated milk between the ages of 3 and 12 years. Whilst for the shorter evaluation period, a control group allowed cross-sectional comparisons, for the longer evaluation period of 10 years, only historical comparisons were possible. This complicated estimation of effect, especially as fluoride-containing toothpastes became more widely available during this period. Favourable features included blind evaluation and monitoring of urinary fluoride excretion.

The above discussion of the main evaluations of milk fluoridation illustrates how desirable features can be included and how, sometimes, less desirable aspects can occur. Recognising these features helps interpretation of findings and also assists those planning their own evaluations; this latter aspect will be considered in more detail in Chapter 7.

2.14.3 *Systematic review*

One way to critically appraise studies is to undertake a systematic review. These are most useful when the unit of 'treatment' is the individual and are less useful for appraising community preventive programmes. Nevertheless, one systematic review of the effectiveness of milk fluoridation has been published, by Yeung *et al.* (2005). This review follows procedures given by the Cochrane Collaboration, which ensure a very thorough assessment of the quality of the evaluation studies and interpretation of the results of the studies which are included for analysis. The objective of the systematic review of Yeung *et al.* (2005) was "To determine the effectiveness of milk fluoridation, as a means of delivering fluoride on a community basis, for preventing dental caries." The criteria used for considering the evaluation studies for the review are given in Table 2.24. As can be seen in this table, the randomised controlled trial (RCT) is favoured: it has a number of attributes, the main ones being random allocation to test and control groups and concealment of group identity from subjects ('blind' subjects) in the study and person(s) assessing the effect ('blind' examiner).

Table 2.24
Criteria for considering studies in a systematic review

Types of studies	Randomised or quasi-randomised controlled trials (RCTs) which may be designed with randomisation at the level of the school (cluster) or the child. Studies with an intervention or follow-up period of less than 3 years were excluded.
Types of participants	General population irrespective of age or risk for dental caries.
Types of intervention	Active intervention: fluoridated milk (all concentrations / dosage were considered). Control: non-fluoridated milk. The milk was provided directly to the children or their family. Any payment for milk should be equivalent in the fluoridated and non-fluoridated groups.
Types of outcome measures	Changes in caries experience, caries increment, as measured by changes in decayed, missing and filled figures on tooth (dmft/DMFT) and surface (dmfs/DMFS).

Note: some details have been omitted. Source: Yeung et al. (2005).

Only two studies were judged to be of acceptable quality. These were the randomised controlled trials of Stephen *et al.* (1984) and Maslak *et al.* (2004). Only one other RCT was identified (Zahlaka *et al.*, 1987) but this was rejected because the control children received no milk rather than non-fluoridated milk. All other clinical evaluations of milk fluoridation were rejected because they were not randomised controlled trials and the risk of bias was, therefore, deemed to be unacceptable. The review of Yeung *et al.* (2005) then proceeds to examine the studies of Stephen *et al.* (1981, 1984) and Maslak *et al.* (2004) in more detail.

Regarding the effectiveness of milk fluoridation in the permanent dentition, the review concluded: "After consumption of fluoridated milk for three years, there was a significant reduction in the DMFT (78.4%, $P<0.05$) between the test and control groups in one trial (Maslak *et al.*, 2004), but not in the other (Stephen *et al.*, 1984). The results, however, could not be pooled because of the difference in concentration of fluoride in the milk; the concentration of fluoride in one study (Stephen *et al.*, 1984) being three times that of the other (Maslak *et al.*, (2004). Whilst the mean DMFT and DMFS were always in favour of the test group in the study by Stephen *et al.* (1984), it was not until the fourth year that a significant reduction of 35.5% ($P<0.02$) was obtained in the DMFT between the test and control groups. By the fifth year, there was a significant reduction in the mean DMFT (31.2%, $P<0.05$) and the mean DMFS (43.1%, $P<0.01$). When only those permanent teeth which were originally unerupted at the baseline examination were considered, by the fourth year, the DMFT and DMFS values were 33.3% ($P<0.02$) and 39.6% ($P<0.05$) respectively in favour of the test group. By the fifth year, the mean DMFT and DMFS differences increased to 35.8% ($P<0.05$) and 48.0% ($P< 0.01$) respectively." For primary teeth, the report concluded: "After consumption of fluoridated milk for 3 years, there was a significant reduction in the dmft (31.3%, $P<0.05$) between the test and control groups in one trial (Maslak *et al.*, 2004). In the other study (Stephen *et al.*, 1984), no significant differences were found between the test and control groups in terms of the modified dmft and dmfs indices at any of these times at the three annual re-examinations. Standard deviations and P values had not been reported. Correspondence with the authors (Maslak *et al.*, 2004; Stephen *et al.*, 1984) has confirmed that no adverse effect was reported."

In the Discussion section of the systematic review, the authors concluded: "Although there was little robust evidence to support fluoridated milk and the external validity of the included studies must be viewed with caution, this does not imply fluoridated milk is ineffective in caries prevention, merely that high quality RCT evidence is lacking in this area."

2.14.4 *The role of systematic reviews in literature appraisal*

Systematic reviews are very valuable to the decision-maker since they provide an unbiased estimate of effect. The criteria upon which studies are allowed into the analysis in a systematic review are often very strict, with RCTs likely to be accepted but community trials rejected: community trials are unlikely to include random allocation of subjects to groups and may not conceal group identity from subject or assessor. Nevertheless, it is not sensible to exclude evidence from such community trials altogether.

Evidence can be seen on several levels, rather than just two levels, and an example of these levels is given in Table 2.25 (Spencer, 2003): the strongest level of evidence is at the top and the weakest at the bottom. Most of the studies described earlier in this chapter could be classed as level III-2, although the two RCTs raise the level of evidence to level II. Since the systematic review of Yeung *et al.* (2005) found "little robust evidence to support fluoridated milk", the level of evidence for fluoridated milk in caries prevention does not reach level I.

Table 2.25
Levels of evidence and study design

Level of evidence	Study design
I	Evidence obtained from a systematic review of all relevant randomised controlled trials
II	Evidence obtained from at least one properly designed randomised controlled trial
III-1	Evidence obtained from well-designed pseudo-randomised controlled trials (alternate allocation method)
III-2 (observational)	Evidence obtained from comparative studies with concurrent or historical control groups, cohort studies, case-control studies or interrupted time series with a control group
III-3 (comparative)	Evidence obtained from comparative studies with historical control
IV	Evidence obtained from case studies
Excluded	Evidence from expert opinion and consensus of an expert committee

Source: Spencer (2003)

2.14.5 *Analysis of information obtained from the clinical evaluations of milk fluoridation*

Earlier sections of this chapter gave descriptions of the clinical studies of milk fluoridation. The design of these studies varied from randomised controlled studies to evaluation of community preventive programmes (or 'field' studies); they were conducted in 10 countries during the period 1962 to 2005. The preceding sections indicated that there were few RCTs. Nevertheless, both of the RCTs and 11 out of the 13 non-RCT studies reported statistically significant reductions in caries increment or caries experience compared with control groups. The purpose of this section is to examine variables relevant to a milk fluoridation programme in order to assist those in public health choose the most effective programme. These variables are: age at which the children enter the programme, the number of years the children are in the programme, background caries severity, fluoride dose per day, number of days that the fluoridated milk is drunk per year, the time of day the fluoridated milk is drunk, and the method of drinking the fluoridated milk (Table 2.26).

When looking at how old the children were when they entered the milk fluoridation schemes, it can be seen that beginning to drink fluoridated milk at an early age was essential to obtain a caries preventive effect in primary teeth. In all five of the studies where children began to consume fluoridated milk before the age of 4 years, caries reduction in primary teeth was observed. For those beginning at the age of 4 years, only two out of five studies reported a caries reduction in primary teeth. To prevent the occurrence of caries in permanent teeth, the children should still be in the programme at the time of eruption of the first permanent molars. If the factors relevant to the two studies which showed no effect are examined, it can be tentatively concluded that: (1) in the Lopes study, the study period was very short to show a caries-preventing effect in permanent teeth (16 mo), and the number of days the children drank the fluoridated milk was no more than 200 per year and may have been much less; (2) in the Ketley study, there was a low background caries experience, the fluoride dose was fairly low, and the number of days was no more than 200 per year. From the information in Table 2.26, it is not possible to make firm conclusions regarding background caries experience, amount of fluoride, days per year or time of day, but it might be tentatively suggested that effectiveness increased with fluoride dose and number of days exposure to fluoridated milk per year.

Table 2.26

Studies ranked or grouped according to six factors which vary between studies and which could influence the effectiveness of milk fluoridation, together with an indication of effect on primary and permanent teeth

	Age at start	Study length	Effect on teeth		Background caries exp.	Amount of F (mg/d)		Days / y	Time of day
	y	y	Prim.	Perm.					
					Low	Low		Low	Mid morning
Mariño	0	4	+	.	Ketley	Bian	0.5	Legett	Bánóczy
Ziegler	1	6	+	+	Riley	Ketley	0.5	Ketley	Pakhomov (B)
Bánóczy	2	4	+	+	Medium	Riley	0.5	Pakhomov (B)	Stephen
						Pakhomov (R)	0.5		
Pakhomov (B)	3	5	+	+	Legett			Zahlaka	Zahlaka
Pakhomov (R)	3	5	+	.	Rusoff	Medium		Stephen	Ketley
						Ziegler	0.5-0.7		
Stephen	4	5	-	+	Weitz	Bánóczy	0.4-0.75	Riley	Riley
Zahlaka	4	3	+	+	Pakhomov (R)	Mariño	0.25-0.75	Lopes	Bian (+ home)
Bian	4	2	+	.	High	Weitz	0.65	Weitz	Weitz
Ketley	4	4	-	-	Bánóczy			Pakhomov (R)	Pakhomov (R)
Riley	4	7	.	+	Stephen	High		Medium	Lunch
Legett	5	3	.	+	Zahlaka	Legett	0.9	Rusoff	Legett
Rusoff	6	3.5	.	+	Bian	Rusoff	1.0	High	Rusoff
Lopes	6	1.3	.	-	Mariño	Stephen	1.5	Bánóczy	Lopes
Weitz	6	3	.	+	Pakhomov (B)	Zahlaka	1.0	Bian	All day
					Ziegler	Pakhomov (B)	1.0	Mariño	Mariño
					Lopes	Lopes	1.0	Ziegler	Ziegler

Pakhamov (B) = Bulgarian study, Pakhomov (R)= Russian study. Note : Values should be taken as approximate.
The classifications into 'Low', 'Medium' and 'High' subgroups are largely pragmatic as severity of background caries experience and amount of fluoride provided in the milk depend on the age of the children. For more precise information, the reader is referred back to the descriptions of the studies.
+ = evaluated, effect; - = evaluated, no effect; .= no evaluation.

2.15 Conclusions

There have been some 20 reports of 15 studies of the effectiveness of fluoridated milk in preventing dental caries. In addition to the reports listed, a number of abstracts have been published as well as papers in languages other than English. These studies took place in 10 countries. Eight of the studies showed a caries preventive effect in primary teeth and 10 studies showed a caries preventive effect in permanent teeth. Two studies showed no effect in either dentition. In addition, one study investigated the effect of cessation of a fluoridated milk programme; this showed an increase in caries incidence in the children who had stopped drinking fluoridated milk. A systematic review identified two randomised controlled trials, both of which showed caries reductions in the children who drank fluoridated milk. At the present time, there has been no study of the effect of fluoridated milk in adults.

From the results of these clinical studies, it would appear necessary that children begin to drink fluoridated milk at an early age, preferably before 4 years, in order to reduce caries in primary teeth. It would also appear necessary for children to be drinking fluoridated milk when their first permanent molars erupt in order to protect these teeth. It is not possible to draw firm conclusions about other variables, such as fluoride dose, number of days per year, background caries experience, time of consumption and method of drinking fluoridated milk, although it would appear desirable to avoid too low a dose of fluoride and the number of days a child receives fluoridated milk should be as many as possible.

3 Basic science studies

W. M. Edgar

3.1 Introduction

The general understanding of the mode of action of fluoride in reducing the incidence of dental caries is that an elevation of the concentration of the ion at the plaque-enamel interface results in a reduction in the rate of demineralisation, an increase in the rate of remineralisation, and a reduction in the rate of acid production in plaque. The required elevation in fluoride concentration is small but prolonged; large increases in intra-oral fluoride following use of dentifrices, mouthwashes or tablets are soon cleared from the mouth, but residual reservoirs release fluoride slowly over a period of hours or even days to exert the above effects. Ingested fluoride is also re-secreted in saliva, and fluoride that is incorporated in the crystals of the surface layers of the enamel during tooth development may be released during an acid attack and re-deposited in the subsurface enamel where the developing lesion may be arrested.

This paradigm forms the background to the present review of the basic science aspects of milk fluoridation. The review is structured as follows: first, evidence on the chemistry of fluoride in milk is considered; then evidence regarding the fate of fluoride after ingestion is examined, including measures of bioavailability; and finally data relating to the effects of fluoride from milk on intra-oral systems – enamel, saliva, plaque and their interactions — are evaluated. Comparison of the results of these studies with those for fluoridated water, whose efficacy is well established, should enable a judgement to be made concerning the biological plausibility of milk fluoridation as a caries-preventive measure.

3.2 Chemistry of fluoride in milk

Concerns about the chemical behaviour of fluoride in milk, and eventual interactions between milk proteins, calcium, phosphate and fluoride initiated studies early after the beginning of milk fluoridation. The aim of these chemical analyses was to determine the free ionisable and total fluoride content of fluoride compounds added to milk, in order to assess the bioavailability or activity, and to draw conclusions regarding the behaviour of fluoride in milk.

Study of the interactions between fluoride and milk components were difficult to perform before the advent of the fluoride ion-selective electrode in the early 1960s. An exceptionally perceptive paper by Ericsson (1958), however, used radioactive fluoride as a tracer to study both chemical interactions and bioavailability of fluoride in standard and homogenised milk. He found that, at 1 and 4 ppm F, no sedimentation of fluoride occurred indicating that insoluble precipitates of salts such as CaF_2 did not occur over 5 hours. Fractionation of the milk showed that 20 to 25% of the fluoride was bound to the casein component, and only traces were found bound to albumin or in the cream. Ultrafiltration of the milk showed that not all of the fluoride was diffusible, consistent with binding to macromolecular component(s).

Konikoff (1974) examined the availability of fluoride in freeze-dried milk using the fluoride electrode. He found that fluoride was well-dispersed in the dried samples. His study purported to show that fluoride was essentially free in milk, as a calibration curve of a range of fluoride concentrations followed physico-chemical theory (a ten-fold increase in fluoride concentration gave rise to an electrical potential difference of 58 mV as predicted by the theoretical 'Nernst equation'). However, he did not show comparable data for fluoride in water, and it would be expected that if a constant proportion of the fluoride was bound, the electrode response should still follow the Nernst equation. In addition, the fluoride electrode requires that the sample be mixed with TISAB (total ionic strength adjustment buffer) at pH 5.0, so any complex formed by fluoride with milk components could have been dissociated. He found that addition of calcium to fluoridated milk did not cause appreciable precipitation of fluoride (as occurs in water); he speculated that this was due to binding of calcium to part of the protein molecule and of fluoride to another part, but the observation could be explained by the presence of TISAB.

The level and chemical form of the fluoride secreted naturally in human and bovine milk, and the effect of drinking fluoridated water on the concentration of fluoride in milk, has been the subject of some controversy, and claims have been made that some of the secreted fluoride is present in a non-ionisable form (Backer-Dirks *et al.*, 1974). Although these data are outside the remit of this review, some of these papers have reported fluoride added to milk to measure its recoverability with the analytical technique used. Duff (1981) found that after addition of 1ppm F to bovine milk, 'ionic F' levels fell on standing (for up to 72 hours) while 'total F' remained constant. He suggested that milk was, therefore, not a satisfactory vehicle for fluoride administration, but his data may have been a result of his non-standard method of analysis of ionic fluoride. Beddows & Kirk (1981) developed a technique for fluoride analysis using a citric acid buffer to precipitate the proteins before analysis of the supernatant with the fluoride electrode; they found complete recovery of added fluoride up to 100 ppm. Similar results were obtained with cold milk, pasteurised milk and UHT milk, using radioactive fluoride as a tracer. Direct analysis of fluoride in milk with the electrode would be feasible for control purposes during manufacture of fluoridated milk, but would not be accurate enough for determination of total fluoride.

Beddows (1982) went on to examine the interaction between fluoride and components of whole, homogenised and skimmed milk. No effect was found on the size or charge of the micelles of casein with up to 10 ppmF. Little evidence of precipitation of fluoride was observed, even with high-speed centrifugation or after addition of calcium ions. All of the added fluoride was dialysable, and all of the dialysate was present as free fluoride ions. He suggested that the fluoride was in simple ionic equilibrium, perhaps forming a reversible ionic complex with milk protein. Heat-treated fluoride-containing milks (Beddows & Blake, 1982) showed similar behaviour on dialysis, except that there was more evidence of complex formation with the protein components, with similar results being obtained with heat treated casein suspension. After precipitation of proteins with citric acid buffer, all of the fluoride was recovered in ionic form. Although removal of calcium and phosphate from milk by dialysis did not prevent complex formation with fluoride, it may not have been possible to remove the ions from inside micelles, or bound tightly to amino-acids in casein such as phosphoserine. In an abstract, Phillips (1991) studied the stability of fluoride

added at 5 ppm to UHT, pasteurised and powdered milk. All of the fluoride added to pasteurised milk could be recovered, using the fluoride electrode, over the 3 day shelf life of the milk; with UHT and powdered milks some reduction in fluoride availability (4 to 12%) occurred indicating complex formation by heat treatment of the milk proteins.

Wieczorek *et al*. (1992) studied the binding of fluoride by purified milk proteins using a gel filtration technique. They found no evidence of binding at normal pH values; only at pH 3.9 did lactalbumin bind fluoride. However, the technique involved dissolving the proteins in an fluoride-free buffer; only when the sample entered the gel did it come into contact with fluoride. If the complex formation is time-dependent, the contact with fluoride may have been too brief. In addition, the purification of the proteins may have changed their conformation and their binding properties. A further publication in Polish by Chlubek (1993) presents the same data on protein binding, and indicates that fluoride is found in both whey and curds; some 11% was found in the lipid fraction, in contrast to the findings of Ericsson (1958) and others.

Kahama *et al*. (1997) described the analysis of non-fluoridated cows' milk by hexamethyldisiloxane-assisted microdiffusion (the milk is acidified forming HF, which in the presence of HMDS diffuses out of the sample into a trap of alkali, from whence it is recovered, buffered and measured with the fluoride electrode). They measured total fluoride by ashing the milk, or by digestion with proteolytic enzymes, followed by microdiffusion. About half of the fluoride was diffusible and thus the remainder was in 'bound' form; but all the total fluoride (measured after ashing) was detected in unashed milk after proteolytic digestion. They concluded that the bound fluoride is sequestered physically or chemically within the milk protein molecular structure. This elegant study answers many of the questions identified in earlier work.

Sharkov & Phillips (2000), in an abstract describing the fortification of fluoride-containing milk with trace metals, distinguish between the availability and activity of fluoride in milk. They suggest that the latter measurement is a better guide to the topical action of fluoride, in that activity describes the ability of the ion to react with enamel or plaque components. This may, however, change in the oral environment and,

in view of the uncertainty as to the interaction between fluoride in milk and the plaque-enamel system, an *in vitro* measurement of ionic activity may not be a good predictor of its efficacy.

Sodium monofluorophoshate (MFP) is well known, from its use in dentifrices, not to bind to calcium salts and has been found to be absorbed rapidly in the stomach in the presence of calcium (Ericsson *et al.*, 1961; Ericsson, 1983; Ekstrand & Ehrnebo, 1980) and at high pH (Whitford *et al*., 1983). There do not appear to be any detailed studies of its chemistry in milk, but Villa *et al*. (1989) found it to be well absorbed from milk in rats and children. The relative importance of the local action of fluoridated milk as compared with absorbed and re-secreted fluoride needs to be determined to allow interpretation of bioavailability data (see section 3.3). If the main anti-caries activity of fluoridated milk is a direct, local effect, then MFP may have one disadvantage, as dentifrice clinical trials indicate that it is slightly less effective than NaF (Bowen, 1995). However, despite this reservation, the use of sodium monofluorophosphate in milk fluoridation has some advantages: first, it is absorbed well (see section 3.3) and second, it mixes well with powdered milk (see chapter 4).

To summarise this section, there is general agreement that a fraction of fluoride added (as NaF) to milk is complexed or sequestered with the protein fraction in an ionisable form, while virtually all of the rest is in free ionic form. More complexation occurs with heat-treated milk but, in the main, there is little difference between fresh, homogenised, skimmed, pasteurised, UHT and powdered milk in this respect.

3.3 Absorption, metabolism and excretion

The favourable clinical results reported from the early milk fluoridation schemes stimulated researchers to investigate more deeply the mode of action of fluorides in milk. Numerous studies examined: the absorption of different fluoride complexes consumed under different conditions; the distribution and excretion of fluorides – pointing to the question of bioavailability, — systemic and/or topical effects; and the usefulness of urinary excretion measurements as indicators of fluoride metabolism.

It is well established that, in fasting subjects, fluoride is absorbed rapidly from the stomach — a process accelerated by the low pH through the conversion of fluoride to uncharged HF, which is transferred across

the stomach epithelium more rapidly than the fluoride ion. Fluoride absorption is retarded by the simultaneous presence of food in the stomach, and by a higher than normal gastric pH. Once absorbed into the plasma, fluoride is distributed throughout the body as free fluoride ions at concentrations ranging from 0.5-1.0 µmol/l (0.01-0.02 ppm) in fasting subjects to, for example, 2.0-4.0 µmol/l (0.04-0.08 ppm) following a dose of 1.0 mg F in water. Fluoride leaves the plasma by crossing the periosteum and being incorporated in new bone; by crossing the reduced enamel epithelium and Hertwig's root sheath during tooth formation; by crossing the periodontium into cementum throughout the life of the tooth; by crossing the secretory epithelia of the salivary, lacrimal, sweat and gastro-intestinal glands; and by filtration in the renal glomerulus (with no reabsorption in the loop of Henlé and distal nephron). Approximately 50% of the ingested fluoride is excreted in the urine in adults. Unabsorbed fluoride (about 10%) appears in the faeces.

Some early fluoride excretion data in children receiving fluoridated milk were reported without supporting description by Ziegler (1956) and the first systematic study of the absorption and metabolism of fluoride from water and milk in rats, and urinary excretion of fluoride from water and milk in human subjects, was published by Ericsson (1958), using radioactive fluoride. In rats, the absorption of fluoride into the blood was much slower from milk than from water over the first 1 hour after ingestion; the increase in blood fluoride content from milk was more protracted and the cumulative uptake was only 6% lower from milk after 10 hours. Femoral fluoride content was about 80% from milk as compared with water; uptake from each peaked at 4 hours, and declined thereafter suggesting loss of fluoride from bone.

In six human volunteers, 1 mg doses of fluoride to which ^{19}F was added as a tracer were dissolved in water or milk and were consumed 3 hours after the last meal. Urine was collected before and after 1, 2 and 4 hours. The rise in urinary fluoride was steepest in the first hour with water as the vehicle, and in the second hour with milk. The 4 hour total excretion was about 80% from milk compared with water. Perceptively, Ericsson comments that, if enamel mottling is caused by excessive peaks in plasma fluoride, the slower but more prolonged absorption of fluoride from milk might make it a safer vehicle than water. On the other hand, if the deposition of fluoride into the teeth is similar to that into the rat

bones, and is important for its anti-caries action, fluoride from milk might be less cariostatic.

At this time, the concept of fluoride bioavailability was based on the then prevailing hypothesis that the mode of action of fluoride was, by being incorporated in the developing enamel, to reduce enamel's solubility in acid. However, König (1960) found that in rats, administration of fluoride to the dams during pregnancy and lactation led to a significant rise in bone fluoride uptake from both water and milk (implying uptake by the developing teeth), but no inhibition of caries on exposure of the weanling rats to a cariogenic diet (for further discussion, see section 3.4.1).

After the publication of the pilot study of milk fluoridation by Rusoff *et al*. (1962) and the 7 year findings of the Winterthur study (Ziegler, 1964; Wirz, 1964), Stamm (1972) criticised the concept of milk fluoridation partly on the basis of a misconception of Ericsson's (1958) findings. He stated that, "unlike fluoridated water, fluoridated milk does not have a significant topical effect on the teeth" because there is a 1 hour delay in the release of fluoride from milk (during which time it would be cleared from the mouth). In fact, the 1 hour delay is in the absorption of fluoride into the plasma, and Ericsson showed that 75 to 80% of the fluoride in milk was not bound and therefore free to exert a topical effect before swallowing.

Shchori *et al*. (1976) measured fluoride uptake in rat molars as well as bone when the animals were administered various fluids containing fluoride at 2.9 ppm as well as controls. Tea, with or without milk, and 2.9 ppmF milk without tea, gave similar bone levels, significantly higher than controls, but tea without milk showed significantly higher molar uptake than controls or fluoridated milk without tea. Tea with milk showed lower, but not significantly different, fluoride uptake in molar surfaces. These findings were confirmed recently by Székely *et al*. (2006) who reported that milk reduced bioavailability of fluoride from tea by about 10%; this *in vivo* study estimated bioavailability by measuring urinary fluoride excretion.

Patz *et al*. (1977) as part of a study on the effect of food intake on fluoride bioavailability, showed that, in beagle dogs, milk and baby formula delayed the rise in plasma fluoride and reduced the 'area under the curve' (of plasma fluoride plotted against time up to 8 hours; AUC) by approximately 25%. In an important study in human subjects,

Ekstrand & Ehrnebo (1979) studied the bioavailability of 3.0 mg F in water, with milk and with milk plus breakfast, measuring plasma fluoride profiles over 9 hours and urinary fluoride excretion over the same period. Bioavailability was measured by comparison with intravenous infusion of NaF in saline over 30 min. The mean values for plasma AUC were: fluoride in water, 100.8% of the fluoride saline infusion; fluoride in milk, 73.8%; fluoride in milk + breakfast 53.7%. For urinary output, the mean values were: 102.2%, 62.2% and 66.1%, respectively. The authors suggested that the reduced bioavailability was brought about either by release of calcium from a bound form in milk in the stomach with formation of CaF_2, or by neutralisation of the gastric acid resulting in the reduction in the proportion of fluoride in the diffusible HF form, and thus the absorption of fluoride. They conclude that fluoride tablets should not be taken with milk or milk products, presumably on the premise that plasma fluoride is the key parameter determining the efficacy of fluoride supplements.

Most discussion of bioavailability studies had hitherto considered that the data related to anti-caries efficacy on the basis of uptake into developing enamel. However, at around this time, evidence was accumulating that the principal action of fluoride in reducing caries was locally at the plaque/enamel interface. Nevertheless, because the levels of fluoride in duct saliva parallel closely those in plasma (Carlsson *et al.*, 1967; Shannon, 1977; Trautner & Siebert, 1986), bioavailability data derived from plasma fluoride profiles could yet be an indicator of efficacy.

Further data were provided by Spak *et al.* (1982) using fluoridated water, milk and reconstituted dried baby formula (each 500 ml at 10 ppmF providing 5 mg F) in four fasting young adults. Bioavailability results (AUC data) were 72% for milk and 65% for baby formula, and 76% and 63% respectively for urinary excretion data. Recoveries of fluoride added to milk and formula *in vitro* were 98 to 100%, and 87 to 91% respectively, and the authors attribute the reduced bioavailability of fluoride from milk to formation in the stomach of a physical barrier to absorption (as also suggested by Ericsson, 1958) in view of the lack of binding of fluoride to milk *in vitro.* In discussing their findings, the authors raised the interesting point that calculation of the fluoride intake of infants and children from analysis of the diet may overestimate the true fluoride exposure because of reduction in bioavailability of ingested fluoride. Reference is also made to the

importance of the fluoride level of drinking water in determining the fluoride intake of bottle-fed infants; breast-fed infants are exposed to very low levels of fluoride as there is in humans little transfer of plasma fluoride across the mammary glands (Ekstrand *et al.*, 1981).

Brambilla *et al*. (1995) measured fluoride levels in plasma, urine, and amniotic fluid in 126 pregnant women receiving milk alone, fluoride in milk (1 and 2 mg F) and 1 mg F as NaF tablets. Plasma levels were 0.015, 0.026, 0.046 and 0.036 ppm F, respectively, all differences being statistically significant. Amniotic fluid levels also rose significantly, being about half those of the plasma; the differences between fluoride groups were not statistically significant. Although it is generally held that fluoride does not cross the placenta easily, the same group had previously shown elevation of amniotic fluid fluoride levels with doses of fluoride in water above 1 mg (Brambilla *et al*., 1994).

Trautner & Siebert (1986) reported reductions in bioavailability of fluoride from foods including milk; the presence of bone meal reduced bioavailability by over 80%. Bioavailability was determined from plasma and salivary profiles (AUC) and from urinary excretion data. Shulman & Vallejo (1990) also found, in human subjects, a reduction in bioavailability of 13% with milk and 47% with foods.

An important finding was described by Trautner (1989) and Trautner & Einwag (1989) who showed that, in six subjects, bioavailability of fluoride was reduced by simultaneous ingestion of solid food. Plasma fluoride peaks after ingestion by fasting subjects of 2 mg fluoride as NaF or NaMFP, in solution or as tablets, were reduced by milk (relative bioavailability in milk 70%) but with milk and food the plasma fluoride profile was flattened but prolonged, and the relative bioavailability was 96 – 99% compared with fluoride from water in fasting subjects. Milk reduced the bioavailability of both NaF and NaMFP, contrary to the findings of Fuchs *et al*. (1982) who found that the bioavailability of fluoride from NaMFP was not reduced by milk compared with water, using higher doses of fluoride (18 mg) and lower volumes of milk (200 ml). The similarity of their results for the two fluoride sources, led Trautner and Einwag to suggest that the reduction in bioavailability with milk is due to the physical barrier provided by the coagulated milk solids, and not to binding by calcium or elevation of stomach pH (in agreement with Spak *et al.*, 1982). The milk then passes from the stomach to the small intestine where absorption is slow. If, however,

solid food is taken concomitantly with milk and fluoride, stomach emptying is delayed and, as the milk proteins are digested in the gastric secretions, the sequestered fluoride is liberated and thus absorbed. The issue is not completely resolved as, in a recent abstract, Tóth *et al.* (2007) reported that fluoride excretion in urine (a measure of bioavailability) is higher when consumption of fluoridated milk is separated from a meal by two hours, compared with simultaneous consumption.

Villa *et al*. (1989) studied bioavailability, as measured by bone fluoride uptake in rats and as urinary fluoride output in pre-school children, of powdered milk fluoridated with NaMFP. They found, in the rat experiment, that with *ad libitum* food access, the bone uptake was approximately twice as much from NaMFP in milk than from NaF in water. When fluids and solids were administered separately, uptake was similar for both fluoride sources. A similar result was found with the children: when food and fluoride were taken together, urinary output of fluoride from NaMFP in milk was more than twice that from NaF in water, but when the fluoride was administered on a fasting stomach, no difference was found. The interpretation of these findings is that NaMFP does not become complexed with milk and is equivalent to NaF in fasting conditions, but also is not affected by the presence of food. The study did not compare milk fluoridated with NaF and NaMFP; from the results of Trautner and Einwag above, bioavailability when taken with food should have been similar.

Marthaler *et al*. (1978) had proposed that the aim of regimes for systemic fluoride supplementation (in particular, salt fluoridation) should be to adjust the dose so that urinary fluoride excretion would match that observed in successful water fluoridation schemes. This rule of thumb could be applicable to milk fluoridation assuming the modes of action of fluoride in water and milk are similar. Subsequently, urinary fluoride excretion has been used to estimate fluoride intake for the purpose of setting the correct dose of fluoride in milk. In an abstract, Marthaler & Phillips (1994) measured fluoride output in the urine of 5-6 year old Bulgarian children prior to the implementation of a milk fluoridation scheme. Because of the difficulty in achieving a 24 hour urine collection, a timed sampling procedure was introduced: after emptying the bladder at about 8 am, the children collected urine under supervision until about 12 noon when they emptied their bladder again. The collection was repeated from noon to about 4 pm. The child's parents were instructed to collect the first urine sample on waking, and

to time the period from going to bed and the first micturition. The volumes and the fluoride concentrations of the samples were measured, and the baseline fluoride excretion rate (µg F/h) calculated. The same collection procedure was repeated next day, but with the consumption of 200 ml milk containing 1 mg F (5 ppm) immediately after emptying the bladder at 8 am. Similar data were obtained from a control group of children. By subtracting the baseline data from the test data, the excretion of fluoride derived from the fluoridated milk was calculated. The results showed that approximately one-sixth of the administered dose was excreted during the daytime collection periods; the fluoride excreted in the nocturnal collections were substantially the same. Using these data for Bulgarian children, comparison was made (Marthaler *et al.*, 1994) with Swiss children consuming fluoridated salt. The excretion rates for the Swiss children were somewhat higher, with occasional high values attributed to consumption of other fluoride-containing items.

In 1997, Kolesnik published the results of urinary monitoring studies in children aged 4-6 years in three Russian cities scheduled for milk fluoridation schemes. The method of urine sampling was similar to the timed collections of Marthaler & Phillips (1994) above, and the data were extrapolated to provide estimates of the integrated daily urinary F excretion (IDUFE: mg/day) and, assuming the fractional excretion of fluoride being 50%, the daily fluoride intake (DFI). The method is described fully in a WHO booklet edited by Marthaler (1999). Data for IDUFE are shown for collections taken before and at 1 week, 6, 12 and 24 months after implementation of milk fluoridation at 0.45 mg F per day (180 ml of milk containing 2.5 ppmF). The values increased from 0.22-0.26 mg/day in the three cities before fluoridation of the milk, to 0.44-0.56 mg/day over the 24 months after introduction of fluoridated milk. No evidence was found for a compensatory adaptation in fluoride excretion, or fluoride accumulation. Similar results were found after 3, 4 and 5 years (Kolesnik & Pereslegina, 2000; Pereslegina *et al.*, 2002). More recently, Székely *et al.* (2007) reported, in an abstract, an increase in fluoride excretion in urine after fluoridated milk was consumed for a week rather than for one day only.

Toumba *et al.* (1996), in an abstract, studied simultaneous plasma and urinary fluoride profiles after consuming 200 ml of milk containing 1 mg F, in eleven adult subjects. The plasma fluoride peaks (mean 0.05 ppm) occurred at 30 min after milk intake, and the urinary peaks

(127 µg F/h) in the 0-60 min collection. After 1 month of fluoridated milk consumption, a spot plasma fluoride measurement showed a significant elevation (value not stated) but surprisingly 24 hour urinary samples did not indicate an increased rate of fluoride excretion. No explanation was given for this anomaly; it is difficult to see what compensatory mechanism could lower the urinary fluoride output despite the evidence of increased plasma levels.

In an abstract, Villa (2000) described the fractional excretion of a dose of 1 mg F as NaMFP in powdered milk and orange juice. Fluoride excretion was measured before and after fluoride administration in 7 or 24 hour samples. The fluoride excreted attributable to the dose, divided by the dose (the fractional urinary excretion of fluoride, FUEF) was 25.9% for milk and 27.9% for orange juice, not statistically significantly different. Similar results were obtained with 7 hour collections. No daily fluoride excretion data were presented. In a subsequent abstract, Villa (2001) reported data for five young adults who provided plasma fluoride profiles and urinary fluoride data for 9 hours after consumption of 3 mg F in water, 3 mg F in water plus breakfast, and 3 mg F in milk plus breakfast. Bioavailability estimates showed no statistically significant differences between the three modes of administration. It is not stated whether or not the fluoride was in the form of NaMFP, as used in previous studies by this author; the finding of Villa *et al*. (1989) that fluoride excretion from NaF was suppressed when taken with food would suggest that the present abstract was describing data for NaMFP. A brief abstract by Wang *et al.* (2001a) stated that the bioavailability of fluoride from milk was 80.2%, by plasma fluoride profile and 84.8% from urinary excretion data.

As part of a milk fluoridation project in Merseyside, Ketley & Lennon (2000) studied the maximum fluoride excretion rate and the daily fluoride excretion in eight 4 – 5 year old children monitored continuously for 55 hours. With consumption of 0.5 mg F in the milk, the mean daily fluoride excretion was 0.33 mg. A second part of the study measured the fractional fluoride excretion of a 0.5 mg dose of fluoride given to the children after removing the fluoridated milk from the diet during a period of 4 days before administering the standard dose; an average of 30% was found. Comparable data were obtained by Villa *et al.* (1999) in Chilean children consuming 1 mg F in orange juice, and other studies in pre-school children have found fractional fluoride excretion values of 20 to 30% (Hargreaves *et al*., 1970; Ekstrand *et al*., 1994). A later

study by Ketley & Lennon (2001) of 24 hour urine samples from 5 – 6 year old children in the Merseyside fluoridated milk scheme, found a mean daily fluoride excretion of 0.30 mg F, and a fractional fluoride excretion, in response to doses in the range 0.5 – 2.0 mg F, of 39%. In this case, the period of low fluoride milk administration was omitted before the fluoride dose period, to avoid the possibility of negative fluoride balance. The estimated total daily fluoride intake during consumption of fluoridated milk at 2.5 ppm was 0.76 mg — considered to be sub-optimal. The fraction of fluoride excreted was higher (50%) from the lower dose of 0.5 mg than from the higher doses of 1.0 – 2.0 mg (30%). An evaluation of the available data on fractional urinary excretion by Villa (2004) revealed that the variation in individual fractional fluoride excretion values is attributable to the inverse of the daily fluoride dose, the rate of urinary fluoride excretion and the age of the child. It is clear that estimation of the fluoride intake of infants from urinary output data must be accompanied by estimation of the fractional urinary fluoride excretion value.

A further study by Ketley *et al.* (2002) compared 24 hour urinary fluoride excretion in 2 to 5 year old children in (a) a low water fluoride area, (b) similar children drinking milk containing 2.5 ppmF, and (c) children receiving optimally-fluoridated drinking water. The daily fluoride excretion values were: low fluoride water, 0.21 mg; fluoridated milk, 0.30 mg; fluoridated water, 0.36 mg. The data support the conclusion that the concentration of fluoride in school milk, at 2.5 ppm, was too low.

A pilot study was reported in abstract (Székely *et al*., 2002) in which the fluoride excretion of 3 to 7 year old Romanian children was estimated from spot samples using the F/creatinine ratio to estimate daily output (Marthaler *et al*., 1995). The mean daily urinary F excretion was 0.32 mg. Using the supervised time-controlled method to estimate the daily fluoride output in the same children (Székely *et al*., 2004), a value of 0.34 mg was obtained. The difference was not significantly different in a paired t-test, indicating the validity of the simpler spot sample fluoride/creatinine ratio method for monitoring fluoride excretion on a community basis, provided the sample is taken before or well after fluoride administration. Zohouri *et al.* (2006) investigated the relationship between fluoride excretion in urine, estimated by the fluoride/creatinine ratio in a spot sample and fluoride excretion measured from 24 hour collection. They reported a statistically significant

positive correlation (0.76), suggesting that the spot sample estimation may be a practical alternative to longer collections of urine, in some circumstances.

In an abstract, 24 hour urinary fluoride excretion was studied in adults consuming a liquid diet consisting of mineral water, vegetable juice and 200 ml milk daily, with or without 1 mg F (Gintner & Bánóczy, 2002a). After 5 days, the mean fluoride concentrations were 0.23 ppm in controls, and 0.58 ppm in the fluoridated milk group. No results for daily fluoride excretion were given. The fractional fluoride excretion was about 55%.

To summarise the literature on fluoride absorption, metabolism and excretion in relation to fluoride in milk: in fasting subjects, the absorption of fluoride is reduced by 25 - 30% when milk is ingested simultaneously. Depending on the nature of the food, solid food administered with fluoridated milk delays gastric emptying and flattens but prolongs the plasma fluoride profile so that more time is available for absorption of fluoride, thus allowing 100% bioavailability. Food without milk also reduces the plasma fluoride peak. In some studies, milk reduces the bioavailability of fluoride from NaMFP, similar to that of NaF, but the evidence is equivocal. The suggested advantage of adding fluoride to milk in the form of NaMFP rather than NaF, because of increased bioavailability, requires confirmation. The delay in absorption of fluoride from milk is probably due to the physical sequestration of the fluoride by curdled milk solids rather than chemical binding.

The results of analyses of urinary fluoride excretion relating to bioavailability largely agree with conclusions drawn from plasma fluoride profiles. Most studies of urinary fluoride excretion are undertaken to monitor the intake of fluoride in community-based fluoride supplementation schemes including fluoridated milk. Fractional fluoride excretion in urine is approximately 50% in adults, but can be as low as 20% in infants, depending on the growth rate, other dietary items, the child’s age and the fluoride dose. Optimal daily urinary fluoride excretion values for 3 to 5 year old children are about 0.4 – 0.5 mg.

3.4 Effects of fluoride from milk on intra-oral systems

The oral cavity represents a very complex domain, where the introduced materials, foods, chemicals etc. may undergo modifications. The surroundings of the dental hard tissues, fluids of the oral cavity (saliva, crevicular fluid) and deposits on the teeth (dental plaque, calculus), including the oral ecosystem, can promote, inhibit or change the effect of fluorides, employed systematically and re-secreted through saliva, or used topically. Therefore, before, or accompanying clinical use, the behaviour of fluorides should be, and has been, studied in order to determine optimal effects in a given situation.

3.4.1 *Intra-oral enamel uptake, and de- and remineralisation of enamel.*

An important factor when determining the mode of action of fluoride in milk was to assess its effect on human teeth. Methods to examine the effect of fluoride on de- and remineralisation of human teeth are manifold: (a) *in vitro* studies on the hard tissues of extracted human teeth, (b) human dental enamel samples built into dental appliances and worn (*in vivo* experiments) by human subjects under different conditions, and (c) *in vivo* experiments on the teeth of human subjects, for example, enamel biopsies under everyday conditions to determine changes in teeth in their usual environment.

Light *et al*. (1958) published a brief report showing data for the fluoride content of the primary teeth of one child who received fluoridated milk from birth and who did not experience decay. This was followed in 1968 by similar data for a second child (Light *et al.*, 1968). These are perhaps the first reports of incorporation of fluoride in human subjects from fluoridated milk, but are of course too slight to be reliable. A rather anecdotal account by Imamura (1959) of his experience of fluoridation of school meals (soup and milk) indicated higher fluoride levels in the enamel of primary molars, but no details of the samples or methods of analysis are given.

In 8 to 10 year old children who had consumed fluoridated milk for 5 years (i.e. before and after eruption of the maxillary incisors), enamel biopsy showed higher fluoride content in incisor enamel than in control children not receiving fluoride supplementation (Tóth *et al.*, 1987). The calcium dissolved during biopsy (acid etch) was lower in the fluoridated milk group than in the controls, suggesting a reduction in enamel solubility, but the difference was not statistically significant. The

children were participants in a clinical caries trial of fluoridated milk, which had demonstrated a 60% reduction in DMFT. A 3 year clinical trial of milk fluoridation (Zahlaka *et al*., 1987) in children initially 4-7 years old, showed a significant reduction in caries in both primary and permanent teeth, but no difference in incisor enamel fluoride content, in contrast to the findings of Tóth *et al*. The post-eruptive exposure of the permanent maxillary incisors to fluoride in milk in this study may have been less than in the Hungarian study, perhaps indicating that uptake into enamel before eruption was not an important factor in this instance.

In a longitudinal experiment in which children were given 1 mg F in milk for 12 months, enamel biopsies were taken before and at 6 and 12 months after introduction of the supplement (Tóth *et al*., 1989). Enamel solubility, measured as the release of phosphorus in the biopsy procedure, was reduced significantly at 6 and 12 months, while the fluoride content rose significantly after 12 months. It would have been of interest to study enamel solubility in children receiving milk without fluoride.

In an intra-oral caries model system, Chandler *et al.* (1995) found, in a preliminary study, that fluoride as NaF and NaMFP did not significantly reduce the demineralisation due to dietary carbohydrate, when added to milk which was administered by immersion of experimental, gauze-covered bovine enamel blocks which were worn in an appliance in the mouths of human subjects (intra-oral cariogenicity test, ICT). The number of subjects involved, the intensity of the caries challenge, and the duration of the experiment were all, however, small.

Tóth *et al*. (1997) studied the uptake of fluoride into the surface enamel, and anti-solubility effects, of the exposure *in vitro* of experimentally demineralised human enamel to milk containing 0, 1 and 10 ppm F for 7 or 14 days. Only with 14 days exposure to 10 ppm F were the enamel fluoride levels raised, and acid solubility reduced significantly, compared with plain milk. A later *in vitro* experiment, reported in abstract by Al-Khateeb *et al*. (1998), found that remineralisation of artificial white spot lesions in human enamel occurred (determined by quantitative laser fluorescence, QLF) after four weeks exposure to milk containing 0, 1, 2.5 and 5 ppm F, but no difference was found between fluoride levels. It is possible that *in vitro* exposure does not take into account factors that are important for fluoride incorporation *in vivo* – for example, the presence of plaque and saliva, which may interact with milk fluoride at the enamel surface.

In an abstract, Rugg-Gunn & Boteva (1997) compared enamel fluoride uptake *in vivo* when enamel samples mounted in intra-oral appliances were worn in the mouth and exposed four times daily for 5 days to fluoridated milk or water. Uptake was greater for fluoridated milk when it was swallowed after rinsing, compared with rinsing alone or fluoride in water. Similar results were found with sound or demineralised surfaces. This preliminary result suggests the importance of the indirect effect of fluoride after absorption and re-secretion in saliva.

Rugg-Gunn & Boteva (2000a) reported increased *in vitro* remineralisation of white spot lesions by exposure for 2 hours/day for ten days to fluoridated milk compared with fluoride in water or milk alone. However, it would appear that the lesions were sectioned prior to exposure (thus the exposed enamel was not the true surface enamel), the baseline demineralisation of the fluoridated milk group was much lower than the other two groups, and there is confusion as to the numbers of sections and their status (natural or artificial). An *in situ* study by Wang *et al*. (2001b) indicated enhanced remineralisation of lesions in bovine enamel with fluoridated milk compared with fluoridated water, but the description of the experimental details is not sufficient to judge the merits of the study.

The effect of fluoridated milk on enamel in an *in vitro* demineralisation model was reported by Arnold *et al*. (2003). The model involved immersion of enamel in a demineralising solution for three days followed by milk, fluoridated milk (at 1 ppm), saline, and a remineralising solution for three days, the two phases of the experiment being alternated for 99 days. After serial sectioning, the zones of the lesion were determined by polarised light microscopy, and their volumes determined by computerised 3-D reconstruction. With fluoridated milk, the volume of the body of the lesion was significantly smaller than with milk alone or with saline. The superficial zone was significantly thicker with fluoridated milk than with the other treatments, and thicker with milk alone than with saline. The calcium content of the superficial zone in the remineralising solution group was significantly higher than in either of the milk groups, while the calcium content of the body of the lesion was significantly higher in the fluoridated milk group than in any of the other groups, and that of the milk group higher than in the saline group. The phosphorus contents broadly followed the calcium data. The fluoride content of the superficial zone in the fluoridated milk group was significantly higher than any of the other groups, but this

difference was not seen in the deeper zones. The authors conclude that fluoride in milk prevents demineralisation in a dynamic 'cycling' model in three ways: first, fluoride binds to calcium to form a reservoir of loosely bound fluoride, second, milk calcium and phosphate contribute to remineralisation and, third, that milk proteins are adsorbed onto the enamel surface to protect the enamel from demineralisation.

The effect of fluoridated milk on root surface lesion progression in an *in vitro* pH cycling experiment was reported by Ivancakova *et al*. (2003). After artificial lesion formation, root tissue sections were cut and coated with acid-resistant varnish except for the initial root surface, which remained exposed to subsequent treatments. The cycling involved 4 hours demineralisation, 6 hours treatment and 14 hours remineralisation for 2 weeks. Lesion progression was measured by polarised light microscopy and microradiography. Treatment groups were water controls, milk alone, milk + 2.5 ppm F, and milk + 5 ppm F. All lesions progressed during the experiment, but the depth of the lesion increased least in the 5 ppm F milk. The increase with milk alone was significantly lower than in the control group. Integrated mineral loss rose in all groups, significantly less in the 2.5 ppm F milk group than the control group. The results provide evidence for a possible protective effect against root caries in the adult population.

A report by Kahama *et al*. (1998) described the effect of variation in intrinsic fluoride levels in cows' milk on calcium loss in a pH cycling system: a reduction of 36% in calcium loss was observed when the enamel samples were demineralised at pH 5 and remineralised with cows' milk containing 0.3 ppm F compared with low-fluoride milk (0.03 ppm F).

Engström *et al.* (2006) reported an investigation into the effect of the addition of fluoride to milk on lesion formation *in vitro,* where lesions were measured by laser fluorescence. Laser fluorescence was considered satisfactory for diagnosis, and lesion formation was significantly reduced by 5ppm F in milk.

Summarising the papers on hard tissues effects, evidence for enamel uptake of fluoride from fluoridated milk is equivocal: this variation in results may indicate the relevance and importance of re-secreted fluoride in saliva on enamel fluoride uptake. However, fluoridated milk promotes remineralisation of lesions in enamel *in vitro* and *in vivo,* and inhibits demineralisation in enamel and dentine. Milk itself

has a protective effect in intra-oral caries models as well as *in vitro* (McDougall, 1977). There is considerable scope for further experimentation with such models to explore further the dose-response concentrations, mode and frequency of administration with the purpose of optimising the delivery of fluoride in milk.

3.4.2 *Intra-oral effects in human subjects: saliva and plaque*

The behaviour of fluoride in the oral cavity is influenced considerably by the presence of saliva and dental plaque. Changes in the secretion rate of saliva and salivary clearance, as well as the mode of consumption (rinsing, drinking and swallowing) may alter the salivary level of fluoride which, in turn, influences the plaque fluoride level. Interactions at the dental plaque/enamel interface are decisive for the incorporation of fluoride. Therefore, numerous *in vivo* studies, prominently in the last decade, have investigated the influence of saliva and dental plaque on enamel fluoride uptake.

The study of the effects of fluoridated milk in saliva and plaque has only recently been attempted. Twetman *et al.* (1998) measured fluoride in whole saliva, parotid and submandibular gland secretions from schoolchildren before and after 7 days in which the children consumed fluoridated milk (at 1 ppm F) daily for 7 days. Fluoride levels were significantly elevated in whole saliva at 1 and 3 hours (approx 0.8 µmol/l, c.f. baseline 0.4), and in gland secretions up to 6 hours after fluoridated milk ingestion. In an abstract, Gintner *et al.* (2000) described an experiment in which adult subjects collected saliva before and after rinsing for 5 min with 200 ml of water or milk containing 5 ppm F. Similar fluoride peaks of approximately 0.5 ppm were found in 5 min stimulated saliva samples immediately after rinsing with both solutions, but the salivary fluoride remained high for 2 hours after fluoridated milk, while it fell to resting levels immediately after the 5 min sample with fluoride in water. This suggested that milk promotes local retention of fluoride after rinsing compared with water. A somewhat different result was found by Rugg-Gunn & Boteva (2000b), in which unstimulated saliva was collected for two, 4 min periods after rinsing for a total of 90 sec with 100 ml of 5 ppm F in milk or in water, with low fluoride milk and water as controls. More fluoride was retained in the mouth, and a higher fluoride concentration seen in the first unstimulated saliva sample after rinsing with fluoridated milk compared with fluoridated water, but the fluoride concentration fell in both tests during

the second 4 min saliva collection. This contrasts with the results of the study by Gintner *et al*., reported above, might be because the samples were unstimulated, and the increased oral activity in collecting stimulated saliva used in the Hungarian study could have dislodged fluoride from reservoirs attached perhaps to the soft tissues into the samples. However, the study of Twetman *et al*. above, found no significant difference between unstimulated and stimulated salivary fluoride levels before and after drinking fluoridated milk.

Boros *et al*. (2001) found that after rinsing with 200 or 500 ml of milk with 5 ppm F, no increase was seen in the fluoride concentration of unstimulated whole saliva at 45-55 min or urinary fluoride excretion at 60 min of young adults, compared with fluoride-free milk. However, when the milk was ingested, both unstimulated whole saliva fluoride concentrations and urinary fluoride excretion rose significantly. This suggests that the increase in salivary fluoride was due to re-secretion of fluoride through the salivary glands after absorption. The fluoride concentration in the saliva after the larger volume of milk (2.5 mg F) was lower than after the smaller volume (1 mg F); this anomaly was explained by a more rapid absorption of fluoride from the larger volume; thus the fluoride peak in the saliva may have occurred before the sample was collected. Labial (minor) gland saliva contained about ten times the fluoride concentration of that of unstimulated whole saliva, and did not alter significantly 45-55 min after fluoridated milk consumption. It was suggested that the higher fluoride concentration in labial gland saliva might be due to reabsorption of water (but not fluoride) via the non-keratinised buccal mucosa.

Petersson *et al*. (2002) studied salivary and plaque fluoride concentrations in 6-8 year old children after consumption of 200 ml portions of water and milk with and without 1 mg F. The fluoride concentration was raised significantly in unstimulated saliva at 15 min following ingestion, but not at 120 min. However, plaque collected 120 min after ingestion contained significantly more fluoride after fluoride in milk or water than at baseline or after fluoride-free milk or water, indicating entrapment of fluoride by plaque components. A later paper from the same group (Engström *et al.*, 2002) examined plaque fluoride concentrations in children, adolescents and adults before and at 30, 120, 240 min, 12 and 18 hours after fluoridated milk ingestion. The milk was administered as a single dose, and as four successive daily doses. With both methods of administration, the plaque fluoride was raised

significantly (approximately 3-fold, from around 6 to around 18 ppm wet weight) at 4 hours but not thereafter.

In a study of the effect of F milk on salivary properties, Gintner & Bánóczy (2002b) administered milk with and without 5 ppm F during 5 days when the subjects received only liquids. Saliva fluoride was 0.57 ppm at the end of the study (controls, 0.096 ppm). No details are given, in this abstract, as to the method and timing of the saliva sampling. The effect of mode of ingestion of fluoridated milk on saliva fluoride levels was studied by Gintner & Bánóczy (2003). Peak saliva fluoride concentrations, from most to least, were observed after rinsing for 5 min (1.62 ppm) > rinsing for 1 min (1.43 ppm) > drinking with a straw between the lips (1.37 ppm) > drinking with a straw positioned at the back of the mouth (0.93 ppm). No statistics are given in this brief report, and the levels at later times after drinking, when re-secretion of fluoride through the glands would be expected, are not reported.

A number of papers have described studies of saliva and plaque properties following use of fluoridated milk. Gombik *et al*. (1992) and Kertész *et al.* (1992) reported in abstracts that, in children consuming milk containing 2.5 ppm F for 8 weeks, plaque fluoride was raised, the total plaque bacterial count remained the same, but counts of mutans streptococci fell significantly: in controls the bacterial counts rose. No effects were observed on salivary bacteria.

In a study by Jentsch *et al*. (1999), adult subjects rinsed for 10 min with 10 ppm F milk, milk alone, 10 ppm F water and water alone, and swallowed the rinse. The volume of the rinse and the total amount of fluoride swallowed were not stated. Unstimulated and stimulated whole saliva samples were collected before and 60 min after rinsing and analysed for a number of salivary components which affect the oral microbial flora. Compared with baseline values, rinsing with fluoridated milk and fluoridated water (but not milk or water) raised the activities of salivary peroxidase and α-amylase in unstimulated and stimulated saliva. No explanation or suggested mode of action of these effects was presented.

In an abstract, Kertész & Vásárhelyi-Peredi (1996) studied the pH response in plaque after rinsing the mouth with milk, milk + sucrose, milk + xylitol, milk + fluoride, milk + sucrose + fluoride, and milk + xylitol + fluoride. Sucrose solution was a positive control. Milk + sucrose reduced acidogenicity compared with the positive control, and

milk + sucrose + fluoride caused a further reduction. Acid formation from lactose in native milk was reduced by fluoride and by xylitol, added separately; together they eliminated acid production from lactose even in subjects with high rates of lactose fermentation. The rate of acid production from lactose is, however, rarely sufficient to lead to significant cariogenicity, and with fluoride present the risk of caries from milk sugar is negligible.

Ivanova & Mateeva (1996b) studied plaque distribution and thickness in 6 year old children receiving no milk, milk alone and fluoridated milk. The authors state that both milk and fluoridated milk have a plaque-inhibiting effect, but the description of the work, especially the method of measuring plaque thickness, is not clear enough to judge its merits.

An *in vitro* model of plaque in the form of an oral bacterial biofilm was grown in artificial saliva and pulsed with 200 ml of milk or 5 ppm F in milk over 30 min each day for 1 month. The presence of fluoride in the milk resulted in a raised pH in the biofilm, and the numbers of total streptococci and mutans streptococci were lower, in the 'climax community' after 13 days growth (Pratten *et al.,* 2000).

A further *in vitro* study of the effect of fluoride as NaF and NaMFP in buffer and in milk on the survival and growth kinetics *of Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans* was conducted by Kamotsay *et al*. (2002). They found that none of the levels of fluoride tested (1 to 1000 ppm) affected survival of any of the microorganisms, but that at high concentrations, fluoride in buffer slowed the exponential phase of growth; milk with added fluoride could not be studied by the method for growth kinetics used.

Engström *et al*. (2004a) did not find significant changes in the salivary microflora after daily intakes of fluoridated milk (250 ml, 5 ppm) for 4 weeks, but the same group (Engström *et al*., 2004b) described a study in children of lactic acid formation in sucrose suspensions of plaque sampled before and at 30, 60 and 180 min after drinking 250 ml of milk with or without 5 ppm F. Without fluoride, lactic acid formation rose in the 30 min samples, but this did not occur with fluoride present. No change was seen in the plaque acidogenicity compared with baseline at 60 or 180 min. The results suggest a direct effect of plaque fluoride derived from milk on acid production.

In summary, the evidence indicates that fluoride levels in whole saliva rise for 30-60 min after fluoridated milk ingestion, and that this rise is due to re-secretion of absorbed fluoride via salivary glands, as well as retained milk fluoride. Plaque fluoride levels also increase, over more prolonged periods than saliva fluoride; while the evidence of a change in salivary and plaque microbial composition due to fluoridated milk consumption is mostly rather negative.

3.4.3 *Experimental studies*

The incorporation of fluoride from milk into dental enamel, and subsequent caries reductions were investigated in numerous studies on experimental animals. The advantage of using animals – mainly caries-susceptible rats – is the short time period required for expected changes in the hard tissues of the teeth, and the more or less free choice and limitation of influencing/confounding factors. Although the results of these studies might be extrapolated to human circumstances with caution, these experiments have brought important knowledge on the possible effects of fluoridated milk.

Milk itself exerts a weak protective effect on dental enamel in animal experiments (Shaw *et al.*, 1959, Bánóczy *et al*., 1990). As noted previously, König (1960) published data showing incorporation of fluoride from milk into rat bone and presumably into the developing teeth: rather less was incorporated from milk than from water. Caries inhibition occurred when fluoridated milk was administered post-eruptively at the same time as a cariogenic diet, but not when administered only during tooth development. The results strongly suggest that the action of fluoride was at the enamel-plaque interface; the effect could be a direct topical reaction or indirectly via salivary re-secretion of fluoride. In the experiments of Poulsen *et al.* (1976) no effect of the vehicle (milk or water) on caries reduction was found, but more fluoride was incorporated into enamel from fluoridated milk than from fluoridated water, with both pre-eruptive and post-eruptive administration.

Results of other rat caries studies (Bánóczy *et al*., 1990; Rotgans 1992; Stösser *et al*., 1995 a and b) similarly found lower caries scores in the fluoridated milk group, than in the fluoridated water group. Stösser *et al*. (1995 a and b) studying milk with different levels of fluoride, found a dose-response effect on caries reduction and a small effect in raising enamel fluoride content. The caries preventive effect was not

dependent on the different fat contents of the milk, or using different fluoride compounds.

Somewhat confounding results – contrary to the previous findings — were reported in an experiment in rats administered during pregnancy and lactation, and in the pups after weaning (Ivanova & Mateeva, 1996a) with the conclusion that the greatest benefit was obtained when fluoridated milk was present during all three phases. Also, Cutress *et al.* (1996), in a study of the incorporation of fluoride into developing ovine incisors, found that administration of fluoride in milk or water resulted in enamel fluoride levels that were independent of the vehicle. These results are in contrast with other findings on the effect of fluoridated milk on enamel of erupted teeth only.

Summarising the information from animal experimental caries studies, reductions were consistently found in rats consuming fluoridated milk, fluoridated water and milk alone compared with water, when the fluoride was administered during caries formation. Milk was a more effective vehicle than water, exerting a protective effect itself. The effect was not seen when the supplementation occurred only during tooth development. The experiments used higher fluoride levels than with human fluoridated milk supplements, but this is consistent with water fluoride data, where protective effects equivalent in percentage terms to water fluoridation at 1 ppm F are achieved in rats with 10 ppm F.

3.5 General summary: the biological plausibility of milk fluoridation

The above review of the action of fluoride in milk and in water demonstrates that ingestion of fluoridated milk increases the concentration of fluoride in saliva, as does the ingestion of fluoridated water. The ingestion of fluoridated milk increases the concentration of fluoride in dental plaque and there is some evidence that it decreases acid production in plaque induced by exposure to sugar, as occurs with the ingestion of fluoridated water. There is some evidence that the ingestion of fluoridated milk increases the concentration of fluoride in enamel, both pre- and post-eruptively, as occurs after ingestion of fluoridated water. The ingestion of fluoridated milk decreases enamel demineralisation and increases enamel remineralisation, as does the ingestion of fluoridated water. It would appear that, at an appropriate concentration (about 1 mgF/l in water and about 5 mgF/l in milk), fluoride in milk exhibits similar anti-caries properties to fluoride in water.

For some of the evidence – for example, caries in animal experiments – these caries-preventive actions of fluoride appear to be greater in milk than in water.

In conclusion, this review has shown that fluoride in milk behaves similarly to fluoride in water in the important actions known to lead to caries prevention. The biological plausibility of fluoridated milk as a caries preventive measure is established.

4 The addition of fluoride to milk

A. E. Villa

4.1 Introduction

Fluoridated milks may be produced in a variety of different forms: liquid (pasteurized, sterilized and UHT) and powder, each containing different fluoridating compounds. Compounds which have been used to fluoridate milk in the earlier clinical trials and laboratory tests included sodium fluoride, calcium fluoride, disodium monofluorophosphate (MFP) and disodium silicofluoride (Stephen *et al.,* 1984; Bánóczy *et al.,* 1985; Villa *et al.,* 1989; Stösser *et al.,* 1995a and b). However, the vast majority of present ongoing international fluoridated milk schemes (in Peru, Bulgaria, China, Russia, Thailand and the United Kingdom), use sodium fluoride as the fluoridating compound. The exception is the caries preventive programme in rural areas of Chile, where the powdered milk and milk derivatives provided to the participating subjects are fluoridated using MFP.

The manufacture of fluoridated milk involves the addition of a fluoride compound to milk in the appropriate amount, such that the resulting product contains the required fluoride concentration. The concentration of fluoride required in the product is dictated by the fluoride dose to be delivered to the recipient children, so as to provide them with the optimum amount in line with the recommendations of the WHO (1994) Expert Committee, i.e. ranging from zero to 1.0 mg F per day according to the age of the child and the fluoride concentration in the local water supply. However, over recent years, the total daily fluoride intake, including other sources of fluoride, is taken into account before establishing the fluoride dose to be delivered by fluoridated milk. The total daily fluoride intake is usually estimated by means of urinary fluoride excretion of the target population (Marthaler, 1999). This issue will be discussed in Chapter 6 (Programme Monitoring).

To calculate the appropriate fluoride concentration, it is necessary to consider the volume of fluoridated milk consumed daily by each child. The volume consumed varies with location: for example, in the UK, a child would typically receive 1/3 pint (189 ml) of school milk per day, whereas in China, kindergarten children each receive 250 ml.

Using these two examples, in order to deliver an appropriate dose of, say, 0.5 mg fluoride per day to children in both areas, the fluoride concentrations in the milk would need to be set at 2.65 ppm and 2.0 ppm, respectively. In Plovdiv, Bulgaria, where 200 ml is the typical volume consumed and the fluoride requirement is 1 mg per day, the concentration of fluoride in milk is set at 5 ppm. Sodium fluoride is generally added to milk in the form of a concentrated aqueous solution using a fixed volume ratio to obtain the required product. The manufacture of fluoridated milk using sodium fluoride is discussed in Section 4.2.

Disodium monofluorophosphate (MFP) is used as the fluoridating agent for manufacturing powdered fluoridated milk and milk derivatives in the current caries preventive programme in Chilean rural areas: the manufacture of these products is discussed in Section 4.3. The choice of MFP in the Chilean scheme was based, partly, on fears that sodium fluoride would not be suitable for the manufacture of fluoridated milk as it was deemed likely to interact with calcium, to its detriment. In practice, these fears have since been shown to be unjustified at the fluoride levels used in milk. This point is dealt with in detail later in this chapter. Monofluorophosphate also reacts with calcium to form a neutral complex Ca MFP; this being more soluble than calcium fluoride (Villa *et al.*, 1992). Another reason for choosing disodium monofluorophosphate for fluoridating milk in the Chile scheme was that it has been shown to give rise to high bioavailability of fluoride in both animal and human tests (Villa *et al.*, 1989). Villa *et al.* (1993) postulated that the high bioavailability was due to the ease with which the neutral complex Ca MFP is absorbed from the gastrointestinal tract.

Currently, calcium fluoride is not used for large scale production of fluoridated milk because of its low aqueous solubility i.e. 16 mg calcium fluoride per litre at 18 °C (Lide, 1995).

Table 4.1 shows a summary of the main fluoridation characteristics of the various international fluoridated milk schemes currently underway.

As can be seen from Table 4.1, the majority of the ongoing international fluoridated milk schemes use sodium fluoride as the fluoridating compound. Disodium monofluorophosphate is only used in the Chile scheme where fluoridated powdered milk or milk derivatives are manufactured.

Table 4.1
Fluoridating agents, final fluoride concentrations in the fluoridated milk or milk derivatives as ingested, and shelf-life of the fluoridated products manufactured in the different international schemes.

Country	Communities with F-milk schemes	Fluoridating agent	Fluoride concentrations in the final product as ingested (mg/l) (*)	Estimated shelf life (♦)
Bulgaria	Bourgas, Plovdiv, Shoumen, Stara Zagora, Varna, Veliko Turnovo	Sodium fluoride	2.5 – 5.0	5 days for fluoridated milk. 10 days for fluoridated yogurt.
Chile	Fifth to Twelfth Regions	Disodium monofluorophosphate	3.13	6 months (powdered products)
China	Haidian district (Beijing)	Sodium fluoride	2.0	N. A. (♠)
Peru	Trujillo	Sodium fluoride	1.0	Product delivered immediately after manufacture
Russian Federation	Voronezh, Volgograd, Gubkinsky, Niznekamsk	Sodium fluoride	2.25-2.75	36 hours
Thailand	Bangkok, Chumphon, Khon Kaen	Sodium fluoride	2.5	10 days for pasteurized and 6 months for UHT F-milk
U.K.	16 districts	Sodium fluoride	2.65	11 days

***** Final product: fluoridated milk, fluoridated yogurt (Bulgaria), and fluoridated milk and milk-cereal (Chile)
♠ Not available.
♦ Refrigerated at 2-6° C.

4.2 Manufacture of fluoridated milk using sodium fluoride

4.2.1 *Fluoridated pasteurized milk*

Fluoridated pasteurized milk is readily produced by adding an aqueous solution of sodium fluoride to milk in a fixed ratio, so as to achieve the required concentration of fluoride in the product. It is convenient to select the concentration of aqueous sodium fluoride such that one litre of solution would be required to treat 1000 litres of milk. In this way the amount of water added to the milk is small (0.1%) and insignificant. The addition of solid sodium fluoride to milk, although feasible, is not recommended because it is more difficult to control and poses a toxic dust hazard for the operator. This problem is reduced considerably when the solid is handled in the laboratory under carefully controlled conditions when preparing the aqueous solution. Fluoridated milk is produced with different concentrations to suit different requirements (Table 4.1), but if a typical value of 2.7 ppm F is considered, the aqueous solution of sodium fluoride should be made by dissolving 5.97 grams of sodium fluoride (extra pure, BP grade) per litre in distilled water.

The sodium fluoride solution may be added to milk in a batch process or by continuous addition, depending upon dairy facilities. However, in the current ongoing fluoridated milk schemes, batch processes are used. When a batch process is used for the manufacture of fluoridated milk, the appropriate amount of sodium fluoride solution is added to the milk in a holding tank, and the mixture is stirred to give a uniform product. Fluoridation of milk may be carried out before or after pasteurization, but the former is the preferred option. When fluoridation is carried out after pasteurization, great care must be taken to ensure minimal risk of microbial contamination. Precautions include: (1) use of sterile sodium fluoride solution; (2) aseptic handling of the fluoride solution with the operator wearing sterile gloves; and (3) decontamination of access ports and any other vulnerable parts of the equipment before entry, by swabbing with alcohol.

Whether the addition of sodium fluoride is made pre- or post-pasteurization, it is recommended that the solution is sterilized at the time of manufacture and maintained sterile during storage. Sterilization is achieved by autoclaving in purpose-made bottles at 121°C for 15 min. When milk is fluoridated before pasteurization, some loss of ionic fluoride availability may occur as a result of the heat-treatment.

The extent of loss is dependent upon the intensity of the process, but would be small when typical pasteurizing conditions (71.7°C for 15 s) are used.

4.2.2 *Fluoridated UHT milk*

UHT milk is a long-life liquid milk which is preserved by ultra high temperature processing to eradicate, as far as possible, all micro-organisms (see Section 1.2 for more details). In order to make the product palatable for children, it frequently needs the addition of flavour and sweetener. Fluoridated UHT milk is manufactured conveniently by addition of the appropriate amount of concentrated sodium fluoride solution to a tank of milk destined for UHT production. The batch is then mixed thoroughly before processing and packaging. The ultra heat treatment does cause some loss of fluoride availability in the product. A study carried out using the UHT facility at The Borrow Foundation, England (Phillips, 1991) indicated a "process loss" of 12%.

4.2.3 *Fluoridated sterilized milk*

Sterilized milk is the term which is given to milk which is preserved by heat-treatment applied when it is in its final container (e.g. a crown capped glass bottle; see Section 1.2 for details). The severe heat-treatment carries the disadvantage of causing flavour and colour changes in the milk which often makes the product a less popular option. Nevertheless, sterilized milk is distributed to school children in some parts of the world (see Section 4.4.4). Fluoridated sterilized milk is manufactured by mixing the appropriate amount of sodium fluoride (preferably in the form of concentrated aqueous solution) into the batch of milk prior to bottling and sterilizing. As with UHT fluoridated milk, the heat-treatment used in the sterilizing process does have a small (12% decrease) effect on the ionisable fluoride content.

4.2.4 *Fluoridated powdered milk*

In order to achieve a homogeneous product, fluoridated powdered milk is manufactured by fluoridating the liquid milk from which the powder is to be produced. The removal of water from liquid milk to give milk powder is carried out in stages. Initially, the liquid milk is evaporated under reduced pressure to give "evaporated" or "condensed milk". This process removes the bulk of the water. Milk, starting with a typical

solids content of 10-12%, is converted to a concentrate with 45-48% solids. The concentrate is then spray-dried to give the powder. In the manufacture of fluoridated powdered milk, the sodium fluoride solution is conveniently added to evaporated milk prior to spray-drying.

Disodium monofluorophosphate (MFP) fluoridated powdered milk and milk derivatives which were used in the pilot study of caries prevention in rural preschool children carried out in Codegua, Chile, were prepared following the same process as the one described above, sodium fluoride solution being replaced by a concentrated MFP solution (Mariño *et al.*, 1999, 2001). However, in the current ongoing caries preventive Chilean programme for rural elementary schoolchildren which provides fluoridated powdered milk and milk-cereal products to approximately 200,000 children, the MFP fluoridated products are manufactured in a different way. The corresponding process is described briefly in the following section.

4.3 Manufacture of powdered fluoridated milk using disodium monofluorophosphate

Based on the successful clinical results obtained in the pilot study carried out in the Chilean Ninth Region, involving approximately 32,000 rural elementary school children (Weitz & Villa, 2004), the Chilean Health Ministry decided to expand the use of fluoridated milk and milk derivatives to all of the country's rural areas where water fluoridation was deemed very difficult to implement. Since in the previous pilot studies carried out in Codegua (Mariño *et al.*, 2001) and in the Ninth Region (Weitz & Villa, 2004) MFP was used as the fluoridating compound, it was decided that this compound should be used in the expanding fluoridated milk programme. However, taking into account that this national programme, which is estimated to be fully implemented by the beginning of 2007 and will include approximately 240,000 schoolchildren, the manufacture of powdered MFP-fluoridated milk by means of the previously described process (see Section 4.2.4) was considered impractical because the fluoridated products were to be provided by four food manufacturing companies that do not produce powdered milk but instead acquire it from different and variable dairy companies. This is why a different process had to be developed for the manufacture of the required MFP-fluoridated powdered milk and milk derivatives in the expanded programme.

When disodium monofluorophosphate (MFP) is used as the fluoridating agent in the current Chilean programme, which delivers flavoured, vitamin-fortified powdered milk and milk-cereal products, it is added as a carefully weighed powder to the so-called "pre-mix" stage that involves an initial dry mixing of the fluoridating agent with flavouring agents and some other minor components together with a small amount of powdered milk. After another mixing at a larger scale, with more powdered milk, this well mixed product is incorporated to the final mixing machine (equipped with two helicoidal mixers), where the remaining components of the formulation for each product are added and mixed.

After optimizing the process conditions, homogeneity of each batch of fluoridated products is quite good and overall differences of MFP concentrations within a batch are not higher than ± 10% relative to target values. Taking into account that these products are consumed after reconstituting with low-fluoride, previously boiled tap water in a 1:10 ratio and mixing thoroughly, the fluoride concentrations of the ingested products are well within specifications. Quality control measurements on the ready-to-drink fluoridated products are frequently performed as described in Chapter 6 of this publication.

4.4 Stability of fluoridated milk

4.4.1 *Milk fluoridated with sodium fluoride*

Although the use of milk as a vehicle for fluoride was considered some 50 years ago, and positive dental caries prevention results were obtained in trials set up at that time (Rusoff *et al.*, 1962), major developments in the concept have occurred during the last three decades as a result of its promotion by The Borrow Dental Milk Foundation (now known as The Borrow Foundation). Since its formation in the early 1970s, the Foundation has supported clinical trials and community schemes which have demonstrated, beyond question, the efficacy of fluoridated milk consumption on caries prevention in children. These studies are detailed in Chapter 2.

Whilst clinical trials were in progress, some scientists (e.g. Duff, 1981) questioned the suitability of milk as a vehicle for fluoride, claiming that ionic fluoride interacts with milk constituents and, as a result, would be irretrievably lost in the milk matrix. The chemical reactions cited ranged from simple combination with calcium ions to form a

precipitate of calcium fluoride to more complex possibilities of fluoride-binding with proteins. Whereas there is little doubt that there is opportunity for interaction between milk constituents and the fluoride ion, previous studies (Phillips, 1991; Edgar *et al.*, 1992) have shown that such interactions have a relatively small effect on the availability of fluoride in milk, when present in the 2 to 5 ppm F concentration range such as is used in practice.

Within the present context, the term "fluoride availability" refers to the chemical availability of the element in its ionic form. This comprises both free fluoride ions and fluoride in the form of other chemical species which readily release free fluoride ion on demand. Disodium monofluorophosphate can be considered an example of this latter case since the covalent anion $(FPO_3)^{2-}$ releases fluoride ions either in a highly acidic medium or through enzymatic action, e.g. that of acid and alkaline phosphatases (Lo Storto *et al.*, 1992*;* Pearce & Dibdin, 1995; Vogel *et al.*, 2000). This latter issue has been described in Chapter 3 and is discussed further below.

Interaction between fluoride and milk constituents is observed, however, when the fluoride concentration greatly exceeds that used in fluoridated milk consumed by children for dental caries prevention. Cutress *et al.* (1996), in a study investigating the deposition of fluoride in ovine enamel and dentine resulting from the consumption of fluoridated milk, used bovine milk containing 300 and 750 ppm F. At these concentrations, they noted that chemical availability of fluoride was only 30% and 20% respectively, of that added.

The nature of the interaction between milk constituents and fluoride at these high concentrations has not been established, but it is likely that calcium would be involved. The typical calcium concentration of bovine milk is approximately 1200 mg/litre (Jenness, 1988), of which some 80 mg/litre exist as free calcium ions (Holt *et al.*, 1981). Calculations based on the solubility product of calcium fluoride — 3.95×10^{-11} mol^3 dm^{-9} at 298°K (James & Lord, 1992) — show this would not be exceeded at a fluoride concentration of 2.5 ppm and, taking into account the reversible interactions between fluoride and other ionic species in milk, may not be exceeded when the fluoride concentration is 5 ppm. However, the solubility product of calcium fluoride would certainly be exceeded when the fluoride concentration in milk is about 300 ppm.

The problem of fluoride availability as a free ion in milk is partly related to its bioavailability or proportion of the fluoride ingested with milk that is actually absorbed from the gastrointestinal tract. Until approximately two decades ago, the anti-caries activity of fluoride was generally believed to be mainly due to a pre-eruptive (systemic) action. Thus, the decrease of fluoride absorption when ingested with milk or milk and breakfast foods (Stamm, 1972; Ekstrand & Ehrnebo, 1979; Duff, 1981) was considered a drawback for using fluoridated milk in caries preventive programmes. However, the currently accepted mechanism of fluoride's anti-caries effect mainly involves a topical action in the oral cavity. A large number of studies have shown that fluoride exerts its predominant cariostatic effect through the liquid phase at the plaque/enamel interface during the de- and re-mineralization processes (Margolis & Moreno, 1990; Ögaard, 1990; Ten Cate, 1990; Rölla & Ekstrand, 1996; Ten Cate & Featherstone, 1996).

Taking into account the above considerations, fluoride bioavailability in milk appears to be less important than the amount of fluoride that can be incorporated into plaque whenever fluoridated milk is consumed. Some studies on this latter subject have been published recently (Petersson *et al.*, 2002; Ivancakova *et al.*, 2003; Engström *et al.*, 2004a and b) and further efforts in this new research area will be necessary in order to improve our knowledge on the mechanisms by which beneficial effects of fluoridated milk are achieved. The anti-caries effect of MFP fluoridated milk has been clinically proven (see Chapter 2 for more details) but the mechanisms through which MFP exerts a beneficial action in the intra-oral cavity are still poorly understood. It appears reasonable to suggest that MFP hydrolysis leading to free fluoride ions can occur through enzymatic action in dental plaque, although the hypothesis that MFP might have a caries preventive action in its own right cannot be disregarded. Currently, there are ongoing studies aiming to elucidate these issues.

The stability of fluoride in the different types of fluoridated milk is considered to be an important issue when considering the practical aspects of caries prevention schemes. Stability studies have been conducted on various forms of fluoridated milk over the period of their respective shelf-lives, ranging from 3 days at 4°C for fluoridated fresh pasteurized milk, to over six months at ambient temperature for fluoridated UHT and powdered milks (Table 4.1). The results are presented below.

4.4.2 *Fluoridated pasteurised milk*

Investigations undertaken by Phillips (1991) and Edgar *et al.* (1992) have demonstrated that the fluoride availability in fluoridated pasteurized milk (5 ppm F) remains virtually constant at approximately 100% of that added, over a typical storage period of 3 days at 4°C. The experiments conducted by the latter group were designed also to investigate the suitability of glass containers for packaging fluoridated milk with this fluoride concentration. Their conclusion was that interaction between such fluoridated milk and glass was very small and of no practical significance (see Section 4.4.4).

4.4.3 *Fluoridated UHT milk*

In contrast to the findings with fluoridated pasteurized milk, Phillips (1991) showed that there is some loss in fluoride availability in long-life UHT milk resulting from both processing and long-term storage. Fluoridated milk with 5 ppm F exposed to ultra high temperature processing (140°C for 4 s), typically showed a 12% drop in fluoride availability in the packaged product at the time of manufacture. The ionisable fluoride in the product (4.4 ppm F) remained fairly constant during three months' storage at an ambient temperature ranging from 5° C and 20° C. Further storage under the same conditions resulted in a steady decline in ionisable fluoride in the product to 3.75 ppm F after five months and 3.1 ppm F after eight months. However, as by far the majority of UHT milk packs are consumed within three months of manufacture, the fall in fluoride availability in fluoridated UHT milk in the latter period of storage, and in particular beyond six months, is of academic interest only.

4.4.4 *Fluoridated sterilized milk*

The stability and availability of fluoride in sterilized fluoridated milk has been investigated as part of a validating study for a milk fluoridation scheme in Russia, where school children are supplied with sterilized milk in glass bottles. The project considered the effect of sterilizing conditions and the use of glass containers on ionic fluoride availability in the milk.

Results showed that milk with 2.5 and 5.0 ppm F, when sterilized at 115° C for 15 min, gave products with ionisable fluoride levels of 2.2 ppm F (88%) and 4.4 ppm F (88%), respectively. In both cases, 80%

of the original fluoride was available immediately in ionic form, whilst a further 8% assumed ionic form over a four hour period of conditioning with TISAB under analytical conditions (see Chapter 6). The slow release of this small amount of fluoride suggests that reversible binding of fluoride to milk constituents had occurred, to a small extent, during sterilization.

The possibility of interaction between fluoride and glass under these sterilizing conditions was investigated by substituting fluoridated milk with fluoridated water at the same concentration. It was observed that there was no loss of fluoride from the water during processing, hence it was concluded that there was no binding of fluoride to the glass (Edgar *et al.*, 1992).

4.4.5 *Fluoridated milk manufactured from powdered milk*

Powdered milk, fluoridated at the time of reconstitution, has also been the subject of a study by Phillips (1991). He observed that the fluoride availability in this product was a function of process conditions used in the manufacture of the powdered milk and, in particular, it related to the pasteurizing conditions used on the liquid milk prior to drying. Pasteurizing conditions commonly fall into three categories: low heat, medium heat, and high heat. The different heat treatments produce variations in the product, particularly in regard to the whey content, which is heat-sensitive and is denatured at temperatures in excess of 65° C (Early, 1992). Fluoride availability in the reconstituted product was found to be inversely related to the intensity of pasteurization (Table 4.2)

Table 4.2
Fluoride availability in powdered milk reconstituted with a 5 ppm F sodium fluoride solution

Pasteurizing conditions used on liquid milk prior to drying		**Ionisable fluoride concentration in reconstituted fluoridated milk (w/v)**	
Low Heat	77° C / 30 s	4.55 ppm	(96%)
Medium Heat	92° C / 2 min	4.40 ppm	(93%)
High Heat	125° C / 5 min	4.20 ppm	(88%)

Source: Phillips (1991).

The percentage values recorded take into account the volume change which occurs when powdered milk is reconstituted.

4.4.6 *Fluoridated powdered milk*

Fluoride availability in reconstituted fluoridated semi-skimmed powdered milk has also been determined by Phillips (1991), using a product which has been exposed to medium heat pasteurization prior to drying. The fluoridated powdered milk containing 50 mg F/kg which, on reconstitution, provided liquid fluoridated milk (10% solids content) with fluoride ion availability of 4.65 ppm F (93% of the target of 5.00 ppm).

The shelf-life of powdered milk relates to the fat content and the storage conditions (packaging and temperature). Degradation of the fat component gives rise to rancidity (an oxidation process of fatty acids leading to deterioration in the quality of the product) and associated off-flavours marks the end of the shelf-life of a particular product. There is, however, a "grey" area in this definition, as people of different cultures accept different levels of rancidity without question. Shown in Table 4.3 are data which give a rule-of-thumb guide to shelf-life defined according to tastes in industrialized countries. A rise in ambient temperature of 10° C generally results in halving of the shelf-life.

Table 4.3
Shelf life of powdered milk

Milk product	Shelf-life stored at 20° C	Shelf-life stored at 30° C
Skimmed milk powder	2 years	1 year
Full fat milk powder ♦	9-12 months	6 months

♦ *Dependent on country of origin, heat classification, use of anti-oxidants and whether instantized or not*

Shelf-life is also influenced by packaging, as deterioration of the product involves an oxidation process which is accelerated by light. Thus, the shelf-life may be improved by packaging under nitrogen and using materials, which exclude light. The shelf-life is adversely affected by additives such as lecithin, which is included in some powdered milk products to improve dispersibility during reconstitution. Also, it must not be overlooked that the quality of the water used to reconstitute the milk affects the overall quality of the liquid product.

4.4.7 *Stability of powdered milk fluoridated with disodium monofluorophosphate*

Stability tests in respect of milk fluoridated with disodium monofluorophosphate have been conducted by Villa & Torti (1987). The results indicated good product stability. Here, fluoridated powdered milk, containing 26.4 mg F/kg packaged in plastic bags inside cardboard cartons, was assayed as reconstituted liquid milk at intervals over a period of up 12 months' storage as powder, followed by up to 4 days storage as liquid milk at 4° C. Results showed that virtually all of the fluoride present remained in the form of monofluorophosphate with only a small amount (approximately 1.2%) as free fluoride. This level of free fluoride equates with that present in food or pharmacopoeia grade disodium monofluorophosphate, indicating that no detectable enzymatic hydrolysis of monofluorophosphate occurred in the powdered and liquid milk during storage.

4.5 Conclusion

The manufacture of fluoridated milk in various forms (pasteurized, sterilized, UHT and powdered) using sodium fluoride or disodium monofluorophosphate as fluoridating agents, involves simple production techniques. All of the products have been shown to be stable with relatively high fluoride availability remaining throughout their shelf-life.

5 The implementation of community based programmes

S. M. Woodward

5.1 Introduction

By the mid-1980s, with a number of publications reporting encouraging results from clinical studies, interest in the potential for using fluoridated milk at a community level began to grow. However, those contemplating the implementation of such programmes were entering into the unknown, facing technical challenges that had never been addressed and the inevitable political obstacles to the proposed use of fluoride as a public health measure, particularly in an unprecedented form. The major breakthrough came in 1988 when fluoridated milk was introduced in the Bulgarian city of Plovdiv and the neighbouring town of Asenovgrad (Pakhomov *et al.*, 1995). This provided the vital experience and inspiration for others to pursue the development of further schemes, both in Bulgaria and other parts of the world.

During the early 1990s the international milk fluoridation programme began to take shape; projects were introduced in the Russian Federation, China, Chile and the United Kingdom. Later on, the programme was extended to include Peru and Thailand. As in Bulgaria, in each country, the intention has been to establish a demonstration or pilot scheme to provide a model for the implementation of further projects in other parts of that country or region. This has largely been achieved although, in China, despite the success of a pilot study in the Haidian District of Beijing, to date, milk fluoridation has not been applied at a community level. The other exception is Peru, where a scheme in the city of Trujillo ceased due to changes in the community's fluoride status.

The evolution of the international programme has demonstrated that milk can provide an alternative vehicle for the delivery of fluoride, and could be particularly appropriate in those areas where it has not been possible to introduce water or salt fluoridation. The aim of this chapter

is to bring together the knowledge gained in the implementation of schemes and in doing so provide guidance to those who may wish to consider the possibility of using this means of fluoride administration.

5.2 Milk distribution systems

Although there are a number of vital "ingredients" for the successful implementation of a milk fluoridation scheme, existing milk distribution systems have, without question, been the major factor. Not only have they proved fundamental when determining feasibility and sustainability, they have also largely dictated the parameters of the schemes.

5.2.1 *Milk provision*

In many countries, milk is provided to children on an organised basis through the educational system. It is, therefore, no coincidence that the majority of the milk fluoridation schemes implemented to date have been established through schools and kindergartens. These schemes provide controlled and targeted delivery of fluoride. They also have the advantage of being supervised by school staff, thereby by-passing some of the compliance problems associated with other interventions, particularly those directed at socially deprived communities.

The schemes also create greater awareness and a wider appreciation of oral health issues within the schools, with related health promotion activities being integrated into the curriculum subjects. The value of this is recognised by Kwan *et al.* (2005) who stated that "schools provide an important setting for promoting health, as they reach over one billion children worldwide and, through them, the school staff, families and the community as a whole"

Although the greatest potential lies with school milk, national nutrition programmes also provide a vehicle for the delivery of fluoride: for example in Peru where, under the government funded Programa del Vaso de Leche, fresh milk is distributed through local community centres, known as 'Mothers' Clubs'. Also, in Chile, a pilot project in the rural municipality of Codegua was implemented under the National Complementary Feeding Programme (PNAC). This programme provides powdered milk products for children aged 0 to 6 years, distributed by nutritionists at local community health centres. An important aspect of these two schemes is that they enable children to consume fluoridated milk on a daily basis, virtually 365 days per year, compared with

those established through educational systems which typically are limited to 200 days or so per year.

Existing milk distribution systems

Regardless of the type of milk programme, schemes are far more likely to be viable, feasible and sustainable where there is access to existing distribution systems and fluoride can simply be added at source. Not only does this offer obvious cost benefits, but there is the further advantage of established logistical and administrative systems that are adapted relatively easily. The implementation of a totally new scheme, involving the introduction of both milk and fluoride, poses a much greater organisational challenge. However, this has been achieved in both Bulgaria and Russia where local authorities in a number of cities have been persuaded to meet the cost of providing fluoridated milk. Although the programmes as a whole become well embedded, progress in the stand-alone schemes depends heavily on the financial status of regional/local governments and more generally the economy of the country.

There is little doubt that the absence of established milk distribution systems makes implementation of certain schemes more fragile, particularly during their formative stages, and at times has proved to be an obstacle to development. By contrast, schemes that have been introduced through established milk programmes have expanded more rapidly; for example, in Chile and Thailand where the rates of growth have been particularly impressive.

The scheme in Chile was introduced in 1999 in the country's 9th region under the National School Food Assistance Program operated by the Junta Nacional de Auxilio Escolar y Becas (JUNAEB). This established a model which has since been "rolled out" to a further six regions covered by the programme, which is targeted at rural schoolchildren. Similarly, the comprehensive national school milk programme in Thailand provides a vehicle for the development of a scheme. First introduced in 2000 with 14,000 children, this has since been extended to include over 400,000 children.

Constraints and viability

Having access to an existing milk supply has considerable advantages and has been essential to the implementation of the majority of

schemes. However, the adoption of such programmes means working within their constraints. This largely determines the scope of the schemes and more particularly it dictates the:

- geographical boundaries,
- age groups that could be involved and consequently the number of years over which fluoridated milk could be consumed,
- number of days per year that fluoridated milk could be consumed, and
- number of children that could be involved.

Consideration has to be given to the coverage of any milk programme that may be used and more particularly whether it reaches the communities in greatest need of fluoride intervention. However, it has been found that such programmes are often targeted at areas of social deprivation and, importantly, uptake in such areas is generally high.

Similarly, the age groups of children receiving milk need to be carefully assessed to establish the age groups involved and consequently the period over which fluoridated milk could be consumed. As illustrated in Table 5.1, this ranges from kindergarten-based schemes, which are limited to children aged 3 to 6/7 years, to a wider community-based programme in Peru which reached children from weaning through to 14 years of age. There are also variations in age groups in different schemes within the same country; for example in the UK where, in some districts, milk is only available in nursery schools (kindergartens), whilst in others provision extends to children in primary schools through to the age of 11 years. In Thailand, in the cities of Chumphon and Khon Kaen, fluoridated milk is provided to children aged 6 to 10 years in accordance with entitlement under the national school programme whereas, in Bangkok, it reaches a wider age group due to the Bangkok Metropolitan Administration's policy of providing free school milk to all children aged 4 to 12 years.

Another important factor is the number of days per year that milk is provided under the existing programme. This is one of the limitations of school based programmes since, typically, school occurs on about 200 days per year. When this aspect of any proposal is assessed, it is important to consider other factors that could impact on potential exposure — for example, the levels of absenteeism. As previously mentioned, the advantage of national nutrition programmes used in Codegua, Chile, and Trujillo, Peru, was that milk was available on a daily basis, virtually 365 days per year.

The number of children required to make a scheme viable from the milk producers' perspective has proved to be an important consideration, as certain proposals have failed to develop when the total volume of fluoridated milk required is too small. Experience suggests that where fresh milk products are to be used, typically 4,000 - 5,000 children are needed to make the scheme viable. However, this will vary according to local circumstances and depend largely on the milk production facilities. Schemes based on supplies of 'long-life' products such as ultra heat treated (UHT) and powdered milk have been introduced for relatively small numbers, because large volumes can be produced, stored and distributed as required over a period of months. For example, the pilot project in Codegua, Chile, extended to only approximately 1,000 children.

5.2.2 *Fluoridated milk products*

The majority of the schemes implemented to date have used fresh pasteurised milk, although powdered and UHT milk products are also fluoridated at a community level. These are detailed in Table 5.1.

Chile was the first, and remains the only country to use fluoridated powdered milk. The manufacture of such products was established in the mid 1990s for the pilot project in Codegua. Under the current scheme, flavoured powdered milk is used.

UHT milk products are also fluoridated. Although the potential for this was demonstrated by Phillips in 1991, it was not until 2005 that its use was applied in a community based scheme. This is in Thailand where, for commercial reasons, regulations require that 30% of all milk supplied under the national school programme must be UHT.

There are other interesting variations highlighted in Table 5.1. First, in Bulgaria where, in addition to milk, yogurt is fluoridated. Second, in Chile where sodium monofluorophosphate is the fluoridating agent, as opposed to sodium fluoride which is used in all other schemes (see Chapter 4).

The type of packaging used across the schemes is predominantly plastic bags which, globally, are the lowest cost option. This is the case in Thailand although the UHT milk is supplied in cartons, due to the need for aseptic packaging. The other exception is the UK, where cartons are also used which is common practise in school milk programmes throughout this country.

The volume of milk provided to children across the schemes is fairly consistent, with 200 ml a day being consumed at the majority of sites. The amount is slightly lower in the UK where 189 ml is supplied due to historic reasons. This equates to a third of an imperial pint, the traditional measure used since 1946 when the School Milk Act provided free milk for children as part of a wider social programme. The only significant variations occur in Bulgaria where the volumes range between 100 to 200 ml per day depending on the site, the age of the child and the type of product.

Table 5.1 also shows that the daily fluoride dose is generally adjusted according to the age of the children, typically 0.25 to 0.5 mg for nursery children. In Chile, where the starting age of six is comparatively high, children receive 0.625 mg fluoride per day. At two of the sites in Bulgaria — Plovdiv and Stara Zagora — a higher daily dose of 0.75 mg is applied for children aged 6 to 7 years. In the project that operated in the town of Codegua, Chile, the daily dose also varied according to age.

The modes of drinking also vary, with the schemes being divided by the use of straws and drinking from cups. There is interest in the effect that this could have in terms of efficacy, as it has been reported that the mode of ingestion of fluoridated milk could influence fluoride concentration and retention time in saliva (Gintner & Bánóczy, 2003) which, in turn, could influence fluoride concentration in dental plaque.

Cost of fluoridated milk

Milk programmes targeted at schools and/or socially deprived communities, often attract subsidies and, in many cases, milk is supplied free of charge. The funding is often provided from local authority/municipality budgets, although in some cases support is provided directly from central government. Such an example occurs in Chile where the Programa de Alimentación Escolar (PAE), operated by the government funded agency JUNAEB, provides the vehicle for the delivery of fluoride.

Table 5.1
Milk fluoridation programme details, worldwide.

Scheme			Age (Years)	Programme	Milk Product					Milk provision (approx no. of days)
Country	Period	Site			Type	Packaging	Volume	F-Dosage	F-Com.	
Bulgaria	**1988 –**	Plovdiv	3 – 5	School	Fresh Pasteurised	Plastic Bags	100 ml	0.5 mg	NaF	200
			6 – 7	School	Fresh Pasteurised		150 ml	0.75 mg		
		Stara Zagora	3 – 5	School	Fresh Pasteurised		100 ml	0.5 mg		
					Yoghurt		200 ml	0.5 mg		
			6 – 7	School	Fresh Pasteurised		150 ml	0.75 mg		
					Yoghurt		200 ml	0.5 mg		
		Varna, Bourgas, Shoumen, Veliko Turnovo	3 – 7	School	Fresh Pasteurised & Yoghurt		200 ml	0.5 mg		
Chile	**1994 - 1999**	Codegua	0 – 2	National	Flavoured	Plastic Bags	200 ml	0.25 mg	MFP	365
			2 – 3	Nutrition	Powdered		(reconstituted)	0.5 mg		
			3 – 6		Milk & Powdered Milk with Cereal			0.75 mg		

	2000 -	6 Regions	6 – 14	School	Flavoured Powdered Milk & Powdered Milk with Cereal	Plastic Bags	200 ml (reconstituted)	0.625 mg	MFP	200
Peru	**1999 - 2005**	Trujillo	0 – 13	National Nutrition	Fresh Milk	N/A	200 ml	0.25 mg	NaF	360
Russia	**1994 -**	Voronezh, Smolensk, Maikop, Tatarstan	3 – 7	School	Fresh Pasteurised	Plastic Bags	200 ml	0.5 mg	NaF	200
Thailand	**2000 -**	Bangkok	4 – 12	School	Fresh Pasteurised & UHT	Plastic Bags / Cartons	200 ml	0.5 mg	NaF	200
		Chumphon	6 – 10	School	Fresh Pasteurised & UHT		200 ml	0.5 mg	NaF	230
		Khon Kaen	6 – 10	School	Fresh Pasteurised & UHT		200 ml	0.5 mg	NaF	200
UK	**1993 -**	1 District	3 – 5	School	Fresh Pasteurised	Cartons	189 ml	0.5 mg	NaF	200
		4 Districts	3 – 7	School						
		11 Districts	3 - 11	School						

The actual process of adding fluoride to milk is relatively straightforward. Consequently, the cost differential between production of fluoridated and non-fluoridated milk is marginal; so much so that it is generally absorbed by the milk producers. However, in the Bulgarian schemes, when fluoride is added, the cost of yoghurt is approximately 20% higher. Conversely, the cost of milk can be 25% lower, largely attributable to the use of basic packaging, since products sold competitively on the open market are commonly packaged in cartons, which are more attractive but also significantly more expensive.

The additional cost of providing fluoridated milk compared with non-fluoridated milk was reported in a publication by Mariño *et al.* (2007) which presented a cost-effective analysis of a fluoridated milk programme in Chile. This programme is described in Section 2.10.1. The cost of the programme per child per year was 1,839.75 Chilean pesos; equivalent, in 1999, to US$ 3.49 per child per year. Eighty per cent of this cost (US$ 2.79) was accounted for by the programme co-ordinator, office facilities, and the additional cost of adding fluoride to the milk feed. The remaining 20% was the cost of monitoring fluoride concentrations in the fluoridated milk and in the children's urine, and for additional dental examinations requested by the health authorities.

The additional cost of providing fluoridated milk compared with non-fluoridated milk in England was estimated, in 2008, to be UK£ 1.25 per child per year (Woodward, *et al*., 2008). This cost included the cost of the programme administrator, travel, supporting literature, and the addition of fluoride to the milk. This figure of UK£ 1.25 is equivalent (in 2008) to Euro 1.56 and US$ 2.43. Thus, information from Chile and England gives the additional cost of providing fluoridated milk, compared with non-fluoridated milk, at about two to three US dollars per child per year.

5.3 Planning and management of schemes

Although those pursuing schemes need to adapt their approach to local circumstances, all proposals essentially follow similar stages of development. This involves an assessment of feasibility, formulation of comprehensive strategic and operational plans, obtaining the necessary consents/approvals from the authorities, and eventually implementation.

5.3.1 *Ownership*

An important aspect of the schemes currently in operation is that they are owned locally, with their management being largely achieved through the reconfiguration of existing resources. Implementation normally involves close collaboration between health authorities and local authorities/municipalities, although the cooperation of other agencies, for example the milk suppliers, is often required.

In Bulgaria, the 'stakeholders' have typically included representatives from the municipalities, Ministry of Health, local dental associations, academic institutions, dairies and those responsible for milk delivery. The engagement of these parties is formalised through their representation on non- profit organisations established at each site. These are known as "Stomatologichno zdrave" (dental health) Associations and the responsibility for the running of the schemes is largely assigned to these bodies.

In Russia and the United Kingdom, responsibility rests firmly with the local/health authorities. This also applies in Thailand, although the schemes there were developed in conjunction with, and under the auspices of, the Dental Health Division of the Department of Health, Ministry of Public Health. Whilst in Chile, local commitment is equally essential, the development of the programme as a whole is 'driven' centrally by JUNAEB, which uses its own national school feeding programme as a vehicle for the delivery of fluoride. In both Thailand and Chile, this centralised support provides obvious political and technical advantages and there is no doubt that this is a key factor behind the rapid expansion of these programmes.

In all countries, good communication between personnel from the respective project sites is encouraged. In Bulgaria and the UK, this is facilitated by the formation of national network groups which ensure that representatives of each scheme, and other key persons, meet regularly to discuss and provide one another with support for the development of their respective individual schemes and consequently the programme as a whole.

5.3.2 *Feasibility*

The first challenge faced by those promoting schemes is to demonstrate that milk fluoridation is feasible in the targeted communities. At such an early stage, it is not possible to confirm this beyond doubt as,

inevitably, some obstacles are encountered only as proposals progress through the detailed planning process. However, preliminary assessments provide a good indication and at least ensure that those investing time and effort in pursuing schemes do so knowing that there is a reasonable prospect of getting them 'off the ground'. As highlighted earlier in this chapter, the major factor is the availability of a suitable milk distribution system; although there are a number of other matters that require careful consideration. Details relating to the preliminary assessment of feasibility, including a questionnaire, can be found in Section 5.4.

5.3.3 *Regulations*

Early consideration should be given to the regulations that may have a bearing on the proposed addition of fluoride to milk and, inevitably, the position varies from country to country. To date, there have not been any absolute barriers, but certain measures are necessary in order to proceed. For example, in Chile, where JUNAEB had to seek permission to add fluoride to milk, this resulted in a decree made by the Ministry of Health. This authorised the fluoridation of powdered milk products under a national food supplement programme, to provide a daily dose of fluoride of 0.65 mg. However, this was conditional on extensive monitoring and evaluation activities being undertaken, including quality control of the products, measurements of fluoride concentration in the water supplies, urinary fluoride excretion studies and surveys of caries experience.

In Thailand, permission to add fluoride to milk was granted by the Secretary of the Food and Drug Administration (FDA). The Department of Health and FDA set the criteria for the dairies that would be engaged in the programme and each production facility was assessed accordingly. Permissions are granted on a temporary basis and are renewable on a twelve monthly cycle, subject to the necessary standards being met. Monitoring is undertaken jointly by the FDA and the Department of Health.

The permission of the Research Institute of Milk Products has been required in the Russian Federation. This involves the issue of specific technical documentation which is subject to review by the National Research Institute of Nutrition and the approval of the Ministry of Health of the Russian Federation. Participating dairies are required to purchase the approved technical documentation.

Labelling requirements

Labelling requirements are primarily the concern of the milk producers. Although there are subtle variations, in general, the requirements are very similar, with a common need to identify clearly that the product is fluoridated and disclose the fluoride concentration. In those countries where fluoridated milk is distributed alongside non-fluoridated products, the producers are obliged to use contrasting packaging to distinguish clearly between the two.

In Bulgaria, the labelling of fluoridated milk is regulated by the 2005 Foods Act. This requires that the products include information on the brand name, nutritional content, shelf-life, storage conditions, net weight, name and address of the producer, serial number of the batch and, where appropriate, directions for use. This is regulated by the Ministry of Health and there are similar requirements at other sites.

European Union Regulation

In 2006, the European Commission adopted a Regulation aimed at harmonising divergent national rules concerning the additions of vitamins and minerals and certain other substances to foods. Fluoride is included as a permitted mineral, although the form in which it can be added is restricted to certain compounds, namely potassium fluoride and sodium fluoride.

5.3.4 *Developing a plan and securing approval*

Having established feasibility, the next stage is to construct a detailed plan to develop the scheme and to seek necessary approvals/consents for its implementation. The planning process requires extensive consultation involving stakeholders and other key parties that have a bearing on the scheme. Although a good 'picture' of the potential for a scheme is created during the feasibility assessments, it is only at this stage that the true potential can be determined, as the practical aspects of implementation are addressed in detail. As these plans develop, they are translated into protocols which set out clearly the:

- basis for the scheme,
- aims and objectives,
- operational plans,

- roles and responsibilities,
- monitoring and evaluation measures, and
- resource requirements.

Not only does the preparation of comprehensive protocols ensure good planning, they are, in effect, a 'contract' between the stakeholders. They also provide the basis for a submission to authorities whose approval is required.

5.3.5 *The implementation process*

In preparing the implementation of a scheme, it is essential that the arrangements for the production and distribution of fluoridated milk and the necessary procedures for child participation are in place. The decision to introduce fluoridated milk into a community is a major undertaking and, although steering committees can play an important role in earlier phases, their engagement is particularly valuable at this stage. They ensure that responsibilities are shared and that there is the necessary communication between stakeholders to facilitate effective delivery.

- The role of the milk supplier

The availability of a suitable milk supply is explored during the early stages of proposed schemes and, indeed, is fundamental in determining feasibility. However, it is not until the detailed planning stage that the practical aspects of manufacturing and distributing fluoridated milk, and the procedures for the monitoring of product quality, are considered in depth. Inevitably, this involves extensive consultation with the milk producers, those responsible for the provision of milk (normally the local authorities) and other agencies engaged in these processes.

The milk producers obviously play a vital role and normally become enthusiastic partners in the development of the respective schemes. When initially approached, however, there are occasions when dairy companies show a degree of reluctance over their possible participation. This is either due to commercial reasons, where it appeared that there is little to be gained from becoming involved or, in some cases, the discovery of adverse publicity on the effects of fluoride — for example, from information obtained from the internet. The approach to dairies and its timing therefore require careful consideration. Where

an existing milk distribution system is adopted, as local authorities are usually responsible for the issue of contracts for school milk, they often have an established relationship with the milk suppliers. Their influence, in this respect, proves extremely valuable and avoids some of the barriers that can be encountered from a 'cold' approach.

Introducing fluoride to the milk supply

The addition of fluoride to milk is a relatively simple process and, although on occasions, some minor modifications to plants have been necessary, it is well within the capabilities of most dairies to supply products to the required specification. However, it is important to firmly establish this at the outset and to ensure that the fluoridation of milk can be achieved with minimal interference to the existing systems — a key factor in terms of scheme development and sustainability. This has largely been accomplished, with the exception of Trujillo, Peru, where some difficulties were encountered as a result of changes made to the distribution process. Milk had originally been delivered directly from farms to a number of community centres, from where it was collected by, or on behalf of, the participating children. All other schemes involve dairy processing, and the absence of this in Trujillo provided no real opportunity to fluoridate the milk. The organisers therefore found it necessary to introduce a mixing plant at a single collection point. Consequently, instead of making direct deliveries to the various centres, farmers were required to transport their milk to the collection point, await the fluoridation process and then continue on to their delivery destination.

During the planning stage, it is important to ensure that the milk producers have the appropriately qualified personnel to undertake the necessary monitoring of fluoridated milk, on which more details can be found in Chapter 6. Generally speaking, staff on site, responsible for the routine testing of milk, find it relatively straightforward to build the necessary procedures into their quality controls. However, the dairies need to acquire specific electrochemical equipment.

Once the engagement of a dairy is secured and the arrangements with regard to the supply of fluoridated milk confirmed, it is helpful to prepare a protocol which provides details of the specification of the products required, laying out clearly the production, storage and handling procedures.

A further consideration is the delivery systems and whether they can be adapted to accommodate fluoridated milk. This requires careful assessment and is particularly important where fluoridated milk is being distributed alongside non-fluoridated products.

- Training

The pressures on the project teams are, not surprisingly, greatest during the preparatory and formative stages of the scheme, which makes 'up-front' investment in training and capacity building vital to ensure they are well equipped to meet the challenge. This has been important, given that considerable responsibility is transferred to those delivering the scheme 'on the ground' and they have generally very limited involvement in the earlier stages of the process. These project staff find it particularly valuable to be informed about the basis for a scheme, plans for its development, the basic principles of fluoride addition and the key issues surrounding its application.

In Chile, to facilitate the rolling out of the programme, JUNAEB organised a series of workshops and produced supporting information. This included self learning modules, promotional brochures, videos developed for food handlers, and manuals providing instructions for reconstituting fluoridated milk.

As part of the training provided under schemes in the UK, project staff are encouraged to visit the dairy where they observe the manufacturing, packaging and distribution processes as well as the quality controls applied to fluoridated milk. This provides them with a better understanding and appreciation of the dairy systems and enables them to be better equipped to deal with issues arising in connection with this aspect of the scheme.

Dairy personnel

Another important consideration is the training required by dairy personnel. It is important to ensure that those responsible have access to necessary technical information and support, and to establish appropriate and robust systems for manufacturing the product. Although milk producers are generally obliged to have qualified staff to carry out quality controls, almost without exception, training is required in the use of fluoride measuring equipment. It is not uncommon for the support of academic institutes to be sought with this aspect of the

schemes, as in Chile where the Instituto de Nutricion y Tecnologia de los Alimentos (INTA) has been engaged under a technical agreement with JUNAEB.

The rapid growth of the scheme in Thailand has resulted in a sudden increase in the number of dairies engaged in the programme. The Department of Health, therefore, made training an absolute priority and courses were run at the Milk Collection Centre at the Royal Chitralada Projects in Bangkok, providing personnel from participating dairies with instruction on the appropriate manufacturing and monitoring methods. In collaboration with the Department of Health, staff from the Royal Chitralada Projects combine the experience gained from running these courses with the knowledge developed from their own engagement in the production of fluoridated milk, to produce an operational manual for the dairies.

- Recruitment and Engagement of Schools

The development of schemes through educational institutions requires the co-operation of school/kindergarten teachers, administrators and other staff. Those participating are informed about the scheme and the reasons for its implementation. They are also given the necessary support to develop their systems for administering fluoridated milk. This varies from site to site but typically involves procedures for ordering, handling, storage, and distribution within the school. For example, in Bulgaria, briefing sessions and workshops are organised for directors and head teachers of the kindergartens. In Thailand, meetings are held for school teachers and other personnel engaged in delivering the scheme to ensure they are well informed.

Another important aspect is the 'recruitment' of children. In school-based schemes, the local/education authorities generally determine whether positive (active) or negative (passive) consent is sought and, most importantly, parents/guardians are provided with the relevant information (informed). This involves the development of materials in appropriate 'language' and, in some cases, authorities also convene open meetings. The dissemination of this information and the 'recruitment' process as a whole is a major undertaking and, in the initial phases, school personnel often require support.

- Scheme Promotion

Consultation with the key persons and organisations in the community is an important consideration. This varies from scheme to scheme, but includes local dentists and other health care professionals who are likely to be providing advice within the local community. One example of this is in Russia where seminars are held for paediatricians.

Health promotional activities have become an integral part of several milk fluoridation schemes, particularly where schools and kindergartens are involved. Games, competitions, plays and other activities are developed and incorporated into core curriculum subjects.

In many sites, publicity is viewed as essential for strengthening schemes, although the degree to which the wider engagement of the community is sought varies from country to country. In Bulgaria, "Healthy Teeth" festivals are held and milk fluoridation is incorporated into other local community initiatives. Promotion is also achieved through various media. For example, in Russia, articles are published in the local press and presentations made on local radio and television. Similarly, the media provide information on milk fluoridation in Bulgaria and Thailand, and play a major role in raising the profile of the programme.

As with other fluoridation programmes, from time to time, the schemes encounter opposition. This is inevitable given the sensitivities surrounding fluoride administration, but organisers are able to demonstrate the sound basis for the intervention and that risks have been assessed carefully.

- Monitoring Scheme Development

It is important for project workers to establish systems for monitoring and reviewing scheme development. Particular emphasis is placed on dairy compliance and uptake. It is essential for these measures to be in place at the outset, as experience has shown that the risk of problems arising is greatest during the early stages of implementation.

Maintaining good communication between the 'stakeholders' is also essential. Where steering groups or similar bodies are established at the outset, it is very helpful for these to remain in place. This encourages regular reviews, measuring progress against the protocol, and provides for prompt reaction and decision-making in response to any obstacles encountered.

5.4 Lessons learnt

As the milk fluoridation programmes have evolved, it has become ever more apparent that the success of such interventions is inextricably linked to the strength and depth of the milk distribution systems under which they are established. The extent to which milk is provided has largely dictated the parameters of the programmes and the stability of such systems is fundamentally important in terms of achieving sustainability. When a proposed scheme is under consideration, it is therefore essential that the milk supply has been carefully examined at the outset.

It is clear that the use of existing milk distribution systems provides the greatest potential for programme development. Also, although national nutrition initiatives are used, it is evident that opportunities to apply such interventions are most likely to arise through school-based schemes.

Experience would also indicate that programmes which are coordinated and driven centrally by government departments or agencies have enjoyed an easier passage compared to those introduced under local initiatives. This offers obvious political advantages, and the implementation process as a whole is greatly eased when milk fluoridation is promoted under a national strategy. This is evident in both Thailand and Chile where rapid growth has been achieved in the respective programmes.

The role of the dairies has also proved to be fundamentally important and their responsibilities go beyond the production of fluoridated milk. This can extend to quality control, distribution and administration although, in some cases, dairies are even more engaged in programmes, playing an active part in programme development. The commitment and compliance of the dairies is therefore of key importance and considerable benefit is gained where project staff are able to establish close working relationships with the milk producers.

The programmes require multi-agency collaboration and, to facilitate the development of proposals, steering groups are often formed. With each of the stakeholders represented, good communication, consultation and efficient planning is ensured. In Bulgaria, the active engagement of the key agencies is maintained through the formation of a national network group which meets regularly to review progress.

5.5 Establishing the feasibility and sustainability of a scheme

Those contemplating the use of fluoridated milk will need to give careful consideration to the feasibility of the proposed scheme, having particular regard for the:

- oral health and fluoride status of the community,
- availability of a suitable milk supply,
- extent of the milk provision, and
- organisational support and resources.

Whilst schemes will inevitably vary according to local circumstances, the same set of principles can be applied in the initial assessment of feasibility. In the first instance, it might be helpful to consider the following questions, which should provide an indication as to the potential for the scheme (Table 5.2):

Table 5.2
Questions to be answered when assessing the feasibility of a milk fluoridation programme.

Milk distribution systems	
Q.1	Is there an existing programme providing milk to children on an organised basis?
Q.2	Is the milk regularly supplied through:
	(a) the formal education system (schools, kindergartens, etc)?
	(b) outside the formal education system (e.g. in nutritional programmes)?
Q.3	How old are the children receiving the milk?
Q.4	How many children are receiving milk?
Q.5	Who is responsible for the provision of milk?
Q.6	Who pays for the milk?
Q.7	Are the long-term future of, and funding for, this milk provision secure?
Q.8	What type of milk is supplied to the children?
Q.9	How often (days per week/weeks per year) is milk provided to the children?
Q.10	What types of packaging material are used?
Q.11	How much milk is given to each child?
Fluoride status	
Q.12	Are there any water or salt fluoridation schemes:
	(a) Locally?
	(b) Other places in the country?
Q.13	Are there plans to introduce fluoridation programmes in the future?
Q.14	What is the level of fluoride in the local water supply (please state as parts per million)?
Q.15	Are there any other significant dietary sources of fluoride for children?
Q.16	Is there any significant use of fluoride dietary supplements (tablets, vitamin-fluoride preparations)?
Q.17	Is fluoride toothpaste available and if so is it commonly used?
Q.18	Is the attitude of relevant authorities (Ministry of Health, local government etc) likely to be favourable to fluoridation?

Oral health	
Q.19	What is the mean dmft of the 6 year old children?
Q.20	What is the mean DMFT of the 12 year old children?
Organisational support	
Q.21	Which are the key organisations/authorities/political bodies whose support would be essential to the implementation of a milk fluoridation scheme?
Q.22	Which organisation/body will take 'ownership' of the scheme to co-ordinate and lead its development?

5.6 Summary

The development of community based schemes since the late 1980s has demonstrated that milk can provide an alternative vehicle for the delivery of fluoride and is particularly appropriate where neither water nor salt fluoridation is possible. Jones *et al.* (2005) described the fluoridation of milk as another example by which public health dentistry can provide the benefits of fluoride without requiring consumers to take on particular responsibilities or change their behaviour.

Milk fluoridation has grown since the first community based scheme was introduced in Bulgaria in 1988. By 2000 programmes were operating in five countries and included over 114,000 children. More recently, particularly due to the expansion of the schemes in Thailand and Chile, this figure has increased to over 800,000. The experience gained in the development of the international milk fluoridation programme has provided considerable knowledge on the practical aspects of implementation. From this it is clear that the availability of a suitable milk supply is the key factor, not only largely determining feasibility but also proving to be the fundamental influence on the scope of the schemes. In most cases, an existing milk distribution system will be a prerequisite, and there is no doubt concerning the advantages to this. However, this means working within the constraints of any such system and careful consideration has to be given to the implications of this in terms of the potential impact of a scheme.

An important aspect of these schemes is the dedication and enthusiasm shown by those responsible for their delivery. Schemes established in the school setting are well received by teachers, whose engagement has the added advantage of raising the profile of health promotion. Milk fluoridation requires the wider collaboration of national governments, local governments, dairies, schools and health care professionals. This example of multi-agency working is consistent with the views of Watt (2005) who recognises that a range of complementary actions delivered in partnership with relevant agencies and the local community are needed to address inequalities in oral health.

6 Evaluating fluoride exposure in milk fluoridation programmes

A. E. Villa

6.1 Introduction

Once the implementation of a fluoridated milk scheme has been decided after careful consideration of the technical and legal guidelines discussed in Chapter 5, it is important to subject the ongoing scheme or programme to periodic assessments. These assessments can be divided into three separate categories:

1. Clinical assessments

It is clearly of interest to evaluate the improvement in dental health of children receiving fluoridated milk, particularly if the scheme is the first in a country and is primarily intended as a demonstration programme. The baseline study provides the starting point in this exercise, whilst periodic dental examination of a representative sample of children in different age-bands will provide ongoing evaluation: these activities are described in Chapters 2 and 7 and will not be discussed further here.

2. Monitoring quality of fluoridated milk

Assessment of the fluoride concentration in representative samples of the milk which the children consume is not only a quality control procedure but also an important safety evaluation measure. In every fluoridated milk scheme, it is necessary to establish tolerance limits on the acceptable variability that the fluoride concentration of milk can have.

3. Biological monitoring

Taking into account that nowadays, in addition to the naturally occurring levels in drinking water, there are many other sources of fluoride ingestion, e.g. fluoride toothpastes which are partly swallowed by young children, it is imperative to estimate the total daily fluoride

intake of a representative sample of children from a given community before launching a fluoridated milk programme. This is achieved by studying the urinary fluoride excretion of the children and comparing the measured or estimated daily amount of fluoride excreted in the urine with the provisional normative values already published (Marthaler, 1999, table 5). Once a fluoridated milk scheme is started, the previously described procedure of biological monitoring must be periodically carried out in order to ensure that the programme is progressing safely in terms of minimising the risk of dental fluorosis.

In this chapter, the above items 2 and 3 will be addressed. In addition, concise guidelines on the measurement of fluoride concentration in fluoridated milk and in urine will be provided.

6.2 Monitoring the quality of fluoridated milk

The target fluoride concentration in milk is generally established by local health authorities in charge of the fluoridated milk scheme or acting as technical advisor to the planners of a community demonstration study. The target concentration is determined considering several factors, such as the age range of the participating children, frequency of fluoridated milk ingestion during a 24 hour period, results obtained in the baseline fluoride urinary excretion studies, previous experience from earlier similar studies, etc. In almost all of the previous and current fluoridated milk schemes, fluoride concentrations in milk are in the range: 2.5-5.0 mg F/l.

Once the 'optimal' fluoride concentration is established, the most important quality control procedure at the manufacturing dairy plant is the assessment of the fluoride concentration of representative samples obtained from the different production batches. It is usually considered that variability in the range 5-10% relative to the target fluoride concentration value is acceptable. Otherwise, corrective measures should be taken by means of either dilution with non-fluoridated milk or addition of concentrated sodium fluoride solution to the 'out-of-range' batch before its distribution.

When the concentration of fluoride in liquid milk at the dairy facilities is well within pre-established values, no other check on this value appears to be necessary since the fluoridated milk will be usually consumed within 24-48 hours after its production. In the case of UHT fluoridated milk, however, it should be advisable to check again

(in randomly selected samples) its fluoride concentration when it is ready for consumption and a relatively long time (1-2 months) has elapsed between its production and consumption. Thus, the number of quality checks depends on the type of product and its shelf-life, an issue which has been discussed in Section 4.4 in Chapter 4.

In the Chilean fluoridated milk programme in which powdered MFP-fluoridated milk is used, the quality control process takes place in two steps. First, periodic assessments of the homogeneity of fluoride concentrations within and between the 1 kilogram plastic bags from randomly selected batches and, second, frequent sampling of liquid fluoridated milk recently prepared from the powdered product by the addition of previously boiled tap water, are carried out. In this way, fluoride concentrations in the ready-to-drink product are closely monitored. Again, 10% variability around the target value is accepted.

6.3 Biological monitoring

Fluoride is a natural constituent of all types of human diet and is present, in varying amounts, in drinking water throughout the world. Thus, fluoride intake varies widely across populations. Optimal fluoride intake provides effective protection against caries causing only a low prevalence and severity of dental fluorosis. Since ingested fluoride from all sources – whether deliberately or unintentionally ingested – is excreted primarily in the urine, studies of urinary fluoride levels are ideal for assessing the intake of fluoride by entire populations (Marthaler, 1999, section 1.3). More particularly, they also provide a basis for decisions concerning community based fluoride programmes for caries prevention within national or sub-national settings.

From previous studies on fluoride metabolism (Whitford, 1990, 1996) it is now well established that:

- Absorption of ingested fluoride from the stomach occurs readily and is inversely related to the pH of gastric contents.
- Fasting plasma fluoride concentrations (micromole/l) in healthy young or middle-aged adults are approximately numerically equal to the fluoride concentration of the drinking water (milligrams/l) they have habitually ingested over the past few years.

- Approximately 10-20% of the daily fluoride intake is not absorbed. Of the fluoride that is ingested, about 50-70% is excreted via the urine during the following 24 hours in young and middle-aged adults, and almost all of the remainder will become associated with calcified tissues (Whitford, 1996; Villa *et al.*, 2004). In the case of children younger than 6-7 years, who constitute the population segment that is at risk of developing dental fluorosis, the proportion of the daily fluoride intake that is excreted in the urine — also called 'fractional urinary fluoride excretion' (FUFE) — appears to be lower than that of adults (Villa *et al*., 1999, 2000; Ketley & Lennon, 2000, 2001; Haftenberger *et al.*, 2001; Franco *et al*., 2005).

On the basis of these relationships, the most reliable means of monitoring the recent total fluoride exposure of populations is by determining fluoride levels in plasma or urine; the latter can be obtained by non-invasive means. In community based surveillance studies, there is general agreement that urinary fluoride excretion assessment is a relatively easy and a more reliable procedure for estimating daily fluoride exposure of young child populations.

6.3.1 *Duration and quality of urinary collection*

Whenever possible, 24 hour urine samples should be obtained, since such a sample will be independent of dietary habits, timing of meals, and periods of maximal fluoride intake. In this type of collection, the amount of fluoride excreted over 24 hours is effectively measured and provides confidence in the estimation of 24 hour fluoride intake.

In certain circumstances, however, it may not be feasible to collect urine over an entire 24 hour period, and if such samples are incomplete, information gained will be unreliable. The lifestyle or level of organization and co-operation of the population concerned may favour or suggest alternative ways to obtain 24 hour estimates. A very useful publication (Marthaler, 1999, section 3) discusses and provides detailed examples on how to estimate 24 hour fluoride urinary excretion using "time-controlled" shorter periods of collection. The interested reader can also consult a typical example of 15 hours urinary collection in a previous paper (Baez *et al.*, 2000).

Not infrequently, the examiner faces the problem of individual urine samples where the volume of urine appears to be unrealistically high or low. Values in Table 6.1, which was partly taken from a previously

mentioned publication (Marthaler, 1999, table 4), provide criteria that help to discard suspected low (or high) volumes samples.

Table 6.1
Cleaning criteria at various ages for various levels of fluoride exposure

	Lower limits	Typical values	Upper limits
Urinary flow (mean for period)			
Age < 6 years (ml/24 hours)	140	500	1200
Age ≥ 6 years (ml/24 hours)	200	1200	3000
Age < 6 years (ml/hour)	5	20	160
Age ≥ 6 years (ml/ hour)	9	50	300
Urinary creatinine concentration			
All ages (mg/ml)	0.1	1	1.5

Source: Marthaler (1999, table 4)

6.3.2 *Evaluation of urinary fluoride excretion results*

From an epidemiological point of view, recording daily total fluoride exposure of a population by directly measuring the amounts of fluoride from foods, beverages, swallowed toothpastes, etc. is very difficult to perform. This is why the assessment or estimation of the amount of fluoride excreted in the urine over a 24 hour period is accepted to be the most reliable marker of total daily fluoride exposure.

The question that arises, however, is how to estimate the total daily fluoride intake (TDFI) (milligrams F/day) and from this value to obtain the daily fluoride dose, a result that can be directly compared with the so-called 'optimal fluoride dose range' (Burt, 1992). The latter calculation can be performed when the body weights of the participating subjects have been previously determined since the daily dose is obtained by dividing the TDFI by the body weight (mg F/kg/day). The question "how to estimate the TDFI from urinary excretion data?" could be answered if the proportion of ingested fluoride that is excreted in the urine, i.e. the fractional urinary fluoride excretion (FUFE) for the age group under study was known, because a simple division of the total amount of fluoride excreted in a 24 hour period by FUFE would yield an estimation of the TDFI.

Over the recent years, several studies addressing the determination of FUFE values in young children have been published (Villa *et al*., 1999, 2000; Ketley & Lennon, 2000, 2001; Haftenberger *et al*., 2001; Franco *et al*., 2005). With the exception of an earlier abstract by Brunetti &

Newbrun (1983) and a more recent study carried out on Iranian children (Zohouri & Rugg-Gunn, 2000), the previously quoted papers point to average FUFE values in the range 30-50%. A discussion of possible reasons for the differences in FUFE values found in these studies can be found in a recent paper by Franco *et al.* (2005).

It might be preliminarily suggested that an average FUFE value of 0.4 (40%) could be used for an approximate estimation of the daily fluoride dose in young children. Considering that this proposed FUFE value might be rather low, it would provide a 'conservative' estimation of the daily fluoride dose, since its use would yield higher daily fluoride intake estimates. However, at this time, a generally agreed upon average FUFE value for preschool children is not available. Until further studies leading to more precise FUFE values are performed, it appears more appropriate to suggest an alternative way to measure daily fluoride exposure from urinary fluoride excretion data.

Presently, the most appropriate way of evaluating daily fluoride exposure is to directly measure (24 hour urinary collection) or estimate through "time-controlled" 8 hour or 16 hour collections (Marthaler, 1999, section 3), the amount of fluoride excreted in the urine over 24 hours and to compare this amount of fluoride with the values proposed on the basis of previous experience obtained for different age-groups from areas "low", "optimal" or "high" in fluoride exposure (principally from water). Table 6.2 extracted from a previously mentioned publication (Marthaler, 1999, table 5) presents the lower and upper limits for either low or optimal amount of fluoride excreted on a daily or hourly basis for different age groups.

Table 6.2
Provisional standards for urinary fluoride excretion (µg)

	Per Day:		**Per hour:**	
Fluoride excretion level (µg)	**in 24-hour collection**		**in 24-hour collection**	
	Lower	Upper	Lower	Upper
Ages 3-5 years				
Low F-intake	170	290	7	12
Optimal F-usage	360	480	15	20
Ages 6-7 years				
Low F-intake	190	310	8	13
Optimal F-usage	480	600	20	25
Ages 10-14 years				
Low F-intake	220	340	9	14
Optimal F-usage	600	820	25	34

Source: Marthaler (1999, table 5)

If the study of urinary fluoride excretion was designed to provide baseline data before the introduction of a fluoridated milk scheme, and values described as 'low F-intake' (Table 6.2; first column) together with a rather high experience of dental caries and low prevalence of enamel fluorosis are found, then it is appropriate to proceed with the planned scheme. It is also recommended that 6 and 24 months after a fluoridated milk scheme is started, follow-up excretion studies be performed.

6.4 Determination of fluoride in fluoridated milk and in urine

6.4.1 *Analytical procedure for fluoridated milk.*

Ionisable fluoride in fluoridated milk, manufactured to contain typically 2 to 5 mg F/l (added as sodium fluoride), is conveniently determined by electrochemical means using a fluoride ion selective electrode, in conjunction with a reference electrode coupled to an ion meter. Modern ion meters are available which provide direct concentration readout. The electrodes (fluoride and reference) may be two single half cells, or a combination-type embodying the sensing and reference electrode in one unit. The latter type can be useful when measuring small volumes of samples. There are a number of companies that supply this equipment. One such organization of international repute is Orion Research, USA. Orion, like many other manufacturers, provides comprehensive instructions on the use of the meter and also full scientific background data. Hence, the user is provided with a good understanding of the technique.

Fluoride determination in fluoridated milk (in the concentration range quoted above) may be carried out by a direct method using the appropriate buffers and conditions. TISAB II (Total Ionic Strength Adjustment Buffer) added in a volume ratio 1:1 in both samples and standards, is recommended for use in this context. TISAB II standardises the ionic strength and pH of the medium, and deals with ions which otherwise interfere. It is also recommended to carry out the calibration process using a calibration matrix essentially identical to that of the real samples, i.e. the calibration standards should be prepared by adding appropriate volumes of a concentrated sodium fluoride solution to a non-fluoridated milk of the same type as that being analysed, and TISAB II mixture (volume ratio: 1:1). Alternatively, a concentrated ionic strength adjusting buffer (TISAB III) can be used, instead of

TISAB II, for preparing standards and samples. The ratio of TISAB III to sample or standard would be 1:10 instead of 1:1 as is when TISAB II is used. In order to encompass the range of measurement to be made and to ensure the reliability of the calibration procedure fluoride standards could be 1 mg F/l, 5 mg F/l and 10 mg F/l.

Fluoride analysis is conducted as a quality assurance measure in fluoridated milk production. The following procedure is employed for a typical fluoridated milk containing fluoride somewhere between 2 and 5 mg F/l, using a meter equipped with direct concentration readout:

1) Set up the meter and electrodes according to the instruction manual.
2) Calibrate the meter with three standards to cover the range to be measured:
   i) 1 mg F/l prepared in a 1:1 volume ratio of a mixture made up of non-fluoridated milk and TISAB II.
   ii) 5 mg F/l prepared in a 1:1 volume ratio of a mixture made up of non-fluoridated milk and TISAB II.
   iii) 10 mg F/l prepared in a 1:1 volume ratio of a mixture made up of non-fluoridated milk and TISAB II.
3) Measure the fluoride concentration in the sample (made up from equal volumes of fluoridated milk and TISAB II).
4) All solutions (standards and samples) should be at a constant temperature e.g. 20 ± 1° C.

The above procedure can also be performed using mixtures made up of standards or samples and TISAB III as discussed previously.

Unlike free fluoride ion, the monofluorophosphate ion $(FPO_3)^{-2}$ cannot be measured directly by a simple ion selective electrode technique. For the purpose of analysis, it is necessary to transform the ion into a species which can be determined readily. This is easily accomplished by hydrolyzing the monofluorophosphate ion in a strongly acidic medium.

In order to determine the total fluoride concentration (which is the value of interest) in a powdered milk using monofluorophosphate (MFP) as the fluoride source, a slightly modified version of a previously published method (Villa, 1988) is proposed:

1) Reconstitute the MFP fluoridated milk dissolving 1.00 gram of powdered milk in 10 ml of de-ionised water.

2) In a screw capped plastic vial, mix 1 ml of the reconstituted fluoridated milk with 10 ml of 1 M Perchloric acid ($HClO_4$), stir gently and hydrolyze overnight at ambient temperature.

3) Measure the fluoride concentration with a combination fluoride selective electrode type Orion 96-09 connected to a digital ion meter. This measurement can be carried out either a) by using a known addition technique, or b) by a direct calibration method:

   a) Details of the known addition technique have been published by Villa (1988).

   b) The calibration curve is built with standards prepared by adding appropriate volumes of a sodium fluoride concentrated standard to a calibration matrix made up of 1 ml of non-fluoridated milk of the same type as that being analysed plus 10 ml of 1 M $HClO_4$. Usually, two standards containing total fluoride concentrations of 1 and 10 mg F/l are used to obtain the calibration curve.

6.4.2 *Determination of fluoride in urine*

The assessment of fluoride in urine (and drinking water) follows the same procedure as that described previously for sodium fluoride fluoridated milk, with only two slight modifications. First, the calibration curve can be prepared using aqueous standard solutions, i.e. by the addition of the appropriate volume of a concentrated sodium fluoride standard solution to a simple calibration matrix made up from equal volumes of de-ionized water and TISAB II (or using TISAB III in a 1:10 ratio v/v). Second, taking into account that the range of fluoride concentrations usually found in young children's 24 hour urine samples lies between 0.3 and 1.5 mg F/l, the concentrations of the three standards might be appropriately fixed in the range 0.2 and 2 mg F/l.

6.5 Conclusions

Monitoring procedures must be considered mandatory with respect to safety and compliance assessment of any fluoridated milk programme. Quality assurance measures should be developed at the fluoridated milk manufacturer's facilities aiming to ensure and guarantee that the

fluoride concentrations of the product to be ingested are within a previously established range of variability with respect to the target value. Daily urinary fluoride excretion is considered to be a reliable marker of recent fluoride exposure within the context of community preventive programmes. Details on how to collect urine samples for planning and monitoring of milk fluoridation programmes have been discussed and criteria for evaluating these results have been provided. Analytical aspects of the assessment of fluoride concentrations in fluoridated milk and urine have been detailed.

7 Programme evaluation

P. E. Petersen and A. J. Rugg-Gunn

7.1 Why evaluate?

We evaluate all the time so that we may progress – we learn from our mistakes and our successes. This often happens automatically without planning. However, if we have a new idea, which we wish to put into action, it is sensible to plan to evaluate it. This is especially so if the procedure is expensive and time-consuming. Without planning the evaluation, we may miss the opportunity to find out whether it is effective and to what degree. Almost all public health programmes introduced into a country will require some form of evaluation. It is usually not sufficient to say that because it has worked in another country, it will work in this country. This is because there is more to evaluation than coming to the conclusion that the programme reduces disease (Petersen & Kwan, 2004). Circumstances differ between countries and we will need to know whether the degree of effectiveness – to what extent the disease is prevented – and, importantly, what factors in the given country may influence the success, or otherwise, of the programme. Under these circumstances, the programme might be termed a 'demonstration programme' and should be evaluated.

The comments above introduce three concepts. First, 'efficacy', which is whether an intervention can prevent disease; second, 'effectiveness' which is the extent to which the programme is effective; and, third, 'process', which is the delivery of the programme or how the programme is delivered. Efficacy differs from effectiveness in that efficacy is established under ideal conditions – giving the intervention or programme the best possible chance to show that it is effective; and, third, process, which is the delivery of the programme or how the programme works. Among the best examples of evaluating efficacy are trials of new drugs. Their efficacy is usually established in randomised controlled trials (RCTs). The design of an RCT will ensure that the

drug has the best opportunity to show its effect as efficiently as possible, ensuring that the chance of bias is as small as possible. Efficiency in efficacy trials is often gained by restricting the subjects in a trial to those most likely to benefit (e.g. those most likely to develop dental caries) and in a restricted age band, so as to reduce variability. Several types of bias can occur and if they are not controlled, it will not be possible to be confident that the effect was due to the drug or programme under test. We will return to the types of bias that can occur in Section 7.3.

Efficacy, therefore, is assessed under rather special circumstances, which may be rather different from the 'real world'. How effective an agent or programme is in the real world is often assessed in what is termed a 'pragmatic trial' or a 'field trial'. Here, the programme is investigated in its usual conditions and, as such, its 'effectiveness' is established (Petersen & Kwan, 2004). These two types of investigations – the randomised controlled trial and the field trial – are not in conflict: they are both valid and test different attributes. The two types of study, though, have different concepts of validity. While RCTs have very good 'internal validity' and often poor 'external validity'; field trials often have rather poor 'internal validity', but good 'external validity' (Black, 1996**)**. High internal validity is achieved by strict control of bias so as to reduce the chance of factors other than the agent under test being responsible for the effect. By high external validity is meant the findings of the study have immediate relevance to the community.

It is obviously best to have high internal validity and high external validity. When planning an RCT, for example, great attention will be paid to achieving high internal validity but this may be at a cost of rather poor external validity – the results of randomised clinical trials may not have immediate relevance to the broad community. Likewise, when planning a community or field trial, high external validity will be sought so that the results are of direct use to planners, but as much attention should be given to achieving good internal validity without compromising external validity. While it is important to be confident that an agent works, it is how well it works in the community which may be particularly important to know (Black, 1996; Green & Tones, 1999; Rychetnik *et al.*, 2002; Petticrew & Roberts, 2003). This applies to milk fluoridation – where the primary concern is to establish effectiveness in field trials.

7.2 What to evaluate

The prime purpose of adding fluoride to milk is to prevent dental caries. Thus, the first objective will be to measure 'clinical effectiveness' (Table 7.1). This has become the standard approach – recording the number of teeth prevented from developing caries. People in public health, though, base their decisions on what preventive schemes to introduce, on several factors, of which 'cost' is one of the most important. They will need to be sure that the proposed scheme is 'good value for money'. Thus, it is sensible to also assess the 'cost-effectiveness' or 'cost-benefit' of a programme (Table 7.1).

Table 7.1
Information which might be included in an evaluation of a milk fluoridation programme.

Clinical effectiveness
Cost effectiveness (economic evaluation)
Safety
Process

While fluoride is very widely used to prevent dental caries, it can cause adverse effects when ingested excessively. Acute fluoride toxicity is very rare and chronic fluoride toxicity is more commonly considered. Dental fluorosis is the first sign of chronic fluorosis in the young child. The aim of any fluoride-based public health programme is to maximise the caries-preventive effect while minimising, if not avoiding altogether, the risk of dental fluorosis. This will have been considered carefully when the milk fluoridation programme was being planned (Chapter 5) so that the amount of fluoride added to milk is appropriate given the age of the children and their background fluoride exposure. Nevertheless, it is important to evaluate safety aspects of any milk fluoridation programme so as to avoid acute and chronic fluoride toxicity (Table 7.1).

Lastly, but not least, 'process' should be evaluated (Table 7.1). Successful public health programmes require action by people at different times and in different circumstances. Some of these actions will progress well and others less so. It is important, therefore, to examine critically each stage in the delivery of the programme to see how well they have performed and how the programme can be improved to maximise its effectiveness at minimum cost and risk. Programmes are usually planned to run for many years, so it is important that there is capacity for training and development of the programme.

There may be circumstances in which other aspects of a milk fluoridation programme might be evaluated. For example, if an increase in the number of days children drink fluoridated milk is seen as desirable, and if a system is introduced to increase the number of days children drink fluoridated milk per year, the success of such an action may need to be evaluated, with the number of days the outcome measured. Another topic might be the provision of fluoridated milk at home. Each of these initiatives may need to be evaluated.

Inevitably, guidance in this chapter is pretty all-inclusive. People planning to evaluate a milk fluoridation programme should not be daunted by the following descriptions and discussion: many sections will not apply to their study. It is better to do a small study well, and these guidelines are intended to assist protocol development.

7.3 Clinical effectiveness

Given the evidence of (a) biological plausibility, presented in Chapter 3, and (b) clinical effectiveness in Chapter 2, it is beyond reasonable doubt that the provision of fluoridated milk is effective at preventing dental caries in children. There is, therefore, little incentive to undertake a study to demonstrate efficacy: it is more important to measure effectiveness in the environment in which the milk fluoridation programme exists. However, much attention is now given, quite rightly, to 'evidence-based' decision-making, both in clinical dentistry and in public health. In Section 2.14.3 and Table 2.24, the systematic review was seen as the best type of evidence, followed by single randomised controlled trial. It was also noted that one systematic review of milk fluoridation had been published (Yeung *et al.*, 2005). This was a formal review "To determine the effectiveness of milk fluoridation, as a means of delivering fluoride on a community basis, for preventing dental caries." The assessment of the quality of the studies which had been undertaken was such that only two studies were judged to have been of acceptable quality (Stephen *et al.*, 1984; Maslak *et al.*, 2004). However, the criteria for judging quality were very largely related to internal validity, thus favouring inclusion of only RCTs, and paid very little attention to external validity, which is concerned with relevance to the community. One problem likely to have confronted Yeung and colleagues was that methods for evaluating RCTs are now well defined (see CONSORT guidelines; Moher *et al.*, 2001) and methods and guidelines for assessing field or community trials are much less

developed. Recently, there have been attempts to correct this with the publication of the TREND guidelines (Des Jarlais *et al.*, 2004; Treasure, 2004). An important message from the above discussion is that the design and conduct of studies to evaluate the effectiveness of milk fluoridation programmes need to pay sufficient attention to internal validity, without compromising external validity. The three most important aspects of internal validity to consider are randomisation, control of confounding factors, and blind assessment.

For evaluation of on-going milk fluoridation programmes, it is not possible to randomly allocate subjects to test and control groups. Random allocation might be considered if planning a demonstration programme. In such trials, it is reasonable to allocate groups of subjects, such as school classes, rather than individual children. This would be known as a 'cluster' design and must be taken into account when estimating 'sample size' (the number of subjects to include in the evaluation study) and in the analyses (Donner & Klar, 2004; Kim *et al.*, 2006). Allocation of subjects, or clusters, must be truly random. Random allocation is a powerful way of diminishing the influence of confounding factors, which are factors (such as social class, use of other fluoride agents, dietary habits) which are known to influence the development of dental caries. Random allocation will help to ensure that test and control groups are equal in these factors and, thus, unlikely to affect the results of the evaluation.

As mentioned above, when evaluating existing milk fluoridation schemes, random allocation to test and control groups is not possible. In this situation, two activities are important. First, control communities need to be chosen with great care and, second, information on known confounding factors should be collected and taken into account during data analyses. Control areas should be selected so as to be as similar to the test (using fluoridated milk) community as possible. An important consideration is that there is a high likelihood of the control community remaining as the control during the course of the evaluation: it is not unusual for pressure to develop in the control community to introduce the preventive measure and not remain as the control. This would seriously compromise the evaluation and, if this could happen, it could be worth considering including a second control community. The usual confounding factors to record are: social class (socio-economic status, educational attainment, or income), use of other fluoride agents (such as fluoride concentration in drinking water, use

of fluoridated salt, fluoridated tablets, toothpastes, mouthrinses, and gels and varnishes), and dietary habits (use of sugar). Dietary habits are difficult to record accurately and it is often adequate to record social class, as there is a strong link between diet and social class in many countries.

A further aspect of control of bias by randomisation is the method by which children are selected for clinical evaluation. If numbers are small, it may be possible to examine all children. Often, though, the numbers of children in a milk fluoridation programme is high and it is necessary to examine a sample. It is very important that this sample is truly random, to avoid selection bias. The method should be written clearly in the study protocol.

Along with randomisation and control of confounding factors, a further important aspect to include within the design of an evaluation is 'blindness'. Another term for it is 'group concealment'. A study can be 'single blind' or 'double blind'. In a single blind study, the person(s) assessing the effect, or 'outcome', is unaware of which group the subject being assessed is from. This removes the possibility that the examiner might be biased – which can occur sub-consciously as well as consciously. In double-blind studies, as well as examiner blinding, the subjects are also unaware whether they are from the test or control group. Double blindness (and triple-blindness, where the person supplying the intervention is also blind to group allocation) has much less relevance in community evaluations than in RCTs, especially if there are elements of health promotion in the programme. Double blindness is unlikely to be achieved in a community programme evaluation, since consent to receive the fluoridated milk would have been required when the programme commenced and there is often an obligation for fluoridated products to be labelled clearly. Single blindness, though, is important and should be planned for. This will require planning and may incur extra expense; for example, if children from both test and control communities are brought to a central location for examination, or bringing in an examiner from outside who is unaware of which communities receive fluoridated milk. When assessing dental fluorosis, one strategy is to take photographs, code them (hiding group allocation) and evaluate them in random order. If numbers are large, this could be done on a sub-sample.

Randomisation, controlling for confounding factors, and blindness are very important aspects in study design. Reviews have shown that studies which do not control for these aspects are more likely to be biased (Juni *et al.*, 2001).

7.4 Design strategy

The simplest design for the evaluation of a milk fluoridation programme is a 'cross-sectional' study (Table 7.2). Here, children from test and control communities are examined once during the same period – usually a few weeks or months. This is known as 'parallel control'. Another design is 'historical control' where the children are examined before the introduction of the milk fluoridation programme and another group of children of the same age are examined after milk fluoridation has been operating for a number of years. Another name for this design is a 'before and after' study. Both of these designs have advantages and disadvantages, as given in Table 7.2.

Table 7.2
Possible designs of studies to evaluate milk fluoridation programmes, and the main advantages and disadvantages of each.

	Advantages	**Disadvantages**
Parallel control (cross-sectional)	Examined at the same time	Communities may be dissimilar
Historic control (before and after)	Same communities examined	Communities may change over time. Very difficult to be a blind study
Combined historic and parallel control	Allows assessment of changes over time, yet allowing the possibility of a blind study	The test and control communities must not change their status (i.e. the control children start to use fluoridated milk and vice versa)

A way of combining the advantages of each design and, to some extent, neutralise the disadvantages of each, is to combine the historical control design with the cross-sectional design (Table 7.2). This will allow an assessment of any change in the background level of dental caries over time (which is the major threat to historical studies) by examining the results from the control community, and yet allow blind assessment of test and control subjects. These are also called 'cohort' studies, as age-groups (but not necessarily the same children) are followed through, year by year. The greatest disadvantage is that the test and control communities must remain test and control for the length of the study. In this respect, it is not unusual for control communities to wish

to introduce the preventive programme when they see that it has been introduced successfully in the test community. A true longitudinal study (or 'follow-up' study), where the same subjects are followed through and caries increments for individuals are calculated, is more suitable for an RCT design study and not a field evaluation.

7.5 Economic evaluation

Economic evaluation measures the efficiency of the programme – is it likely that the programme saves money? The principles of economic evaluation are well known (Drummond *et al.*, 1997) and are, basically, a comparison of costs of implementing a programme with the saving in costs of dental care resulting from the programme. An example (Mariño *et al.*, 2006) of an economic evaluation of a milk fluoridation programme has been published. They evaluated the milk fluoridation programme in Codegua, Chile (see Section 2.10.1), where children received fluoridated powdered milk and milk derivatives from birth to 6 years of age. Dental caries development was recorded in a sample of children in this town and in the control town of La Punta.

The costs associated with the milk fluoridation programme are summarised in the first column of Table 7.3. It was a requirement of the scheme that dental examinations and urinary analyses were undertaken, so the cost of these was included. Supervision costs and laboratory costs were also included, as well as the almost negligible cost of adding fluoride to the milk and milk derivatives.

Table 7.3
Economic evaluation of a milk fluoridation programme: (a) cost of the milk fluoridation programme, (b) cost of dental care, calculated for study and control communities separately.

Cost of programme	Cost of dental care
Staff — programme coordinator — dentist, dental assistants Data analysis Laboratory services — milk — urine Office rent Consumables	Restorations Extractions Transport to dental centre Productivity loss

Source: Mariño et al., (2006)

The effectiveness of the programme was indicated by calculating the difference in dental caries increment between children in Codegua who

received the fluoridated milk and milk derivatives and the children in La Punta who received milk and milk derivatives without added fluoride. This difference (or saving) in dental disease was multiplied by the cost of treating the disease, according to the Chilean Ministry of Health fee-scale. The cost of travel to the dental clinic for treatment and the loss of productivity by the parent were also calculated and included (Table 7.3). Economic evaluation took the form of cost-effective analysis (CEA). The authors discuss the many assumptions which it was necessary to make, and they concluded "While the analysis has inherent limitations as a result of its reliance on a range of assumptions, the findings suggest that there are important health and economic benefits to be gained from the use of fluoridated milk products in non-fluoridated rural communities in Chile." Those requiring further information on economic evaluation, particularly related to milk fluoridation, can consult a work manual by Mariño & Morgan (2006).

7.6 Evaluation of safety

Chapters 4 and 6 have described the addition of fluoride to milk and the safeguards that are necessary to ensure that milk is delivered to the children with the appropriate quantity of fluoride in an available form. Whoever supervises the children drinking the fluoride milk should ensure that each child receives this quantity each day, and no more. This will ensure that the risk of acute toxicity is virtually non-existent.

These safeguards will also go a long way in ensuring that the opportunity for chronic toxicity to occur is also very low. Before the milk fluoridation scheme is introduced, the background fluoride exposure of the children will have been assessed in order to determine the appropriate amount of fluoride to be added to the milk. These assessments would have been made by enquiry and by questionnaire (see Section 5.5 and Table 5.2) and possibly urinary fluoride analysis. After the programme is introduced, chronic toxicity can be assessed by: (a) continuing to monitor urinary fluoride excretion, and (b) examination of teeth for dental fluorosis.

The principles and method of monitoring urinary fluoride excretion has been given in Chapter 6. For continuing evaluation, it is probably sufficient to monitor a small random sample of children each year, when the children are receiving the fluoridated milk. Dental fluorosis only occurs when an excessive amount of fluoride is ingested at a critical time when teeth are forming. If the fluoride intake of children in a

programme increased markedly (due to some new source of fluoride becoming available) it could be detected by urinary fluoride monitoring much earlier than any increase in dental fluorosis. Thus, the chance of dental fluorosis occurring as a result of a milk fluoridation programme is very small. Nevertheless, it is probably sensible to include an assessment of it as part of the children's clinical assessment; it is probably sufficient to limit this to the aesthetically important upper front permanent teeth (the maxillary incisors).

There are several indices of developmental defects of enamel or dental fluorosis to choose from. The two most common would be the Dean's index (Dean, 1942) and the TFI (Fejerskov *et al.*, 1988). Most milk fluoridation schemes presently in operation are school-based, so that the children are aged 4 to 6 years when they join the programme (Table 5.1): this age is generally considered to be well passed the critical age for fluorosis development in incisor teeth. Thus, the chance that the introduction of milk fluoridation being the cause of dental fluorosis is very small. Some fluoridated milk programmes begin soon after birth (see Chapter 2 and Table 5.1) and, for these programmes, monitoring dental fluorosis is sensible.

An example of this situation occurred in Codegua, Chile. Fluoridated milk and milk products were provided from birth to 6 years of age (Mariño *et al.*, 2001) and children in Codegua and the control community of La Punta were examined for dental fluorosis both before the milk fluoridation programme began and five years later (Mariño *et al.*, 2003). The Dean's index was used as this allows calculation of the community fluorosis index (CFI). The children aged 6 to 9 years who had received fluoridated milk and milk products had a CFI of 0.18, while the children in the control town had a CFI of 0.16. Both values were well below the CFI threshold level of 0.6, which is the level above which there is likely to be public health concern.

7.7 Process evaluation

Many people and organisations contribute to the success of public health programmes. To launch a programme requires much work, but it is equally important to ensure its sustainability and development. Many of these aspects were described in Chapter 5. Process evaluation is the formal and critical examination of how the fluoridated milk is delivered, and factors which are likely to influence the long-term survival of the scheme. These are listed in Table 7.4 under two headings.

The first column contains information which might be collected in order to find out if the children are, in fact, drinking the fluoridated milk and, if so, for how long, while the second column contains information which might be collected to decide how well the programme is accepted, and whether it is sustainable or could be improved. If the children do not drink all their milk and do not drink it on the days on which it is provided, then the effectiveness of the programme will be compromised (Chapter 2). Also, if there is much movement of children in and out of the programme, it will be harder for any clinical evaluation to show an effect: it may be necessary to limit clinical evaluation to children whose attendance rate has been high. If children are choosing not to drink milk, this needs to be investigated.

Table 7.4
Process evaluation. (a) Factual information on the consumption of fluoridated milk, and (b) attitudinal information which might affect the long-term success of the programme.

Factual information	**Attitudinal information**
Number of days each child drank milk	Attitude of children
Was all the milk drunk?	" parents
Delivery and storage of milk	" school
Movement of children	" dairies
	" dentists
	" other health workers

All the people in the second column of Table 7.4 play a part in ensuring the success of the programme. There may be others, depending on the organisation of the scheme. There are several ways of assessing what they think of the scheme, such as telephone, interviews, focus groups, meetings, and questionnaires, and they can be used appropriately. It is best if the interviewers are not directly involved with the organisation of the programme, so as to avoid biased responses.

7.8 Protocol preparation

7.8.1 *Introduction*

A Protocol is a formal description of what needs to be done to undertake and complete an evaluation. It includes much detail of each stage of the evaluation and is essential if misunderstandings and missed opportunities are to be avoided. Any alterations to, or deviations from the protocol must be agreed and recorded. In many countries, local research ethics committees require to see and approve the study protocol.

The protocol is, also, very useful when it comes to writing the full report and scientific articles. The several sections of a study protocol are given in Table 7.5. This is given for guidance only and can be modified to suit local needs and circumstances. It is common for protocols to run to 20 pages or so, with several appendices.

Table 7.5
Outline of information which might be included in a protocol for a study to evaluate a milk fluoridation programme

Background	overview of present milk fluoridation programme reason for the study results of any previous study
Organisation of study	responsibilities for organising the study
Aims	purpose of study aims objectives
Study design	outline of design
Subjects	locations (study and control communities) age groups duration of study estimation of sample size sampling method ethical approvals
Clinical examination	location what to examine criteria examiners training and concurrent appraisal of examiners blinding (group concealment)
Economic evaluation (if being undertaken)	personnel to collect information costs related to fluoridation of milk costs of dental disease in test and control children comparison of costs
Urinary fluoride measurement (if being undertaken)	locations age-groups and numbers required collection procedure personnel to be involved validation of samples and estimation of fluoride concentration and excretion
Process evaluation	delivery and use of milk attitudes of stakeholders
Data handling	methods of recording information, data validation and creation of database
Statistical analyses	personnel to be involved tests to be used
Timescale	timeline
Reporting and dissemination	personnel to write report dissemination of report and findings
Appendices	map details of sampling, numbers of subjects examination recording form, and equipment required examination criteria in detail questionnaire(s)

7.8.2 *Background*

This should provide a brief overview of the present or proposed milk fluoridation programme: only details relevant to the evaluation need to be included. The reason for the evaluation should be given as well as any results of previous evaluations of the programme.

7.8.3 *Organisation of the study*

This is a very important section. Many different people and organisations are involved or affected by a public health programme: the role of these stakeholders in the evaluation needs clarification. A steering committee should be formed, which may be widely representative, although not all stakeholders need be on it. It is sound practice for those responsible for the evaluation (the evaluation team) to be different from those involved in the day-to-day organisation of the milk fluoridation programme (the implementation team). The steering committee should be small enough to get the job done but yet include appropriate expertise. For example, statistical advice is almost always required. If an economic evaluation is planned, a suitable expert should be invited to participate. The same might apply for urinary analyses, process evaluation, and use of questionnaires. Some of these experts may be found in WHO Collaborating Centres: WHO could advise on this issue. It is common for subcommittees to be formed, which report to the steering committee. The roles of those participating in the evaluation should be set out clearly in the protocol.

7.8.4 *Aims of the study*

The three important points to include are: the purpose of the study, the aims of the study, and the objectives. There may be several aims – these are often useful to the statistician as the statistical analyses are geared to answering the questions posed in the aims. Objectives are more focused – they are objectives to be achieved; almost to be ticked off.

7.8.5 *Study design*

A number of study designs which might be suitable were discussed in Section 7.4. The advantages and disadvantages of each need to be considered, in the present context — for example, available resources. It is better to do a small study well than to take on a study that is too large. The broad outline is given here, as details of age and number of subjects etc. will be given in detail in the next section.

7.8.6 *Subjects*

In this section, details relating to the subjects are given.

- Locations – study and control communities

If the evaluation relates to an existing milk fluoridation programme, the study area will be defined already. If the milk fluoridation programme is extensive, it may be economic and sufficient to limit the evaluation to one community, which might be chosen as typical. The control community should be chosen with care, for reasons given in Section 7.4. The more similar it is to the test community, the less reliance on statistical adjustment for confounding factors will be necessary.

- Age-groups

This will be determined largely by the age at which children receive a regular supply of milk – usually at school, each school day. The ages at which many schemes begin can be seen in Table 5.1 and, from the remarks in Section 2.14.5, there are likely to be advantages in effectiveness of beginning at a young age. The youngest age group to be examined should be those about to enter, or who have just entered the milk fluoridation programme. The oldest age group might be those who are leaving the milk programme. It may not be necessary to examine every age between the youngest and oldest ages – may be every other year. If the intention is to evaluate dental fluorosis, children whose maxillary permanent incisor teeth have erupted (at least 8 or 9 years) should be included.

- Duration

If the design were a cohort design, it would be usual for the study to last until the children examined first on entry to the milk fluoridation programme, reach the age when they leave the milk programme. This might mean the full evaluation lasts some six or more years. In which case, interim objectives might be set, and examinations might take place every two years, or even less frequently.

- Estimation of sample size

This formal process is likely to require the advice of a statistician. The study should have sufficient ‘power’ so that it is able to show an effect if an effect exists. The power of a study depends heavily on the number

of subjects in the evaluation. On the other hand, it is wasteful to include too many subjects. If the study is going to be a longitudinal study (where the same child is followed for a number of years), allowance must be made for children leaving the programme (study 'drop-outs'). If the design is a cluster design, the statistician will have to allow for this, as clustering reduces study power.

- Sampling method

Having defined the communities and, thus, the children and schools involved, a decision is required whether to examine all children or a sample of them. If a sample is decided upon, then this is likely to be chosen in two stages. First, by sampling schools or school classes and, second, by sampling within schools or classes. If all the children in a school or school class are chosen, this is known as a cluster, and the school or class is the sampling unit. Each stage of sampling must be random, using random number tables. Weighting sampling towards any sub-group is possible but probably unlikely: if it is required, the statistician can advise.

- Ethical approvals

Requirements will vary between countries. It is usually necessary to obtain approval from education authorities if the evaluation is to involve schools or schoolchildren. Communities usually have a local Research Ethics Committee, and approval of the study protocol will be necessary. After obtaining the approval of the Research Ethics Committee, the subjects themselves will be invited to give their consent: in the case of children, this will usually be the parents or carer. Consent usually has to be 'positive', 'informed' and written. A positive consent is when the subject has to give their consent to participation, while negative consent is when a subject has to opt out of the study or else they are included. Negative consent is often not approved by ethics committees.

7.8.7 *Clinical examination*

- Location

The location has to be suitable and convenient, but a central location should be considered if the intention is to mix children from study and control communities before examination.

- What to examine

The two diseases likely to be included are dental caries and dental fluorosis. For dental caries, a decision will need to be made on whether to include primary and/or permanent teeth, and whether to record information at the tooth level or at the tooth surface level. Recording surfaces increases examination time slightly, but it increases the sensitivity in analyses. It may also indicate which type of tooth surfaces benefit most, if large caries increments are expected. For dental fluorosis, it is probably necessary to only examine the labial surface of maxillary permanent incisor teeth.

The above are related to recording disease (caries and fluorosis). In field evaluations, there is now greater emphasis on recording health, or wellness. If this is considered desirable, it would be wise to seek advice from WHO, *inter alia*, to see if such information can be recorded on WHO forms and how it might be analysed.

- Criteria

Criteria for recording dental caries and dental fluorosis are given in the WHO manual 'Oral Health Surveys; Basic Methods' (WHO 1997).

- Examiners

A good rule is 'the fewer examiners the better' as long they will be available for all the examinations (that might be over a number of years). On the other hand, the examination period does not want to be too long, and clinical evaluation should be completed within a month or two. Previous experience in surveys would be desirable, as examiners need to develop the mindset of a computer not a clinician: a clinician is inclined to think of clinical outcomes, while a computer does the same thing every time in a given situation.

- Training and concurrent appraisal of examiners

Examiners have to record the same way throughout the evaluation. Training and calibration is required before the study, and consistency (or repeatability) should be appraised during the study. These aspects are discussed in the above-mentioned WHO manual (WHO, 1997).

- Blinding

The importance of this has been emphasised in several previous sections. Details of the methods used to ensure that the examiners do not

know the group allocation of the subjects being examined, need to be given. If it is possible to perform a double blind study, details should be given.

7.8.8 *Economic evaluation*

This form of evaluation was discussed in Section 7.5 and attention was drawn to a publication describing such an evaluation (Mariño *et al.*, 2006). A more detailed description is given by Mariño & Morgan (2006). Decisions will need to be made on who will collect the information, and what information needs to be collected relating to the costs of the milk fluoridation programme and the benefits of the programme. The advice and assistance of a health economist is likely to be required. The protocol should list all the information required as much of the information will need to be collected as the milk fluoridation programme progresses, which is usually preferable to retrospective collection. It may be sensible for Economic Evaluation to be a stand-alone protocol.

7.8.9 *Urinary fluoride measurement*

Section 7.6 discusses when it might be appropriate to monitor urinary fluoride excretion, as part of the evaluation of safety. Details of methods used to collect urine and measure fluoride concentration and amount excreted per day, are given in Chapter 6. In this part of the protocol, details of the selection of subjects, collection and storage of urine should be given, under the headings given in Table 7.5. As with all data collection, great care is needed to ensure that the collections of urine are complete, and the requirements and training of staff should not be underestimated. Further information on methods and results of previous studies can be found in the references given in Chapter 6.

7.8.10 *Process evaluation*

The ideas behind an evaluation of process have been given in Section 7.7, with key aspects listed in Table 7.4. Chapter 5 provides information about the organisation required to implement a milk fluoridation programme. Some of the information required for process evaluation will be collected on a regular basis by staff implementing the programme, such as the number of days per year each child drunk their milk. This applies to other aspects too, and it may be that the protocol for the evaluation of Process, can be stand-alone, rather than linked to

other aspects of evaluation. Nevertheless, it should be a formal document, giving timetables for data collection and arrangements for reporting the findings.

7.8.11 *Data handling and statistical analysis*

With the exception of information collected in the economic and process evaluations, almost all the information will be converted into numeric form for statistical analysis. In the 'data handling' section, methods for converting information so that it is suitable for statistical analysis needs to be given. For example, how will the various socioeconomic and education attainment information be grouped; how will use of fluoride toothpaste be classified? Almost certainly, some data will be missing or incompatible, and arrangements for 'cleaning' the data will need to be described.

Once data sets are created, statistical analyses can be performed. This may be done by a statistician. The two main types of statistics produced are: (a) descriptive, and (b) analytical. Describing the data is important, and leads on to the analytical stage, as the type of test may depend on the distribution of the data. An outline of these stages should be given in the protocol, so that it can be seen clearly that the analyses will answer the questions posed in the Aims of the study (Section 7.8.4).

7.8.12 *Timetable*

A timetable, or timeline, will assist staff in knowing when they are required; so ensuring efficient working.

7.8.13 *Report and dissemination*

Reporting is essential and, indeed, it is an ethical requirement if subjects have been invited to participate voluntarily. In the protocol, it is useful to give thought to the type of report likely to be required and who might prepare them. Most research funders and ethics authorities require dissemination of the findings, whatever the outcome: it is useful to put in the protocol how dissemination might be achieved.

7.8.14 *Appendices*

Appendices should include detail of aspects such as: numbers of participants in each community and sampling intentions, details of examination methods and criteria, and questionnaires (Table 7.5).

7.9 Summary

Almost all public health programmes need to be evaluated to ensure that they are performing the task they were designed and implemented to do. Evaluation may be of a demonstration programme, when milk fluoridation is newly introduced into a country, or of an on-going scheme. The cost of this evaluation should be allowed for in the budget. Aspects which are likely to need evaluation are: clinical effectiveness, cost effectiveness, safety, and process. The design of any study to evaluate clinical effectiveness needs careful consideration: there are several possible designs, each with advantages and disadvantages. Key aspects of design are randomisation and control of confounding factors, and blind assessment. A full study protocol is essential. The outcome measured is almost always dental caries reduction, quantified as per cent caries-free and dmft/DMFT. Economic evaluation is of considerable interest to decision-makers: it may be necessary to seek expert assistance for such evaluations to ensure that costs and benefits are measured appropriately. Safety considerations include monitoring urinary fluoride excretion; usually of a small sub-sample, annually. The decision whether to measure dental fluorosis will depend on the age at which children begin to drink fluoridated milk. Process should be evaluated so as to ensure maximum benefit to the children and the programme's development. Information in this chapter is fairly all-inclusive, and potential evaluators will need to choose which sections are appropriate for their needs.

8. Conclusions

The story of milk fluoridation is now more than 50 years old. During this time, much work has been published on both laboratory and clinical aspects of the addition of fluoride to milk. It is clear that it has an important role in preventing dental caries, and milk fluoridation takes its place alongside other well-tested fluoride-based preventive measures. This World Health Organisation manual has described the many aspects of milk fluoridation, so that the reader can become familiar with the scientific basis for milk fluoridation and how it might be implemented and evaluated. The large number of references provided, is testament to this research, and forms a valuable source of further reading for those wishing to delve deeper.

The first chapter highlighted the value of milk as a food. Many health authorities subsidise the provision of milk to children, and school milk programmes exist in many countries; these programmes are supported by the WHO and the FAO. World consumption of milk is forecast to rise; dramatically so in some countries. The effect of milk (without the addition of fluoride) on dental health was discussed in detail as the literature on this subject is extensive: it was concluded that cow's milk is non-cariogenic. Despite containing about 4% sugars, other factors in milk ensure that it is not a threat to oral health; indeed, it is more likely to be protective.

The long history of investigations into the clinical effectiveness of fluoridated milk was presented in Chapter 2. Early studies in Switzerland, Japan and the United States, were followed by 15 clinical evaluations in Scotland, Hungary, Israel, Bulgaria, Chile, Russia, England and China. Eight of the studies showed a caries preventive effect in primary teeth and 10 studies showed a caries preventive effect in permanent teeth. Two studies showed no effect in either dentition. In addition, one study investigated the effect of cessation of a fluoridated milk programme; this showed an increase in caries incidence in children who

had stopped drinking fluoridated milk. At the present time, there has been no study of the effect of fluoridated milk in adults.

The large quantity of non-clinical research concerning the addition of fluoride to milk was summarised in Chapter 3. The type of investigation ranged from laboratory studies to animal studies, *in vivo* remineralisation experiments to the accumulation of fluoride in plaque, saliva and enamel. This large quantity of research has allowed the biological plausibility of milk fluoridation to be established. The chemistry of fluoride in milk was described, as well as the absorption, metabolism and excretion of fluoride when added to milk.

The practical aspects of milk fluoridation were discussed in Chapters 4 and 5. The mechanisms for adding fluoride to milk, and the stability and storage of fluoridated milk were considered in Chapter 4. The fluoride compounds discussed were sodium fluoride and sodium monofluorophosphate, and the types of milk were pasteurised, ultra-heat-treated, sterilised, and powdered milks. The availability of fluoride in milk and its stability over time, were both reported to be high. The many aspects concerned with the implementation of fluoridated milk community programmes, were discussed in Chapter 5. The presence of an existing milk distribution system was shown to be very important in ensuring the success and expansion of the schemes. This chapter presented information on WHO milk fluoridation programmes operating in Bulgaria, Chile, Russia, Thailand and the UK. The programmes in Chile and Thailand were reported to be expanding rapidly due to national policy decisions and good milk distribution systems. Regulatory aspects were discussed, as well as the different roles played by the dairies, the schools and public health authorities. The need for training was emphasised.

The final chapters, Chapters 6 and 7, described programme monitoring and programme evaluation, respectively. Monitoring the quality of fluoridated milk, and determination of fluoride concentration in milk and urine, were described in detail. The need for programme monitoring was highlighted in Chapter 7. Most newly-introduced public health programmes should be evaluated. There can be several aspects to programme evaluation, such as clinical effectiveness, cost effectiveness, safety, and evaluation of 'process', and the need for each will depend on local circumstances. Possible designs for evaluations were discussed, and aspects of the study protocol were described in some detail.

The information given in these chapters will be useful to several types of reader. Those concerned with already functioning milk fluoridation programmes will find information which will assist with evaluation and possible expansion of the scheme. Those considering introducing a milk fluoridation programme should find information in this manual which will guide them through the various stages to full implementation. Those wishing to learn more about fluoride in milk from a scientific viewpoint will find a wealth of information concerning this aspect of population oral health.

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Around the globe, dental caries is a public health problem and the disease burden is particularly high among under-privileged groups. In several low-income countries, the WHO anticipates that the incidence of dental caries will increase as a result of growing consumption of sugars and inadequate exposure to fluorides.

The good news is that dental caries is preventable through the effective use of fluoride. WHO emphasizes the importance of automatic administration of fluoride as part of public health programmes. Substantial research has provided evidence of the effectiveness of milk fluoridation in the prevention of dental caries. As milk fluoridation mostly targets the child population, such schemes have been established within the context of school health programmes and programmes for healthy diet and nutrition. This publication describes the justification of milk fluoridation as an effective public health measure and experiences from community health programmes are highlighted.

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ISBN: 978 92 4 154775 8