Milk fluoridation for the prevention of dental caries

Editors

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Preface

The WHO Global Oral Data Bank (GODB) demonstrates wide varieties of dental caries levels. During the last 10 years dental caries prevalence in most industrialized countries has decreased from “very high” and “high”, to “moderate” and “low” levels. Hence, in 1995, of the 178 countries for which the WHO GODB has data available, 25 were rated at a “very low” level, 75 as “low”, 54 as “moderate”, 22 as “high” and only two were recorded as having a “very high” caries level. These data, based on the weighted mean DMF in 12-year-olds, have been obtained from national surveys, or collected from published papers on oral health surveys conducted in selected areas of the countries. Often these texts indicated an increase (or decrease) in caries of people living in different areas of a particular country, whereas the DMF weighted mean at the national level was still without change. Thus, in China, one of the most populated countries in the world, the DMFT weighted mean is still around 1.7. However, there is clear evidence from several recent epidemiological studies that the caries level in Chinese urban populations is increasing persistently. Furthermore caries still remains one of the most common diseases, affecting a substantial number of children and adults in many eastern European countries.

Laboratory and clinical research suggests that fluoride is the most effective caries-preventive agent, particularly when a low level of fluoride is maintained constantly in the oral cavity. During the last few decades, fluoride has been used on a global scale, for the most part with much benefit. Since 1969, WHO has approved three resolutions recommending Member States “... to examine the possibility of introducing and where practicable to introduce fluoridation of those community water supplies where the fluoride intake from water and other sources for the given population is below optimal levels” (WHA22.30, 1969). Worldwide, fluoridated water is now available to approximately 220 million people. It has also been proven that salt fluoridation is a similarly effective method of dental caries prevention with respect to its efficacy, safety and cost. However, there are some communities where fluoridation of neither water nor salt could be implemented, due to technical and/or political problems. This has
encouraged WHO to promote and support programmes aimed at demonstrating the feasibility, for community level use, of fluoridated milk as an alternative to water or salt fluoridation.

From the mid-1950s, several papers were published concerning the possible use of fluoridated milk as a dental caries preventive measure for children. However, since the 1970s, studies sponsored by the Borrow Dental Milk Foundation (BDMF), Portsmouth, U.K., and carried out among small groups of children in various countries, have confirmed these earlier results of a decrease in caries levels due to fluoridated milk consumption. Although these investigations have shown promising results in caries prevention, they were not based on large-scale surveys. Therefore more clinical trials were required before milk fluoridation could be recommended as a true community-based substitute to water or salt fluoridation.

In 1986, WHO and BDMF, using the WHO Standard Methodology for oral health preventive programme planning, and with experience gained from previous research projects, decided to extend the implementation of milk fluoridation to community-level projects. Intensive work to investigate the chemical availability of fluoride in various forms of fluoridated milk, with particular emphasis on long-life varieties, has been conducted at the BDMF laboratory in Portsmouth, U.K. It was found that fluoride availability remains high in such systems over the whole of their shelf-life, an important feature if this vehicle were to be used as a means of controlled fluoride delivery to children for caries prophylaxis. The conclusion drawn from the study was that liquid (both fresh and UHT) milk is suitable for such purposes. Taking into account the positive results obtained in the above-mentioned clinical and laboratory studies, the exclusive nutritional value of milk for children, and its availability on a regular basis in many countries, it was proposed that the use of milk as a means of delivering community level fluoridation was attractive, but would benefit from further long-term investigations.

With experience gained on the development of milk fluoridation, WHO is now able to define three categories of projects which have already been introduced and which could be implemented in the near future. These are constituted by the WHO International Milk Fluoridation Programme as follows: (a) community level demonstration projects to evaluate the efficacy and organizational pattern of milk fluoridation for dental caries prevention as an alternative to other non-voluntary means of fluoride delivery; (b) feasibility studies to explore
whether milk could be used as a vehicle for fluoride intake at country or regional level; (c) clinical or laboratory studies relating to a specific research project whereby data obtained would facilitate the implementation of milk fluoridation for caries prevention.

The first large-scale project was implemented in Bulgaria, the purpose of which was to investigate the caries-reducing effect of a community-based milk fluoridation project over five years. Results obtained from this study have confirmed previous data, i.e. that the caries-reducing effect of fluoridated milk appears comparable with that of other fluoride vehicles. Similar objectives were proposed for community projects in Chile, China, Russia and the U.K. where milk fluoridation has been recognized by national health authorities as a potential anti-caries measure for children. Other research investigations of varying types have also been implemented in Germany, Hungary, Italy and New Zealand. Results from all these studies are presented in this monograph, in the belief that those who are involved in implementing community programmes for oral health will obtain the necessary scientific and practical data required of milk fluoridation.

Dr G.N. Pakhomov
Manager
WHO International Programme for Milk Fluoridation
1

Nutritional value of milk

D. Benbouzid, J. Ramanathan

1.1 Introduction

The nutrients supplied by milk and dairy products are essential components of the human diet throughout life. Indeed milk is the first and only food infants need during the initial 4–6 months of their lives. Lactation in its simplest form is an extremely ancient physiological function, probably dating back some 200 million years, certainly antedating the evolution of placental gestation (Jelliffe & Jelliffe, 1978). Breast feeding, before gradually being replaced by other milks such as cow's milk, is considered a universal imperative, ensuring infant survival and health (WHO, 1989).

Milk is one of the most complete single food items in the diet, only egg being considered as another complete food. However, in the diet of the adult, milk and dairy products are used rarely as single food items, but in combination with other food components. Thus milk has to be considered as a supplement of nutrients from the weaning period up to old age. Hence, interest should be devoted to dietary components in which dairy products are especially rich, or may have a specific nutritional or physiological implication as a supplement to the conventional diet (Hambraeus, 1994).

The quality of the diet is very important for linear growth, particularly for infants between 18–30 months of age. An early growth-faltering occurs even before, at around 3 months after birth, for infants in most developing countries. To illustrate the relative importance of nutritional factors and their relationship to linear growth, Allen (1994) has developed a hypothetical model for nutritional influences on children’s length, from pregnancy to 32 months of age (Figure 1.1). This illustrates the role of maternal nutrition during pregnancy and the building of foetal stores, and indicates that problems of growth-faltering may be related to suboptimal foetal endowment with nutrients during pregnancy. The impact of foetal stores are also difficult to evaluate.
Figure 1.1 Hypothetical model of nutritional influences on children's length, from pregnancy until 32 months of age

The nutritional value of milk, whether from human or other origin, should be considered in relation to foetal stores at an early age. Nevertheless, the characteristics of diet and feeding practices also have a significant role, including the effect of the dietary sugar content which has cariogenic potential and most probably leads to obesity, these being the two major nutritional/dietary public-health problems in industrialized societies, according to Jelliffe & Jelliffe (1978).

1.2 Milk consumption

In the European Union, it has been reported that liquid milk consumption in all its forms represents between 200 and 500 gms per person per day, i.e. 45 times the consumption of powdered, condensed or evaporated milk; 22 times the consumption of all types of cheeses, and 15 times the consumption of yoghurt (Mayor Zaragoza, 1994).
1.3 Milk composition

Hambraeus (1994) in his review of milk composition in humans and animals, has discussed the nutritional aspects of milk from two sides, (a) a macronutrient aspect which includes the energy and essential nutrient content, these playing a major role in growth in particular, and (b) a micronutrient aspect with the physiological importance of trace elements, vitamins, hormones and growth factors.

1.4 Milk as a source of macronutrients

1.4.1 Energy

The energy content of human milk is around 66 Kcal/100 ml, equivalent to cow's milk content. Hambraeus (1994) has compared the energy content of human and cow's milks, and of the average diet in industrialized countries (Table 1.1). It is interesting to note the low protein content, but the implication of this for the future outcome and metabolic programming of the offspring is not entirely clear.

Table 1.1 Energy distribution in human and bovine milk versus adult diet in a typical industrialized country

<table>
<thead>
<tr>
<th>Energy in % from:</th>
<th>Human milk</th>
<th>Cow's milk</th>
<th>Diet in industrialized country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>6</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>Fat</td>
<td>52</td>
<td>50</td>
<td>38</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>42</td>
<td>29</td>
<td>50</td>
</tr>
</tbody>
</table>

Adapted with permission of the publisher from Hambraeus (1994).

1.4.2 Protein

As a protein source, the nutritive value of milk is related to its amino acid content. The reference amino acid pattern for estimation of protein quality is close to that of breast-milk, showing a relatively low content of the sulphur amino acid methionine, and a higher content of the branched chain amino acids, leucine, iso-leucine and valine.

1.4.3 Calcium

Calcium is the other macronutrient found in high concentrations in milk. The calcium in milk occurs essentially bound to casein, which is a very specific phosphoprotein (Table 1.2). As calcium is an essen-
tial building material for bone and tooth tissues, the intake of calcium, its relation to the future development of bone mass, and the occurrence of osteoporosis has been a matter of concern. Most scientists seem to agree that the calcium intake during infancy, childhood and adolescence is of importance in the prevention of osteoporosis. In industrialized societies, dairy products constitute about 65–75% of calcium intake.

**Table 1.2 Casein, calcium and phosphorus content in human and bovine milk**

<table>
<thead>
<tr>
<th></th>
<th>Human milk</th>
<th>Cow's milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (g/L)</td>
<td>0.3</td>
<td>2.8</td>
</tr>
<tr>
<td>% of total protein</td>
<td>35</td>
<td>80</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td>340</td>
<td>1200</td>
</tr>
<tr>
<td>Phosphorus (mg/L)</td>
<td>140</td>
<td>950</td>
</tr>
<tr>
<td>Ca/P ratio</td>
<td>2.1–2.4</td>
<td>1.2–1.3</td>
</tr>
</tbody>
</table>

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Human milk has a low casein content and, as the calcium content is related to casein content, this means there is also less calcium in breast-milk. Consequently the calcium intake during infancy and childhood is far higher in those given artificial products based on cow's milk when compared to those who are breast-fed. Whether or not this is positive or negative in the long-term, is impossible to say.

Dequeker (1988), in his review, confirms that to allow normal bone growth, calcium intake during childhood should meet the requirements of net calcium retention and obligatory calcium loss. It seems that the mean net calcium retention for the entire growth period is in the range of 100–200 mg/day, with a peak retention during the pre-pubertal growth spurt of 300–400 mg/day. It should be noticed that both in utero and during the first year of life, modest shortages in the supply of calcium or vitamin D, not severe enough to cause rickets, reduce the rate of increase of cortical bone mass. It is not known whether the deficit can be regained in later childhood, or if it persists to reduce peak adult bone mass.

1.4.4 Lipids

A recent expert consultation on Fats and Oils (FAO/WHO, 1993) has revised the role of lipids in human nutrition. The meeting's recom-
mentations regarding infant and young child feeding were the follow-
ing:

(a) Infants should be fed breast-milk if at all possible.

(b) The fatty acid composition of infant formulae should correspond
to the amount and proportion of fatty acids contained in breast-
milk.

(c) During weaning and at least until two years of age, a child’s diet
should contain 30–40% of energy from fat, and should provide
similar levels of essential fatty acids as are contained in breast-
milk.

Lipids constitute about 3–5% of milk and 98% are triacylglycerols. The
growth and development of a child depends on both the amount and
quality of dietary fat consumed. These influences are mediated
through energy levels and through the specific action of fatty acids
and various non-glyceride components of the fat. Breast-milk pro-
vides between 50–60% energy from fat, and during the weaning pe-
riod, care needs to be taken to prevent dietary fat intakes from falling
too rapidly or below the required levels. The use of fats, especially
vegetable oil, in the foods fed to weaning infants and young children,
is an effective way to maintain the energy density of their diets.

For normal growth and development it is also important to ingest
adequate amounts of essential acids. Arachidonic acid and
docosahexaenoic acid (DHA) are particularly important for brain de-
velopment, and breast-milk is a good source of these fatty acids. Pre-
term infants who have an insufficient intra-uterine supply of
arachidonic acid and DHA, and who are born with low fat reserves,
may face particular problems.

Milk is a significant source of fat compared to other foods groups.
Based on FAO’s food balance sheets, Table 1.3 data show the geogra-
phical distribution with regard to lipid contribution to the diet.

Overall, 50–60% of energy is provided from human milk as lipid, of
which about 5% of the energy is from essential fatty acids and 1%
from long-chain polysaturated fatty acids. Studies on lactation
showed that in well-nourished mothers, milk fat contribution in-
creased from 40–50 g/litre at 3 weeks, to 60–70 g/litre at 4–6 months.
In developing countries, the rise in milk fat content was less with lower
energy intakes. Therefore, it seems inappropriate to wean infants on
low fat diets. In a Committee Report (1991), the European Society of
Table 1.3 Contribution of fats and oils by food groups in 1990

<table>
<thead>
<tr>
<th>Region</th>
<th>Total fat g/cap/day</th>
<th>Vegetable</th>
<th>Animal</th>
<th>Meat</th>
<th>Milk</th>
<th>Cereal</th>
<th>Oilerop</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>68.3</td>
<td>36</td>
<td>11</td>
<td>23</td>
<td>9</td>
<td>8</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Africa</td>
<td>43.1</td>
<td>48</td>
<td>4</td>
<td>9</td>
<td>5</td>
<td>16</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Far East</td>
<td>44.6</td>
<td>35</td>
<td>6</td>
<td>24</td>
<td>6</td>
<td>13</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Near East</td>
<td>72.3</td>
<td>49</td>
<td>7</td>
<td>11</td>
<td>7</td>
<td>13</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Latin America</td>
<td>75.4</td>
<td>43</td>
<td>9</td>
<td>22</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>USSR</td>
<td>106.8</td>
<td>25</td>
<td>22</td>
<td>26</td>
<td>12</td>
<td>5</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Oceania</td>
<td>137.8</td>
<td>20</td>
<td>18</td>
<td>40</td>
<td>13</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Europe</td>
<td>142.8</td>
<td>30</td>
<td>20</td>
<td>28</td>
<td>12</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>North America</td>
<td>151.0</td>
<td>39</td>
<td>9</td>
<td>27</td>
<td>14</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>


Paediatric Gastroenterology and Nutrition recommended that 40–55% of the dietary energy be provided as fat (4.4–6.0 g/100 Kcal) for follow-up formula. During weaning, the fat component should provide 30–40% of dietary energy and similar levels of essential fatty acids as found in breast milk from appropriate foods, until at least 2 years of age. In this period, complementary food should include adequate amounts of fats and oils as the breast milk component of the diet declines.

1.4.5 Carbohydrates

Lactose, or milk sugar, is the predominant carbohydrate in milk, its concentration varying among species, and being highest in human milk. (Hambraeus, 1994). This milk sugar is a disaccharide compound of glucose and galactose. Compared with bovine milk the oligosaccharide concentration is ten times greater in breast-milk. These carbohydrates and protein- or amino acid-bound sugars are able to induce Lactobacillus bifidus activity. More important is the fact that lactose has a specific role in enhancing calcium absorption and preventing rickets, even if there is a low calcium concentration in human milk. Furthermore, there are correlations between relative brain size and lactose and fat contents of milks. Lactose is found only in milk and not in animal or plant sources, thus showing the importance of its high concentration in human milk.
Table 1.4 Mineral and trace elements in human and cow’s milk

<table>
<thead>
<tr>
<th>Minerals (mg)</th>
<th>Human milk</th>
<th>Bovine milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>30</td>
<td>120</td>
</tr>
<tr>
<td>Magnesium</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Sodium</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>Potassium</td>
<td>51</td>
<td>150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trace elements (μg)</th>
<th>Human milk</th>
<th>Bovine milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>42</td>
<td>11</td>
</tr>
<tr>
<td>Iron</td>
<td>74</td>
<td>60</td>
</tr>
<tr>
<td>Zinc</td>
<td>251</td>
<td>337</td>
</tr>
<tr>
<td>Selenium</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Chromium</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Cobalt</td>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td>Manganese</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Iodine</td>
<td>20</td>
<td>8</td>
</tr>
</tbody>
</table>

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1.5 Milk as a source of micronutrients

1.5.1 Trace elements

In Table 1.4, the trace element content in human and cow’s milk is detailed. It is not the high content but the bioavailability of the trace element which matters, particularly with respect to iron (Hambræus, 1994). Its binding with lactoferrin, of which human milk is rich, gives it a nutritional role to play as a source of protein, and as a bioavailability-increasing factor. Also, it is not the zinc content, but its bioavailability which is of significance. Fomon & Ekstrand (1993) have published a comprehensive review of fluoride in infant nutrition, and Table 1.5 summarizes the fluoride concentration of infant foods and, more interestingly, the estimated fluoride intakes from milks and formulae, respectively.

1.5.2 Vitamins

The vitamin content of human and cow’s milk differs greatly as can be expected from the macronutrient concentrations and, amongst
the fat soluble vitamins, vitamin E content differs the most. The higher level of this vitamin in human milk is probably in relation to its high content in polyunsaturated fatty acids. There is more vitamin K in cow's milk than in breast milk but, from the nutritional standpoint, it is the vitamin E content which matters in the adult diet.

The water-soluble vitamins are in lower concentrations in human milk than in bovine milk, except for vitamin C and niacin. As to these vitamins, the bioavailability depends on the protein to which they are bound. However, more research is needed to determine the content of various milk specimens to the physiological needs and functions (Hambraeus, 1994).

1.5.3 Other natural components

In human milk, and milks from other mammalian species, components such as enzymes and hormones are found. Some enzymes are synthesized in the mammary gland while others are produced outside and transferred to milk by the bloodstream.
1.6 External components or contaminants

The nutritional value of milk is linked to its natural composition but may be modified by exogenous components. Hence, for breast-fed infants, drugs which pass to the mother’s milk represent a potentially dangerous hazard, as do chemical contaminants from various sources, these being stored, generally in adipose tissues. In human milk the storage time is longer than in bovine milk and, because of the relatively higher production in the dairy cow, contaminants (e.g. pesticides) in human milk are found at a concentration 5–10 times higher than in cow’s milk (Hambraeus, 1994).

1.7 Conclusion

Milk is an essential component of the human diet throughout life, both as a source of micro- and macronutrients, as well as being a carrier for undesirable contaminants e.g. drugs and extraneous chemical pollutants.
2 Physico-chemical studies on milk fluoridation
A.G. Kolesnik, P.C. Phillips and A.E. Villa

2.1 Introduction

Fluoridated milks may be produced in a number of different forms; liquid (pasteurized, sterilized and UHT) and powder, each containing a variety of fluoridating agents. This chapter covers three aspects; (i) the manufacture of fluoridated milks, (ii) the stability of fluoridated milks with regard to the chemical availability of fluoride, and (iii) the analytical techniques used in the monitoring and quality control of the product.

Compounds which have been used to fluoridate milk include sodium fluoride, calcium fluoride, disodium monofluorophosphate and disodium silicofluoride, successful clinical trials and laboratory tests having been conducted with the respective milk preparations (Stephen et al., 1984; Bánóczy et al., 1985a; Villa et al., 1989; Stösser, Kneist & Grosser, 1993).

Of the above compounds, sodium fluoride is by far the most commonly used agent for large scale production of fluoridated milk, currently serving community schemes in Bulgaria, China, Russia and the United Kingdom, whilst disodium monofluorophosphate is used in a project in Chile.

The manufacture of fluoridated milk involves the addition of a fluoride compound to milk in the appropriate quantity, 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 Expert Committee (1994), 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.
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 ½ pint (189 ml) of school milk per day, whereas in China, kindergarten children each receive 250 ml.

In order to deliver a (say) appropriate dose of 0.5 mg fluoride per day to children in both areas, the fluoride concentrations in the milk require to be set at 2.65 ppm and 2 ppm respectively.

In Bulgaria, where 200 ml per day 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.

When disodium monofluorophosphate is used as the fluoridating agent (in Chile), it too is added to pasteurized liquid milk in the form of a concentrated aqueous solution but, in this case, as the milk is to be converted to and dispensed as powder, the important point is to use the calculated quantity of the monofluorophosphate for the batch of milk to be fluoridated, taking into account the total solids content of the milk.

The choice of disodium monofluorophosphate in the Chile scheme was based, partly, on early 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, but this is 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, 1989). Villa, Rosenkranz & Garrido (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/litre
at 18 °C (Lide, 1995), although this may be remedied by use of complexing agents, e.g. potassium aluminium sulphate (alum).

2.2 Manufacture of fluoridated milk using sodium fluoride

2.2.1 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 to the operator. This problem is reduced considerably when the solid is handled in the laboratory under carefully controlled conditions when formulating the aqueous solution.

Fluoridated milk is produced with different concentrations of fluoride to suit different requirements, but a typical value may be 5 ppm F. In order to manufacture this product, the aqueous solution of sodium fluoride is made by dissolving 11.06 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. When operating the continuous addition option, dosing equipment utilizing proportional-flow pumping is used to feed in the sodium fluoride solution.

The equipment, which is commercially available (e.g. from Fluid Management Technology Ltd, Didcot, Oxfordshire, UK), controls dosing to match the milk flow so as to maintain the set dosing ratio 1:1000, even though the milk flow-rate may be fluctuating. The fluoride solution enters the milk in the line en route for processing or packaging. The dosing equipment is generally designed to be “cleaned in place” (CIP).

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 conducted 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; (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 subsequent 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. Losses of fluoride availability due to heat-treatment are dealt with later in this chapter.

2.2.2 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 microorganisms. It is packaged in hermetically-sealed cartons under totally aseptic conditions.

UHT processing conditions are typically 140 °C for 4 s. Exposure of milk to this temperature is very effective in destroying microorganisms, but does have the drawback of reducing flavour quality due to induced structural changes in some milk constituents (Basset & Acosta, 1988). In order to make the product palatable for children, it frequently needs the addition of flavour and sweetener.

By the very nature of the UHT process, it is clear that any additives required in the product must be introduced prior to heat-treatment, in order to achieve complete product sterility. Fluoride is no exception in this respect.

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 on the UHT facility at the Borrow Dental Milk Founda-
tion, England (Phillips, 1991) indicated a "process loss" of 12% (vide infra).

2.2.3 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). Typical conditions used for the process are 115–120 °C for 15–20 min (Early, 1992). The severe heat-treatment deals very effectively with micro-organisms, but also 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.

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%) effect on the ionizable fluoride content.

2.2.4 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.

A variation on this process is to make the fluoridated milk concentrate by reconstituting milk powder with sodium fluoride solution of the appropriate concentration, then spray-dry the product. Fluoridated milk powder made in this way would typically contain 50 mg F/kg and would reconstitute to liquid milk with 10% solids content containing 5 mg F/kg.
2.3 Manufacture of fluoridated milk using disodium monofluorophosphate

A manufacturing process for the production of powdered milk fluoridated with disodium monofluorophosphate has been developed in Chile at the Milk Technological Research Centre of the Austral University, Valdivia. It involves the following steps.

Powdered milk (26% fat) is reconstituted and pasteurized at 73 °C for 15 s. The milk is then treated with the appropriate amount of a concentrated solution of disodium monofluorophosphate during transfer to the evaporation facility using a proportional-flow pumping system. In the evaporator, water is removed until the solids content reaches 48%. The evaporated fluoridated milk is then fed to the drying tower (operating at 150 °C) to give the powdered fluoridated milk product.

The process was developed so as to meet the following criteria (CORFO 1987):

a) The fluoridating agent must be distributed evenly in the product and should be at the required concentration;

b) The modification to existing technology must be minimal and easy to implement;

c) Additional cost of fluoridating the powdered milk must be minimal;

d) The organoleptic characteristics of reconstituted fluoridated milk must be almost identical with those of the non-fluoridated product;

e) The stability of the fluoridated milk powder must equate with that of the non-fluoridated product.

2.4 Stability of fluoridated milks

2.4.1 Milk fluoridated with sodium fluoride

Although the use of milk as a vehicle for fluoride was considered some 40 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 two decades as a result of its promotion by the Borrow Dental Milk Foundation. Since 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 elsewhere in Chapter 4 of this text.

Whilst clinical trials were in progress, some scientists (eg. 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 protein.

Whereas there is little doubt that there is opportunity for interaction between milk constituents and the fluoride ion, recent studies (Phillips, 1991; Edgar, Lennon & Phillips, 1992) have shown that such interactions have a relatively small effect on the availability of fluoride in milk, when present in the 2–5 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 the ionic form F. This comprises both free fluoride ions and fluoride in the form of other chemical species which readily release free fluoride ion on demand. An example of the latter type is the complex ion formed between aluminium and fluoride. Milk frequently contains traces of aluminium with a typical concentration being 0.5 ppm (Jenness, 1988). Aluminium forms a number of complex ions with fluoride, of which AlF$_2^+$ is an example, but these complex ions are formed in a reversible fashion and free fluoride ion is readily available from them:

$$\text{AlF}_2^+ \leftrightarrow \text{Al}^{3+} + F^-$$

When fluoride is determined in milk using standard electrochemical techniques employing an ion selective electrode and TISAB buffer (detailed later in this chapter), the sum total of free fluoride plus fluoride available from such complexes, is evaluated automatically.

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. (1995), in a study investigating the deposition of fluoride in ovine enamel and dentine resulting from the consumption of fluoridated milk (see Chapter 3), used bovine milk containing 300 and 750 ppm F. At these concentrations, he noted that the chemical availability of fluoride was only 30% and 20% respectively, of that added.
The nature of the interaction between milk constituents and fluoride at this high concentration has not been established, but it is likely that calcium would be involved. The typical calcium content of bovine milk is approximately 1200 mg/litre (Jenness, 1988), of which some 80 mg/litre exists as free calcium ion (Holt, Dalgleish & Jenness, 1981).

Calculations based on the solubility product of calcium fluoride $3.95 \times 10^{-11}$ mol$^2$ dm$^{-6}$ at 298K (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 of the type cited above, may not be exceeded when the fluoride concentration is 5 ppm. However, the solubility product of calcium fluoride would certainly be exceeded when 300 ppm fluoride is added to milk.

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.

2.4.2 Fluoridated pasteurized milk

Investigations undertaken by Phillips (1991), and Edgar, Lennon & Phillips (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 content. Their conclusion was that interaction between such fluoridated milk and glass was very small, and of no practical significance.

2.4.3 Fluoridated UHT milk

In contrast to the findings with fluoridated pasteurized milk, Phillips (1991) has found 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 ionizable fluoride in the product, 4.4 ppm F, remained fairly constant
for three months' storage at an ambient temperature ranging between 5 °C and 20 °C. Further storage under the same conditions resulted in a steady decline in ionizable 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.

2.4.4 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 ionizable fluoride levels of 2.2 ppm (88%) and 4.4 ppm (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 (Vide infra). 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 there was no loss of fluoride from the water during processing, hence it was concluded there was no binding of fluoride to the glass.

2.4.5 Fluoridated milk manufactured from powdered milk

Powdered milks, fluoridated at the time of reconstitution, have also been the subject of a study by Phillips (1991). It was observed that the fluoride availability in this system 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 whey protein 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 related inversely to the intensity of pasteurization (Table 2.1).

<table>
<thead>
<tr>
<th>Pasteurizing conditions used on liquid milk prior to drying</th>
<th>Ionizable fluoride concentration in reconstituted fluoridated milk (w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Heat 77 °C / 30 s</td>
<td>4.55 ppm (96%)</td>
</tr>
<tr>
<td>Medium Heat 92 °C / 2 min</td>
<td>4.40 ppm (93%)</td>
</tr>
<tr>
<td>High Heat 125 °C / 5 min</td>
<td>4.20 ppm (88%)</td>
</tr>
</tbody>
</table>

*Source: Phillips (1991) Fluoride availability in fluoridated milk systems*

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

2.4.6 Fluoridated powdered milk

The fluoride availability in reconstituted fluoridated semi-skimmed powdered milk has also been determined by Phillips (1991), using a product which had been exposed to medium heat pasteurization prior to drying.

The fluoridated powdered milk containing 50 mg F/kg, on reconstitution to provide a liquid fluoridated milk (10% solids content), gave a product with fluoride ion availability of 4.65 ppm F (93%).

Repeated tests, carried out over the shelf-life of the fluoridated powdered milk, stored at average ambient temperatures of 15 °C, showed that the fluoride availability of the reconstituted product remained at 4.65 ppm F throughout.

The shelf-life of powdered milk relates to the fat content and the storage conditions (packaging and temperature). Degradation of the fat component giving rise to rancidity 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 2.2 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 2.2 Shelf-life of powdered milks

<table>
<thead>
<tr>
<th>Milk product</th>
<th>Shelf life stored at 20 °C</th>
<th>Shelf life stored at 30 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skimmed milk powder</td>
<td>2 years</td>
<td>1 year</td>
</tr>
<tr>
<td>Full fat milk powder*</td>
<td>9–12 months</td>
<td>6 months</td>
</tr>
</tbody>
</table>

* Dependent on country of origin, heat classification, use of anti-oxidants and whether instantized or not.
Source: Renyard (1996)

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, using materials which exclude light.

The shelf-life is adversely affected by additives such as lecithin, included in some powdered milk products to improve dispersibility during reconstitution. Also, it must not be overlooked that the quality of water used to reconstitute the milk features in the overall quality of the liquid product.

2.4.7 Stability of powdered milk fluoridated with disodium monofluorophosphate

Stability tests in respect of milk fluoridated with disodium monofluorophosphate have been conducted by Villa. The results, (CORFO, 1987) 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 to 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 pharmacopeia grade disodium monofluoro-phosphate, indicating that no detectable enzymatic hydrolysis of monofluorophosphate occurred in the powdered and liquid milk during storage.

2.5 Measurement of fluoride concentration in fluoridated milk

2.5.1 Milk fluoridated with sodium fluoride

Ionizable fluoride in fluoridated milk, manufactured to contain typically 2–5 ppm F (added as sodium fluoride), is conveniently deter-
mined by electrochemical means using a fluoride ion selective electrode, in conjunction with a reference electrode coupled to an ion meter. Ion meters are available which provide a direct concentration readout or a millivolt output from which concentration may be computed.

The electrodes (fluoride and reference) may be two single half cells, or a combination-type embodying the sensing electrode and reference electrode in one unit.

There are a number of companies which supply this equipment. One such organization of international repute is Orion Research of Boston, 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 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 volume ratio 1:1 in both samples and standards, is recommended for use in this context. It standardizes the ionic strength and pH of the medium, and deals with ions which would otherwise interfere. The basis of the technique is to calibrate the instrument using fluoride standards containing TISAB II which encompass the range of measurement to be made (for example in the above case, fluoride standards could be 1 ppm F and 10 ppm F), and then conduct direct measurement of the fluoridated milk sample containing the appropriate amount of TISAB.

2.5.2 Preparation of standards

Water-based standards of 1 ppm F + TISAB, and 10 ppm F + TISAB, are commercially available and may be used in routine situations where high precision is not required (i.e. up to 5% deviation acceptable). However, in order to obtain results with greater precision, it is recommended that standards are made up in milk of the same type as that being analysed. This technique deals with the minor interferences which occur due to components in the milk matrix which result in small changes in the response of the electrode. It has been used in the past by Konikoff (1974) and is recommended by Orion Research Inc (1978). Milk-based standards are prepared by diluting the corre-
sponding concentrated water standards in the appropriate proportion. Standard solutions containing 100 ppm F and 0.1 M NaF (1900 ppm F) are commercially available.

The difference between using water standards and milk-based standards has been studied in detail by Kolesnik (unpublished). In his investigation, ten combination fluoride electrodes (Orion, model 9609), in conjunction with an Orion 920 pH/ISE meter, were used to obtain statistical validity for the results.

Here, the electrode slopes per decade in fluoridated milk were, on average, 2.6 mV lower than in fluoridated water, in the range 1–10 ppm F. In the analysis of fluoridated milk (2.2–7.5 ppm F), using water-based standards, he observed that the recorded value deviated from the real value by 2.6% – 3.6%. Data in Table 2.3 show the results obtained in the study.

<table>
<thead>
<tr>
<th>F-Concentration in milk (ppm)</th>
<th>Range of deviation from real value (%)</th>
<th>Variation Coefficient V (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>-0.5 to -4.1</td>
<td>3.2</td>
</tr>
<tr>
<td>2.5</td>
<td>+1.2 to -3.6</td>
<td>2.6</td>
</tr>
<tr>
<td>2.8</td>
<td>+0.7 to -4.2</td>
<td>3.0</td>
</tr>
<tr>
<td>5.0</td>
<td>-2.0 to -4.4</td>
<td>3.6</td>
</tr>
<tr>
<td>7.5</td>
<td>-0.5 to -3.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

2.5.3 Function of TISAB

TISAB II contains the following:

- Ethanoic acid 1.4%
- Sodium ethanoate 8.2%
- Sodium chloride 5.8%
- Cyclohexyldiamine tetracetic acid (CDTA) 0.4%
- De-ionized water 84.2%

(Data sheet available from Orion Research Inc)

The sodium chloride and sodium ethanoate are the major components which adjust the ionic strength of the sample (or standard) to the required value for measurement.
The necessity to standardize the ionic strength relates to the function of the fluoride electrode. In reality, the response of the fluoride electrode relates to the activity of fluoride in the medium rather than the concentration.

By standardizing the ionic strength, the ratio of fluoride activity to fluoride concentration (activity coefficient) will be fixed and constant. Under these conditions the electrode response will also relate to fluoride concentration in the same way as it does to fluoride activity, and it may be used to measure concentrations.

TISAB II also acts as a pH buffer which will bring the pH of samples and standards into the required range of 5–6. The ethanoic acid/sodium ethanoate combination is responsible for the buffer action. Control of pH is necessary because, should it be allowed to fall to lower values, then progressively more fluoride would be in the form of HF and would not be recognized by the electrode. On the other hand, if the pH value gets too high, particularly above pH 8, the hydroxyl ions in the medium interfere with electrode performance and distort the response.

TISAB II also contains the powerful complexing agent, CDTA, to deal with metal ions in the sample which otherwise would interfere with fluoride measurement. Ions which typically come into this category are Al³⁺, Fe³⁺, Ca²⁺ and Mg²⁺.

2.5.4 Analytical procedure

Fluoride analysis is conducted as a quality assurance measure in fluoridated milk production. The following procedure is employed for a typical fluoridated milk containing 5 ppm F, 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 two standards to cover the range to be measured:
   i) 1 ppm F with TISAB II (1:1)
   ii) 10 ppm F with TISAB II (1:1)

3) Measure the sample (made up from equal volumes of fluoridated milk and TISAB II).
During these procedures the following must be observed:

i) Electrodes should be washed with distilled or de-ionized water and blotted dry between each measurement, taking care not to damage the sensor of the fluoride electrode.

ii) All solutions (standards and samples) should be at a constant set temperature eg. 25°C +/- 0.5 °C when measurements are made.

iii) Solutions should be stirred to assist in reaching equilibrium. If the stirring is continued during measurement, it should be done so at a uniform rate for all solutions. Alternatively, once equilibrium has been reached, the stirrer may be stopped before recording the reading. Equilibration time is inversely related to the concentration of fluoride in the milk. It would typically be 1-2 min with 2-5 ppm F in milk, using a new electrode. Older electrodes take progressively longer.

If the meter used does not give concentration readout, but does give millivolt output, then measurements are made using the same procedure. A calibration graph is compiled using the standards, by plotting millivolt reading versus log concentration, which is linear over this range.

Extra calibration points may be inserted if desired. The graph is then used to convert millivolt readings obtained from the sample (flouridated milk + TISAB) into fluoride concentration values in the milk.

2.5.5 Milk fluoridated with disodium monofluorophosphate

Unlike free fluoride ion, the monofluorophosphate ion (FPO₄²⁻) cannot be measured directly by a simple ion selective electrode technique. For the purpose of analysis, it is usually necessary to transform the ion into a species which can be determined readily. A thorough discussion of analytical techniques for fluoride and monofluorophosphate has been presented by Lindhal (1983). These include volumetric, colourimetric, gas chromatographic, ion chromatographic and non-destructive nuclear methods. In addition, it is possible to hydrolyse quantitatively monofluorophosphate to free fluoride ion and determine this by use of the fluoride ion selective electrode. This latter method is popular due to its simplicity and relative economy when compared with the more sophisticated methods. It also has the advantages of low detection limit and good precision which are the char-
acteristics of the fluoride ion selective electrode technique. There is, however, the disadvantage that it is not specific to monofluorophosphate and it is necessary to ensure that the sample under investigation does not contain other fluoro compounds which would hydrolyse under the conditions to give free fluoride, and thereby interfere with the technique.

The method for analysis of powdered milk fluoridated with disodium monofluorophosphate, summarized below, is described in detail by Villa (1988) and Villa et al. (1989). Disodium monofluorophosphate is generally determined as the difference between ionic fluoride before and after acid hydrolysis of monofluorophosphate using perchloric acid (Gron, Brudevold & Aasenden, 1971). In order to determine the total fluoride concentration in a powdered milk using monofluorophosphate as the fluoride source, the following procedure is suggested:

(a) Weigh 2 g of sample into a 30–50 ml screw-capped plastic vial.
(b) Add 20 ml of 1 M HClO4.
(c) Hydrolyse overnight at 50 °C in a thermostatically-controlled bath with gentle shaking.
(d) After cooling, dilute 10 ml of the hydrolysate to 100 ml in a graduated flask.
(e) Measure fluoride concentration with a combination fluoride selective electrode type ORION 96-09 connected to an ORION EA-940 digital ion meter against a calibration curve previously constructed in a 0.1 M perchloric acid matrix. It is suggested that results of such measurements be checked against those obtained also by employing a double known addition technique. Further details of this method have been published by Villa (1988).
(f) Calculate the final fluoride concentration in the solid sample applying the corresponding dilution and blank corrections.
(g) Subtract the initial value of free ionic fluoride in the powdered milk from the total value obtained in Step (f), to obtain a value for fluoride present in the solid in the form of monofluorophosphate.

If the necessary apparatus is available, the method may be improved by modifying Steps (b) and (c) as follows:
Step (b) – Use IM HCl instead of IM HC104.
Step (c) – Use a Microwave Laboratory System type Milestone (Italy) Model MLS-1200 Mega.
2.6 Conclusion

The manufacture of fluoridated milk in various forms (pasteurized, sterilized, UHT and powdered) using sodium fluoride or sodium monofluorophosphate as fluoridating agents, involves simple production techniques.

All of the products have been shown to be stable with relatively high fluoride availabilities remaining throughout their complete shelf-lives.

Laboratory methods for monitoring product quality in terms of fluoride content using ion selective electrode techniques are rapid, reliable and easy to perform.
3
Bioavailability of fluoride from milk

J. Bánóczy, E. Brambilla, T.W. Cutress,
P.C. Phillips and L. Stösser

3.1 Introduction

Bioavailability of fluoride from cow’s milk has been studied, both in animal and human experiments which also sought to clarify the systemic or topical mode of action of fluoridated milk. However, as Flynn (1992) revealed that such results could be influenced by the natural fluoride content of the consumed milk (approximately 20 μg/litre), this fact has to be considered when undertaking and evaluating the studies discussed in this chapter.

3.2 Experimental Studies (laboratory animals)

The objectives of the animal experiments which have been pursued with respect to fluoridated milk were to investigate the caries-preventive effect of milk: (i) with different fat contents; (ii) with different fluoride dosages and sources, and (iii) their effect on dental plaque composition, and (iv) the fluoride uptake from fluoridated milk in dental hard tissues.

3.2.1 The caries preventive effect of milk and the influence of different fat contents

In an attempt to understand the possible cariostatic properties of milk, several studies have been conducted in animals, albeit contradictory results have arisen. Rats receiving fresh milk as a sole source of nutrients either remained essentially caries-free or showed a reduced caries challenge (Shaw, Ensfield & Wollman, 1959). The latter observation was supported by Reynolds & Johnson (1981) who suggested that supplementation of a cariogenic diet with milk also reduced caries incidence in rats.
Findings from animal experiments with milk as the only drinking fluid (Stösser et al. 1995), showed the importance of standardized dietary administration prior to any judgement of nutritional effects. Shown in Figure 3.1 is the high caries prevalence amongst control animals water-fed under *ad libitum* conditions (T-lesions = 9.2) as compared to the results following administration of raw milk (T-lesions = 5.7), the most likely explanation being an uncontrolled higher consumption of diet by control animals when provided solely with water as their fluid source. The lower molar caries rate in the milk group was caused partially by the reduced food intake of these animals as a result of their milk consumption. The identical weight development of the water control animals and the experimental milk animals indicated a higher, and probably more frequent, food consumption level by the former group which, in turn, was the likely reason for their higher caries score.

*Figure 3.1 Effect of raw milk on experimental caries challenge in OM rats (N/group=12) under ad libitum or programmed feeding of a cariogenic diet. The cumulative demonstration of A, T, and B grade lesions according to König, Morthaler & Mühlmann (1958) is shown with a trial period of 3 weeks, the weights of animals representing values at the end of the trial.*

<table>
<thead>
<tr>
<th>Diet MIT 200</th>
<th>ad libitum</th>
<th>programmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet/sucrose [%]</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>weight [x (g)]</td>
<td>137</td>
<td>138</td>
</tr>
</tbody>
</table>

*Reproduced with permission of the publisher from Stösser et al. (1995)*
However, as also noted in Figure 3.1, under programmed administration of the same dietary sucrose quantities to all groups, via a feeding machine, milk-fed animals produced larger weight gains. Standardization of eating patterns caused overall reduced food intakes of water-fed rats which resulted also in a decreased experimental caries challenge. None the less, the differences between the milk and water sucrose-fed programmed groups was non-significant (T-lesion scores = 6.3 and 6.6 respectively).

Hence, a possible dependence of the caries-protective effect of milk upon its fat content, despite high variation of individual findings, was not apparent (Table 3.1). Furthermore, Stösser et al. (1995) showed that, with an increasing fat content and different pasteurization methods, no correlation existed between the experimental caries challenge, the type of milk treatment, or its fat content.

The addition of 5% sucrose to milk caused a lower caries increase (40%) than did the same amount of carbohydrate in drinking water.

### Table 3.1 Caries reduction by milk with different fat components in DM rats (Summary of 5 experiments [Stösser et al., 1995])

<table>
<thead>
<tr>
<th>Trial No</th>
<th>Groups</th>
<th>Control Sum of lesions : A+T+B+C</th>
<th>Milk</th>
<th>Caries reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/88</td>
<td>raw milk, ad libitum diet</td>
<td>22.9</td>
<td>16.8</td>
<td>37</td>
</tr>
<tr>
<td>2/88</td>
<td>raw milk, programmed diet</td>
<td>15.0</td>
<td>14.6</td>
<td>3</td>
</tr>
<tr>
<td>3/88</td>
<td>raw milk, ad libitum diet</td>
<td>17.8</td>
<td>15.4</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>raw milk, programmed diet</td>
<td></td>
<td>16.9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>milk 2.2% fat</td>
<td></td>
<td>15.1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>milk 0.4% fat</td>
<td></td>
<td>18.9</td>
<td>--</td>
</tr>
<tr>
<td>1/92</td>
<td>milk 1.5% fat</td>
<td>25.0</td>
<td>12.1</td>
<td>51</td>
</tr>
<tr>
<td>2/92</td>
<td>UHT 0.3% fat</td>
<td>20.6</td>
<td>10.4</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>UHT 1.5% fat</td>
<td></td>
<td>11.8</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>UHT 3.5% fat</td>
<td></td>
<td>12.2</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>fresh milk (pasteurized) 3.8% fat</td>
<td></td>
<td>13.1</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>raw milk 4% fat</td>
<td></td>
<td>10.6</td>
<td>39</td>
</tr>
</tbody>
</table>

**Mean of caries reduction:** \(\bar{x} = 27 \pm 20\) 

\(n=12\)

Reproduced with permission of the publisher from Stösser et al. (1995)
(Stösser et al. 1995). Similar observations were made by Bowen & Pearson (1993) in a comparison of animal groups fed milk or water, with and without sucrose additives. As a result, it would appear that a caries-protective effect of milk does exist.

3.2.2 Caries-preventive effect of fluoridated milk with different fluoride dosages and sources

When fluoride is added to milk, it may interact partially with the intrinsic calcium, or else be incorporated into the milk proteins. According to some reports, this leads to a deactivation of the fluoride (Hattab, 1985), and Duff (1981) considered milk as a "poor" carrier for fluoride. However, animal findings do not support a decreased bioavailability of fluoride from milk. In the reports cited below, a dependence of the observed caries-preventive effect could not be found upon the type, concentration or dissociation of the fluoride compounds employed. Here, caries-preventive effects of 40–50% were recorded (Bánoczy et al., 1990; Poulsen, Larsen & Larson, 1976; Stösser et al. 1995), such reductions having been achieved in rats, with low fluoride concentrations in water, only in few cases (Spuller et al., 1986).

Even the use of calcium fluoride, which was only incompletely ionized despite adding alum (6.5 ppm F instead of 15 ppm F), caused a clear caries-inhibiting effect in the study of Stösser et al. (1995). With regard to any caries benefit obtained, no differences could be detected between the various sodium fluoride concentrations employed (5–15 ppm F). Monofluorophosphate, which released little ionized fluoride (1.09 ppm F from 15 ppm F), turned out to be just as effective, as did fluorosilicate which is hydrolysed rapidly (13.49 ppm F from 15 ppm F) in an aqueous environment (Figs. 3.2, 3.3, 3.4).

The caries-preventive effect achieved in the following animal experiments was most likely connected with the permanent presence of low F-concentrations being maintained in the oral cavity by steady and frequent drinking. The fluoride uptake and elimination during an 18-day metabolic experiment with rats fed on sodium fluoride-, monofluorophosphate-, or silicofluoride-enriched milk (all at 15 ppm F) showed that independent of fluoride source, approximately the same F retention was determined (55–60%) in young, growing rats (Stösser et al. 1995). The same positive F-balance (65%) was found in the F-water group (again at 15 ppm F) in spite of a much lower liquid intake. Thus, any impairment of fluoride bioavailability due to milk components can be discounted.
Figure 3.2 Caries-preventive effect of CaF₂, alum-milk in comparison to CaF₂, alum-milk, milk, water + NaF and water, as well as weight development of DM rats (N/group = 10; 3 week trial period). Multiple comparison of mean values of the carious T-stages according to Duncan (1957) are shown. The stages with the same letter - X, Y or Z - do not differ significantly.

<table>
<thead>
<tr>
<th>ppm F</th>
<th>weight [x(g)]</th>
<th>control</th>
<th>water</th>
<th>milk control</th>
<th>milk 15 ppm F (CaF₂)</th>
<th>milk 15 ppm F (NaF)</th>
<th>milk Aik(SO₄)₂</th>
<th>milk 15 ppm F (CaF₂ + Aik(SO₄)₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>115</td>
<td>7.9</td>
<td>X</td>
<td>6.7</td>
<td>6.7</td>
<td>6.1</td>
<td>5.7</td>
<td>4.9</td>
</tr>
<tr>
<td>15</td>
<td>115</td>
<td>6.7</td>
<td>Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Duncan's Multiple Range Test for Carious Lesion 'T'
(N/group = 10, a = 0.05)

3.2.3 The influence of fluoridated milk with different fluoride dosages and sources on dental plaque composition

An influence by systemically administered F-concentrations on dental plaque has been described rarely, as experimental animal plaque is influenced mainly by dietary composition and less by drinking fluid (Beighton, McIntosh & McDougall, 1979). Where any bacterial count reductions have been observed at such low F-dosages, these have been associated with low numbers of carious lesions, thereby leaving less room for bacterial retention (Beiraghi et al. 1989; Spuller et al. 1986). Stösser et al. (1995) reported that plaque samples provide no evidence...
that fluoridated milk can influence dental plaque composition, as bacterial counts early and late in the trials of Stösser, Kneist & Gabdjiel (1985), and Kneist & Stösser (1988), indicated increases of CFU by both the sugar-rich cariogenic diet and the use of milk.

The administration of the latter probably had an additional growth-promoting influence on the flora since milk was the only fluid provided. Here, Streptococci, Lactobacilli and Actinomycetes dominated in the plaque samples isolated. The percentage of caries-inducing Streptococci (20–40%) was equally high in all groups, and not influenced by milk or fluoride administration.
Actinomyces constituted <10% at the beginning of the experiments and was altered little by sugar-containing diets. However, in most cases, this increased to 20–40% following milk administration and probably indicated "more neutral" intra-oral environmental conditions due to their minor aciduric properties. The phenomenon of superior buffering by milk components in the mouths of experimental animals was described by Beighton, McIntosh & McDougall (1979). As a result, altered plaque composition and a lower cariogenic challenge were recorded. However, an additional antibacterial influence of milk could neither be observed in the experiments of Stösser et al. (1995), nor by others (Bowen & Pearson, 1993).

Figure 3.4 Caries preventive effect of 15 ppm F as NaF, Na₂MFP and Na₂SIF₆ in milk, as compared to milk, or water and water + NaF (N/group = 12; 4 week trial period); the animals' weight development and multiple comparison of mean values of T-stages according to Duncan (1957) are indicated by the letters X, Y and Z, where similar letter-sharing reveals no-significant differences.

<table>
<thead>
<tr>
<th>ppm F:</th>
<th>0</th>
<th>15</th>
<th>0</th>
<th>15</th>
<th>15</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>weight (g):</td>
<td>107</td>
<td>107</td>
<td>132</td>
<td>139</td>
<td>129</td>
<td>131</td>
</tr>
</tbody>
</table>

Duncan's Multiple Range Test for Carious Lesion 'T'
(N/group = 12; a ≤0.05)

<table>
<thead>
<tr>
<th>control</th>
<th>water</th>
<th>7.0</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>milk</td>
<td>4.5</td>
<td>Y</td>
</tr>
<tr>
<td>milk</td>
<td>15 ppm F (NaF)</td>
<td>3.3</td>
<td>Y</td>
</tr>
<tr>
<td>milk</td>
<td>15 ppm F (Na₂SIF₆)</td>
<td>2.8</td>
<td>Y</td>
</tr>
<tr>
<td>milk</td>
<td>10 ppm F (Na₂MFP)</td>
<td>2.6</td>
<td>Y</td>
</tr>
<tr>
<td>milk</td>
<td>15 ppm F (NaF)</td>
<td>2.2</td>
<td>Z</td>
</tr>
</tbody>
</table>

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3.3 Fluoride accumulation in the dental hard tissues

3.3.1 Fluoride uptake in the enamel of experimental rats

Administration to rats of fluoride with milk has resulted in a significant increase in the fluoride concentration of enamel in treated animals, with Poulsen, Larsen & Larson (1976) reporting greater fluoride uptake in enamel from milk (357 ppm F) than from similarly dosed water (248 ppm F). Nonetheless, the increase varied considerably from approximately a 50%–200% enrichment, although there was evidence of high variation even amongst the milk and water control groups which received no fluoride. Similar low-uptake findings were reported by Stösser et al. (1995).

In non-F-treated animals, surface enamel concentrations ranged from 42 to 76 ppm F, while, for fluoridated rats, uptake varied slightly with dosages of 5, 10 and 15 ppm F via NaF. The different dissociation of the fluoride compounds used also proved important, significantly less fluoride being incorporated from sodium monofluorophosphate than from either sodium fluoride, or sodium silicofluoride which hydrolyses in aqueous solution. The calculated F-uptake of enamel from drinking fluids, also reported by Shchori et al. (1976) had no quantitative correlation with any caries-reduction observed, and is probably indicative only of its incorporation during the remineralization process.

3.3.2 Fluoride uptake from fluoridated milk and water into the developing enamel and dentine of sheep incisors

At present, the most commonly accepted explanation for the caries-preventive action of fluoride is a raised level of fluoride at, or in, the surface of tooth enamel, particularly when porous due to early caries attack. An anti-cariogenic role for fluoride, incorporated as fluorapatite into the tooth substance during tooth formation, remains a possible alternative or complementary mechanism. If so, regular ingestion of fluoride during tooth formation would be beneficial.

Fluoride forms stable molecules with a number of cations, in particular calcium, as well as complexing with proteins and other components in milk. The mix of ionized calcium and fluoride has long been considered undesirable, i.e., such as occurs when milk is supplemented with fluoride and, as a result, fluoride's biological availability has been questioned previously (vide supra). However, the level of "complexing" depends on the levels of fluoride added, the higher
above the "optimal", the more fluoride is complexed, this chemical problem being discussed extensively in Chapter 2. None the less it seemed worthwhile to attempt to determine the concentrations and actual site(s) of fluoride deposition within the dental hard tissues.

The aim of this study was, therefore, to compare the uptake of fluoride ingested in milk or water, into the developing incisor teeth of sheep. Here, 25 sheep, aged approximately 6 months, were randomized into five groups and farmed under identical conditions. Over 22 weeks, they were orally dosed, daily, with fluoride (as NaF) in 20 ml water or bovine milk. Daily doses were divided as follows:

Group 1 received 15 mg F in 20 ml milk (HF - M).
Group 2 received 6 mg F in 20 ml milk (LF - M).
Group 3 received 15 mg F in 20 ml distilled water (HF - W).
Group 4 received 6 mg F in 20 ml distilled water (LF - W).
Group 5 (control) received no supplement.

Intra-muscular tetracycline was given as a marker to identify the start of fluoride dosing. After sacrificing, incisors were removed, sectioned and analysed for fluoride and calcium, by multi-proton microprobe scans from the enamel surface to pulp (Figure 3.5).

The fluoride concentrations across the enamel and dentine are summarized in Figure 3.6. The distribution of fluoride in the mineralized tissues is typically characterized by a high concentration at the outermost enamel layer, i.e. approximately 900–1400 ppm. Away from the surface, fluoride levels drop abruptly to about 100 ppm until the dentine is entered. However, control surface enamel peak levels were lower, and have a narrower base than those in teeth formed with fluoride. In dentine, fluoride levels are raised to about 300 ppm, but with significant peaking which becomes a marked trend as the pulp region is approached. At the dentine/pulp surface, fluoride levels were found to correspond to those in surface enamel.

Scans assessed at similar distances from the tetracycline line were averaged (+/− SD) for surface, enamel and deep dentine for each group. The influence of ingested fluoride on the distribution of fluoride in the tooth tissues was very evident. With the exception of deep enamel in the incisally-located scan (Scan 1), all teeth of sheep which received fluoride showed raised fluoride levels compared with control subjects. The relative magnitude of fluoride levels in teeth was Controls <LF-M, <LF-W, <HF-M, <HF-W. Statistical analyses (ANOVA) confirmed that no significant differences existed between LF-M or -W; or be-
tween HF-M or -W; and that both HF- and LF- M and -W were much higher than in the control teeth (Cutress et al. 1995).

With respect to the fluoride levels attained in milk, water and milk aliquots from weekly preparations of the dose regime, prepared from respective stock solutions, were analysed for free fluoride ion levels immediately following preparation, and on return of residual supplies one week later. Ionized fluoride levels (Table 3.2) showed a consistent pattern. Fluoride in water samples were identical to calculated levels of dissolved NaF whereas in milk, only a small proportion (depending on the amount) of the dissolved fluoride was in the ionized phase. Proportionately lower ionized fluoride was identified with
Figure 3.6 Fluoride deposition in developing sheep teeth following ingestion of fluoridated milk, or fluoridated water, or no fluoride supplement. Fluoride concentrations (PPM) are shown for surface enamel, deep enamel, and dentine, as means and standard deviations.

Experimental groups:
- High fluoride in milk
- High fluoride in water
- Low fluoride in milk
- Low fluoride in water

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Table 3.2 Mean (SD) concentrations of ionized F in water and milk, over the dosing period, compared with total added F

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>F by electrode [F ± SD] ppm</th>
<th>F, theoretical [F] ppm</th>
<th>% Ionized</th>
</tr>
</thead>
<tbody>
<tr>
<td>High F-water (HF-W)</td>
<td>16</td>
<td>741.2 ± 5.0</td>
<td>750</td>
<td>98.8</td>
</tr>
<tr>
<td>High-F milk (HF-M)</td>
<td>16</td>
<td>154.3 ± 15.6</td>
<td>750</td>
<td>20.5</td>
</tr>
<tr>
<td>Low F-water (LFW)</td>
<td>16</td>
<td>298.0 ± 2.4</td>
<td>300</td>
<td>99.3</td>
</tr>
<tr>
<td>Low F-milk (LFM)</td>
<td>16</td>
<td>101.0 ± 10.0</td>
<td>300</td>
<td>33.7</td>
</tr>
</tbody>
</table>

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higher added fluoride levels. No change in ionized levels of fluoride in milk occurred over a one week period.

Evaluating the results, it is a recognised fact that the sheep is a suitable animal model for studying the deposition and distribution of fluoride in tooth tissues. Experimental procedures have been reported in previous studies (Suckling, Thurley & Nelson, 1988; Nelson et al. 1989; Richards, Coote & Pearce, 1994), and from a recent experiment (Suckling et al, 1995) on the association between fluoride uptake and stages of amelogenesis in developing sheep incisors, evolved the experimental design used in the present study.

Hence, by using the sheep model and proton microprobe analyses, it was shown that, irrespective of the interaction of fluoride when mixed with milk, fluoride is absorbed and becomes deposited into developing tooth tissues at a level which approximates to that via fluoride in water.

Complexing of fluoride with milk components is pronounced (70–80%), with only a small proportion being detected by electrode. The nature of the complex and the transformations that occur to facilitate absorption, transportation and deposition as fluorapatite, are not known. The stability of the form (ionized or otherwise) of fluoride added to milk was constant over the seven-day period of use, and supports the findings of Phillips (1991). Fluoride analysis at the time of addition, and at the end of seven days, showed unchanged fluoride ion levels.

The above study investigated directly the question of ingestion and deposition of fluoride into developing tooth tissues. It was not concerned with the question of fluoride's action and if "bound" fluoride becomes available and absorbable during digestion, the original ionization levels of fluoride have no particular relevance.

Several other reports also concluded that fluoride added to milk, despite low ionization, becomes biologically available following its ingestion. Ionic fluoride levels in blood and amniotic fluid were found similar irrespective of the carrier source, i.e. milk or NaF tablets (Brambilla et al. 1996). While Duff (1981) concluded that milk was not a suitable carrier for fluoride because of the strong interaction between the fluoride ion and milk components, Phillips (1991) showed that availability of ionized fluoride is high (>80%) at low fluoride concentration (5 ppm). Identifying fluoride deposition into enamel and dentine gives a definitive answer on fluoride absorption and trans-
port to the target tissues, although other reports do not support this finding. Hence, while it is not easy to reconcile the contradictory findings of Phillips (1991) and Duff (1981), it is clear from the present study, and that of Ericsson (1958) and Brambilla et al. (1996), that the ionization levels in milk are not relevant to fluoride absorption. From a systemic point-of-view, therefore, milk can be considered an appropriate, convenient and effective carrier means for fluoride intake of children, where water and other sources of fluoride are inadequate. None the less, direct extrapolation of the above sheep-derived findings to humans requires caution because of the obvious differences in diet and digestion between these species.

3.4 Human studies

3.4.1 Fluoride in human maternal milk

The mean fluoride content of mature human milk is 16 μg/litre, reported values ranging from 5 to 25 μg/litre, these reflecting maternal intake (Esala & Vuori, 1982; Spak, Hardell & DeChateau, 1983; Flynn, 1992). Following ingestion of high fluoride doses, an elevation in the plasma fluoride level occurs. However, the milk fluoride level in nursing mothers’ breast-milk has been found to be only moderately increased (Ekstrand et al. 1984; Opinya et al. 1990; Simpson & Tuba, 1968). Hence, due to self-regulatory mechanisms, breast-fed infants receive less than an optimal fluoride dose from their mothers (Ericsson, 1960), and Flynn (1992) recommended such infants might receive fluoride supplements.

3.4.2 Factors influencing the bioavailability of fluoride from milk in humans

The influence of gastric contents on the bioavailability of F⁻ from milk was first investigated by Konikoff (1974), results indicating that none, or very little, of the F⁻ binds to the food constituent. However, for the consumption of fresh fluoridated milk to be effective, this has to occur within four hours of preparation. Several other studies, including investigations of plasma fluoride levels and urine analyses, reported that the absorption of F⁻ given with milk is reduced by 13–30% depending largely on the gastric content, its acidity and sequence of consumption of milk, fluorides and other foods (Ekstrand & Ehrenebo, 1979; Trautner & Einweg, 1989; Schulman & Vallejo, 1990). Comparing in vivo the bioavailability of fluoride added to infant formula feeds and milk, Spak, Ekstrand & Zylberstein (1982) showed 72% absorp-
tion of all F\(^-\) from the milk, but only 65% from water-diluted baby formulae.

3.4.3 Fluoride accumulation in dental plaque

The ionizable fluoride content of dental plaque has been found to be elevated significantly after eight weeks' fluoridated milk consumption (Kertész, Gombik & Bánóczy, 1992).

The influence of F\(^-\) milk on plaque pH changes was investigated by Jenkins & Ferguson (1966), who reported less pH decrease than occurred with glucose, lactose, fruit juices or sweetened beverages (Frostell, 1970). While other intra-oral methods ranked milk as less acidogenic (Mor & McDougall, 1977), after an adaptation period, more pronounced plaque pH decreases were noted to occur by Birkhed, Imlerd & Edwardsson (1993). Furthermore, human milk caused a greater fall in plaque pH than did bovine milk (Rugg-Gunn, Roberts & Wright, 1985), and the addition of 4 ppm F\(^-\) to cow's milk was found to have no appreciable effect on plaque or sediment pH changes (Mor & McDougall, 1977).

3.4.4 Enamel biopsy studies in children consuming fluoridated milk

In order to assess possible cariostatic mechanisms resulting from fluoridated milk consumption, changes in the acid-solubility and fluoride content of dental enamel have been determined, mainly by using enamel biopsy methods.

Few data exist on the fluoride content of enamel surfaces following fluoridated milk consumption. According to in vitro studies, the fluoride content of extracted human teeth, and of enamel and dentine ash of deciduous teeth, increased on exposure to fluoridated milk, pointing to F\(^-\) incorporation into these tissues (Light et al. 1968; Konikoff, 1977).

Zahlaka et al. (1987), performing labial enamel biopsies on upper permanent central incisors of children after 30 months' fluoridated milk consumption, found no difference in the mean F\(^-\) content (2500 ppm) between test and control groups, in spite of clinical benefit accruing.

However, these workers presumed the milk F\(^-\) supported early lesion remineralization, in spite of a lack of its incorporation into sound enamel.
In connection with the Hungarian milk fluoridation study (Bánoczy et al. 1985), clinical details of which are reported elsewhere in this text, a horizontal comparative and longitudinal follow-up enamel biopsy study was undertaken on subjects in the programme.

The aim of the first study (Tóth et al., 1987) was to establish in vivo whether there was any difference in the F\(^{-}\) content and acid dissolution rate of enamel in the upper incisors of children who had consumed fluoridated milk for five years, as compared to respective data of those who had received non-fluoridated milk. Here, members of two closed child communities aged 8–10 years were examined, the experimental group being composed of 41 boys and girls, while the control had 47 subjects. In these children, tooth 11 was examined by the acid-etch enamel biopsy method of Hotz et al. (1970).

The fluoridated milk group results showed a significantly higher F\(^{-}\) content (P<0.01) than did those of the control group, and the amount of dissolved calcium was lower in the experimental group, but not significantly so (Table 3.3). Thus the higher F\(^{-}\) concentration in teeth of children who consumed fluoridated milk for five years was in accord with the clinical caries reductions achieved.

As the literature contained no guidance as to the time-period necessary for the decrease in acid-solubility, and increase of the fluoride content of enamel after continuous consumption of fluoridated milk, a second, longitudinal enamel biopsy study was undertaken to as-

### Table 3.3 Enamel biopsy studies on Tooth 11 after five years' consumption of fluoridated milk

<table>
<thead>
<tr>
<th>(a) Fluoride content</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>N</strong></td>
<td><strong>ng F(^{-})/7.1 mm(^2)</strong></td>
<td><strong>S.D.</strong></td>
</tr>
<tr>
<td>F-milk</td>
<td>41</td>
<td>12.54</td>
<td>±0.76</td>
</tr>
<tr>
<td>Control</td>
<td>47</td>
<td>7.30</td>
<td>±0.36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(b) Calcium content</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>N</strong></td>
<td><strong>µg Ca/7.1 mm(^2)</strong></td>
<td><strong>S.D.</strong></td>
</tr>
<tr>
<td>F-milk</td>
<td>41</td>
<td>4.57</td>
<td>±0.51</td>
</tr>
<tr>
<td>Control</td>
<td>47</td>
<td>5.60</td>
<td>±0.53</td>
</tr>
</tbody>
</table>

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sessed any change six months or one year after the beginning of F⁻ milk consumption (Tóth et al. 1989).

The investigations were carried out on 79 children aged 9–10 years living in the same community. The results showed that the amount of dissolved phosphorus became significantly lower ($P<0.05$) after only six months, and a further significant decrease ($P<0.01$) was observed by the end of the one-year follow-up period. The fluoride content of the enamel biopsy samples showed an increase, but only became statistically significant ($P<0.01$) by the end of the first year (Table 3.4).

### Table 3.4 Milk fluoridation and changes in enamel surface fluoride content in relation to length of fluoride consumption period

<table>
<thead>
<tr>
<th>Time</th>
<th>$N$</th>
<th>μg F⁻ / 7.1 mm²</th>
<th>S.D.</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>79</td>
<td>19.37</td>
<td>±0.85</td>
<td>n.s.</td>
</tr>
<tr>
<td>6 months</td>
<td>79</td>
<td>20.01</td>
<td>±1.00</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>12 months</td>
<td>79</td>
<td>25.08</td>
<td>±1.21</td>
<td>$&lt;0.01$</td>
</tr>
</tbody>
</table>

**Phosphorus content of enamel biopsy samples**

<table>
<thead>
<tr>
<th>Time</th>
<th>$N$</th>
<th>μg F⁻ / 7.1 mm²</th>
<th>S.D.</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>77</td>
<td>2.95</td>
<td>±0.14</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>6 months</td>
<td>77</td>
<td>2.47</td>
<td>±0.13</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>12 months</td>
<td>77</td>
<td>1.92</td>
<td>±0.07</td>
<td>$&lt;0.01$</td>
</tr>
</tbody>
</table>

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None the less the changes observed, mainly from the longitudinal study, indicate the importance of fluoride's topical benefits on erupted permanent teeth.

### 3.4.5 Fluoride levels in amniotic fluid and blood of pregnant women resulting from fluoridated milk and tablet consumption

There have been a number of reports which suggest that pre-natal exposure to fluoride leads to improvement in the dental health of the child. Clinical studies which address the question of pre-natal fluoroprophylaxis efficacy have been conducted by Glenn (1977), Glenn, Glenn & Duncan (1982), and Le Geros et al. (1985). The subject has also been covered in reviews by Stephen (1993) and Fassman
(1993), although both of these report that clinical and statistical evidence to support such benefit is inconclusive.

Caldera et al. (1988) and Forestier et al. (1990) have studied transplacental transfer of fluoride by measuring fluoride concentrations in maternal and foetal blood. The latter group investigated women who were 22 weeks into pregnancy at a time coinciding with the formation of embryonic dental enamel. Their investigation revealed that the concentration of fluoride in foetal blood was approximately 40% of that in maternal blood. In order to assess the pre-natal benefit of fluoride derived from fluoridated milk consumption, Brambilla et al. (1996) determined the efficiency of transplacental fluoride transfer. This was achieved by measuring F⁻ concentrations in maternal blood and amniotic fluid following ingestion of controlled amounts of fluoride, when taken as either fluoridated milk or sodium fluoride tablets. The concentration of fluoride in amniotic fluid was considered representative of foetal exposure.

Here, 126 pregnant women, all of whom were scheduled for routine amniocentesis, gave their consent to participate. Their ages ranged from 26 to 38 years and all were 13–17 weeks pregnant. In choosing participants, exclusion criteria employed included (i) those with any systemic disease and (ii) those consuming long-term drugs which were likely to interfere with the study. All subjects were instructed to abstain from the use of fluoridated dentifrice and systemic fluorides, other than those employed in the study, i.e. with the exception of domestic water (0.4 ± 0.22 ppm F⁻) consumption during the experimental period. The women were divided into four groups:

1. the Control Group (N=31) who received milk but no supplementary fluoride;

2. Test Group A (N=33) receiving 1 mg fluoride per day in 200 ml UHT milk (Dentamilk, Borrow Dental Milk Foundation, Portsmouth, U.K.);

3. Test Group B (N=33) receiving 2 mg fluoride per day in 400 ml (i.e. 2 x 200 ml packs, each with 1 mg F⁻) UHT milk (Dentamilk);

4. Test Group C (N=29) receiving 1 mg fluoride per day as NaF tablets (Zymafluor, Zyma, Spa Saronno, Italy).

After seven days, each woman provided samples of amniotic fluid and blood taken three hours after the last ingestion of milk, fluoridated milk or sodium fluoride tablets. The fluoride concentrations in amni-
otic fluid and plasma samples were measured using an ion selective electrode technique (SA 720 fitted with a 94-09 Fluoride electrode, Orion Research Inc., Boston, USA).

The results obtained regarding the fluoride concentrations observed in plasma and amniotic fluid in all four groups are shown in Table 3.5. Here, the fluoride concentrations in both fluids were elevated from an ingestion of fluoride, whether in the form of fluoridated milk or sodium fluoride tablets. However, the data indicated that the F⁻ concentrations achieved could be ranked as: fluoridated milk 2 mg F > NaF 1 mg tablet > fluoridated milk 1 mg F. The differences observed were statistically significant in every case (P<0.05). Thus it would seem that the F⁻ levels in plasma were dependent upon total intake, as well as the vehicle used for fluoride delivery.

**Table 3.5 Mean fluoride concentrations observed in blood sera and amniotic fluid in the four groups studied**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Blood µg/litre</th>
<th>Amniotic fluid µg/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test group A¹</td>
<td>33</td>
<td>25.82</td>
<td>12.67</td>
</tr>
<tr>
<td>Test group B²</td>
<td>33</td>
<td>46.01</td>
<td>16.14</td>
</tr>
<tr>
<td>Test group C³</td>
<td>29</td>
<td>35.69</td>
<td>14.81</td>
</tr>
<tr>
<td>Control group</td>
<td>31</td>
<td>14.62</td>
<td>7.10</td>
</tr>
</tbody>
</table>

1, 1 mg F in milk daily; 2, 2 mg F in milk daily; 3, 1 mg F tablet daily  
* P<0.05

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The fluoride concentrations in amniotic fluid in all three test groups were significantly higher than in the control group (P<0.05), but they were not statistically significantly different from each other. They were, however, some 50–65% lower than the F⁻ concentrations in the plasma of the corresponding test group.

As the fluoride concentration in amniotic fluid provides an assessment of foetal exposure, the conclusion drawn from the study was that daily consumption of fluoridated milk by pregnant women resulted in fluoride levels in the amniotic fluid sufficient to benefit the
oral health of their offspring to the same degree as that achieved by taking sodium fluoride tablets.

In practical terms, fluoridated milk would therefore seem to be an appropriate and effective vehicle for possible pre-natal fluoride supplementation.

3.5 Conclusion

To summarize, the caries-protective effect of milk or fluoridated milk on experimental animal caries under programmed feeding conditions, showed a moderate caries-preventive effect which did not depend on its fat content. Fluoride concentrations of 5–15 ppm F as CaF₂, NaF; Na₂-monofluorophosphate or Na₂-silicofluoride caused a significant caries reduction of 40–50% and did not depend on the compound or concentration of the fluoride used. The bioavailability of fluoride was not reduced by milk, and a low accumulation of fluoride was measured in the enamel. The composition of dental plaque was not influenced by the fluoride dosage, but milk probably effected an increase in Actinomyces. It was concluded that fluoridated milk keeps a permanently low level of ionized fluoride within the oral cavity, promoting remineralization. It is likely that this topical mechanism contributes to the caries-preventive effect of fluoridated milk. On the other hand, fluoride uptake from milk into the developing enamel and dentine of sheep incisors supports also a systemic effect, and points to the finding that ionization levels of fluoride in milk are not relevant to subsequent fluoride absorption and physiological availability. Finally, human fluoridated milk enamel biopsy and amniotic fluid studies have confirmed fluoride's dual mode of action i.e. topical and systemic.
4
Clinical studies
J. Bánóczy, K.W. Stephen, G.N. Pakhomov

4.1 Introduction

The beneficial effects of milk and milk supplements on physical status (and the teeth) of children were described in the early 1930’s (Roberts et al. 1931; Sprawson, 1932). The use of fluoridated milk as a possible dental caries prevention medium was first proposed by the Swiss paediatrician Ziegler in 1953. In a review, Ziegler (1956) described his method of fluoridating milk in Winterthur, by adding 1 ml of a 0.22% NaF (1000 ppm F⁻) solution to 1 litre of fresh milk. Based on his extensive research, he stated “the addition of fluoride in dosage of 1 mg F⁻ per litre (1 ppm F⁻) to milk, in cases where the fluoridation of drinking water is not possible, is justified on the grounds of physiological and toxicological considerations. The advantages of this mode of administration as compared with fluoride prophylaxis with tablets, salt or even with water, seem to be significant. The fluoridation of milk provides the best means of dosage for all age groups to meet the prophylactic requirements. The practical use in households and in large collective concerns, such as the school milk in Winterthur, is feasible.”

Epidemiological studies in Winterthur had shown a very high caries prevalence (Ziegler, 1959), which justified the introduction of fluoridated milk on a wider basis in 1958.

Since then, the caries-inhibiting characteristics of fluoridated milk have been investigated in several studies. Supporters of the methods claimed that fluoride added to milk does not alter its taste or other characteristics. Furthermore, it is absorbed well, although more slowly than from water (Ericsson, 1958). It has also been considered advantageous as the fluoride is added to a staple food for children and that its consumption, being voluntary, can be confined to those groups needing it most (Borrow & Davis, 1975; Rusoff, 1975). The slowly fermentable lactose is less cariogenic than sucrose (Rusoff, 1975; Birkhed et al., 1981), and the proteins and fats contained in milk may have a cariostatic effect (McBean & Speakman, 1974).
Some views have been expressed concerning the binding of ionic fluoride to milk in the form of insoluble milk/calcium or milk/protein, and Duff (1981) considered milk as an unsatisfactory fluoride carrier. However, such binding is significant only four or five hours after addition (Konikoff, 1974; Duff, 1981). Therefore, it was argued that fluoridated milk, consumed more-or-less immediately (i.e. within 30 min of mixing) might be clinically effective.

More recent studies, detailed in Chapter 3, have shown that the availability of fluoride is high in fluoridated milk. In the most commonly used pasteurized milk, virtually all added fluoride remains available throughout its shelf-life, extending over several days when stored below 6 °C. However, ultra-heat-treated (UHT) fluoridated milk does suffer some loss of fluoride availability during processing and subsequent storage at ambient temperatures over the six month shelf-life.

In the first clinical trial, Imamura (1959) in Yokohama, Japan, added 2–2.5 mg NaF to milk or soup served daily with school meals. After 4 years, a 36.3% caries reduction was observed in the permanent teeth of the fluoride group, which consisted of 167 11-year-olds who consumed the above dose of F⁻ in milk for 150–180 days a year.

In the USA, Rusoff et al. (1962) enrolled 65 children, aged 6–9 years at outset, in a 3½ year study where milk containing 3.5 ppm F⁻ was provided daily at school. The overall caries reduction achieved was 35%, but for subjects aged 6 years at the beginning of the investigation, there was a 78% difference between the test and control groups. Wirz (1964) and Ziegler (1964) published reports on a large experiment in Winterthur, Switzerland. Here, they distributed milk containing 1 ppm F⁻ to 749 test children, using 553 others as controls, all of whom were aged between 9–44 months at the beginning of the trial. The caries reduction after 6 years ranged from 14.6% to 31.5% in primary teeth, and from 64.2% to 65.2% in permanent molars.

A major change in interest and promotion of the widespread use 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 with the above purpose. 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 objective via 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 implementa-
tion by grants, equipment, lectures, scientific papers and every pos-
sible means”.

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 then support clinical schemes, there seemed to be justification for further investigation of this means of providing community-based fluoridation for children.

4.2 Scotland

Although 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), in Glasgow in 1976, it was deemed worthwhile to assess the potential caries-inhibiting benefits of daily fluoridated school milk in a pilot study which employed adequate pre-
trial stratification and double-blind methodology.

In addition, one aspect of human metabolism which had received litt-
le consideration in any previous fluoride study was the possibility
that an increased fluoride intake might have had an adverse effect on
skeletal mineralization in a vitamin-D deficient area. This suspicion
was based on the fact that when fluoride is used in pharmacological
doses for management of osteoporosis then, even in vitamin-D re-
plete areas such as the USA, a 10 to 20-fold increase in daily fluoride
intake has to be matched by around a 100-fold increase in vitamin-D
intake to prevent the appearance of significant quantities of
unmineralized bone matrix (Jowsey et al., 1972). By virtue of its geog-
raphy (latitude 56°N), Glasgow is a relatively vitamin-D deficient area
since it is well-recognized that unless vitamin supplements are taken,
dietary sources of this so-called vitamin are trivial, and almost all vi-
tamin-D is synthesized in the skin by the action of ultra-violet light
on 7-dehydrocholesterol (Boyle, 1980). In the West of Scotland, how-
ever, latitude determines that virtually no radiation of wavelength less
than 300 nm reaches the earth’s surface during winter and even fit,
mobile and well-nourished laboratory staff in this area have a range
of serum 25-hydroxy vitamin-D levels (the principal circulating form
of vitamin-D), of only 5 to 24 ng/ml in early spring (Tezic, 1980). Pa-
tients with osteomalacia usually have values below 5 ng/ml (Mawer,
1980). Thus the Glasgow population has little margin for tolerating
any further environmental influences which might adversely affect bone mineralization.

Conventional investigations used to detect the presence of impaired mineralization or rickets in children include skeletal X-rays, blood analysis for calcium, inorganic phosphorus, alkaline phosphatase, parathyroid hormone, vitamin-D metabolites and, where indicated, bone biopsy. While such invasive procedures were deemed impractical for a school-based investigation, it was felt that some indirect indication of impaired mineralization or anti-vitamin D effect might be demonstrated by either a rise in the fasting urine hydroxyproline level, or a fall in the fasting urine calcium level respectively, the former value increasing in rickets as a result of increased bone collagen turnover.

4.2.1 Materials and methods

In view of the follow-up problems which were encountered in a previous fluoride tablet clinical pilot study (Stephen & Campbell, 1978), where, due to very close age and social class stratification, children who had been distributed through 24 schools at baseline were eventually placed in 38 schools three years later, it was decided to offer fluoridated milk trial participation to all children in first grade classes at only 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 (Sutherland & Stephen, 1973; Todd & Whitworth, 1974).

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. Here, information was given regarding the trial format and the need for an annual set of bitewing radiographs, these to be made available to general dental practitioners for later treatment purposes if required. As a result of a letter of explanation, parental permission was obtained for 187 subjects, 83 per cent of those eligible.
In addition to the local and education authority committees mentioned above, officials from the Department of Agriculture and Fisheries for Scotland had to be informed as milk (a "food") was being used as the vehicle, and permission was also sought from the Department of the Environment (Glasgow District). Furthermore, the offices of the Chief Medical and Chief Dental Officers of the Scottish Home and Health Department were advised of the project in advance.

As milk was to be distributed to these 4½/5½-year-olds 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 and colour data were 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 (Dr J.G. Davis & Partners, Reading, RG1 5AS, U.K.), the contents of one container being added to 5 gallons of milk daily, giving a final concentration of approximately 7 ppm F⁻ per 200 ml test pack.

Milk distribution and all clinical examinations were on a double-blind basis and, for the former, a grant-funded milk distributor was employed to ensure minimum classroom disruption as the necessary daily documentation required for the study would have involved teachers in additional classroom duties. The milk distributor was also 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. Baseline clinical and radiographic examinations were performed in the University of Glasgow Dental School's Mobile Research Unit (Stephen, 1969). Here, methods identical to those described by Stephen & Campbell (1978) were used, and radiographs read separately from the clinical data. Thereafter, subjects were graded into three groups, then divided equally between "test" and "control" according to baseline mean modified dmft scores of 0–4, 5–8 or 9–12 (Stephen et al, 1976). In addition, within the 4½ to 5½ year age band, children were subdivided into four groups by 3-month wide spans, these then being allocated equally between test and control. The initial and repeat clinical examinations were carried out annually by the one examiner (K.W.S.) whose repeat caries penetration examination variations had been shown to be insignificant (P>0.9; Bennie et al, 1978).
To comply with the requirements of the U.K. Department of the Environment, fluoride monitoring, using an Orion specific ion electrode (EIL, Chertsey, KT16 9LF, U.K.) was carried out daily at the dairy, and on a random basis by that Department. In addition, a weekly analysis was also performed at the University Department of Oral Medicine, 96.4 per cent of samples tested being within the desired range.

Collected clinical and radiographic information was coded for computation by SPSS package, statistical analyses being completed using the Wilcoxon Rank Sum test (Armitage, 1977).

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.

Urine was assayed for calcium by atomic absorption spectrometry; for inorganic phosphorus by a phosphomolybdate method based on that of Fiske & Subarow (1925); and for creatinine by a method based on that of Hare (1950). Hydroxyproline was estimated using a Hypronostican kit (Organon Technica, St. Neots, Huntingdon, U.K.), and fluoride measured by Orion specific ion electrode. Statistical comparison of the means employed the Student ‘t’ test.

4.2.2 Clinical 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 (6.2%) 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.

As reported previously by Stephen et al (1984), the data in Table 4.1 show the number of participants in both groups after each of the five annual re-examinations, there being 50 in the test group and 56 in the control group at the fifth inspection. By that time, 20 test and 19 control subjects had moved from schools involved in the project, but 24 of those remaining in the former group (32.4%) and 18 of the latter (24.3%) had decided they did not wish to continue with school milk by the age of 10. This Table also indicates the mean milk consump-
tion, in terms of percentage provided each year, there being no difference between the quantities taken by either cohort.

Table 4.1 Mean consumption (expressed as a percentage of total milk provided) for those test and control children available for re-examination after 1, 2, 3, 4 and 5 years of the study

<table>
<thead>
<tr>
<th></th>
<th>Test N</th>
<th>% consumption</th>
<th>Control N</th>
<th>% consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976–77</td>
<td>82</td>
<td>91%</td>
<td>83</td>
<td>92%</td>
</tr>
<tr>
<td>1977–78</td>
<td>80</td>
<td>93%</td>
<td>78</td>
<td>93%</td>
</tr>
<tr>
<td>1978–79</td>
<td>72</td>
<td>92%</td>
<td>71</td>
<td>93%</td>
</tr>
<tr>
<td>1979–80</td>
<td>49</td>
<td>95%</td>
<td>59</td>
<td>95%</td>
</tr>
<tr>
<td>1980–81</td>
<td>50</td>
<td>91%</td>
<td>56</td>
<td>93%</td>
</tr>
</tbody>
</table>

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Details of the primary caries status in terms of the modified dmft and dmfs indices after the 1976 baseline stratification had been completed, and at the three annual re-examinations which followed, are given in Table 4.2. No significant differences were found between the test and control data at any of these times.

Table 4.2 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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>C</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>ð dmft</td>
<td>4.3</td>
<td>4.5</td>
<td>5.4</td>
<td>5.2</td>
</tr>
<tr>
<td>ð dmfs</td>
<td>12.4</td>
<td>12.1</td>
<td>17.6</td>
<td>16.3</td>
</tr>
</tbody>
</table>

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The DMFT and DMFS values relating to all erupted teeth, along with their respective differential data, are shown in Table 4.3. Thus, while both groups started with 1976 baseline DMFT and DMFS scores of zero, only after 4 years of milk consumption did results differ significantly, when the mean DMFT of the test group was 1.65 (median 1.36) compared to a 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
### Table 4.3 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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>C</td>
<td>Δ%</td>
<td>T</td>
<td>C</td>
<td>Δ%</td>
</tr>
<tr>
<td>n (teeth)</td>
<td>23</td>
<td>23</td>
<td>304</td>
<td>278</td>
<td>716</td>
<td>687</td>
</tr>
<tr>
<td>DMFT</td>
<td>0</td>
<td>0.33</td>
<td>0.35</td>
<td>0.79</td>
<td>1.15</td>
<td>1.54</td>
</tr>
<tr>
<td>DMFS</td>
<td>0.35</td>
<td>0.36</td>
<td>2.8</td>
<td>1.40</td>
<td>1.56</td>
<td>11.4</td>
</tr>
</tbody>
</table>

*P<0.05; ***P<0.01

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### Table 4.4 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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>C</td>
<td>Δ%</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>n (teeth)</td>
<td>284</td>
<td>266</td>
<td>702</td>
<td>671</td>
<td>224</td>
</tr>
<tr>
<td>DMFT</td>
<td>0.40</td>
<td>0.43</td>
<td>7.0</td>
<td>0.69</td>
<td>1.04</td>
</tr>
<tr>
<td>DMFS</td>
<td>0.43</td>
<td>0.45</td>
<td>4.4</td>
<td>1.09</td>
<td>1.26</td>
</tr>
</tbody>
</table>

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

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fifth year the mean DMFT of the fluoridated group had risen to 2.14 (median 2.20) compared to a rise of 3.11 (median 3.17) in the non-fluoridated, 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 4.4 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 fluoridated DMFS index was 3.29 (median 2.1) compared to the mean surface score of 6.33 (median 5.0) for non-fluoridated, a difference of 48.0% (P<0.01).

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

After two years, 57 of the 80 children still taking the fluoridated milk provided urine samples together with 53 of the 78 remaining in the control group, giving compliance rates of 71% and 69% respectively. In order to correct for any small differences in renal function the concentrations of calcium, hydroxyproline, phosphate and fluoride were all evaluated in Mm/l and then expressed as a Mm ratio to the creatinine concentration. The results, reported by Stephen et al. (1984), are shown in Table 4.5, where it can be seen there is a wide scatter of data and no significant difference between the means for the two groups with respect to the calcium/creatinine, inorganic phosphate/creatinine or hydroxyproline/creatinine ratios. Not surprisingly, the mean for the fluoride/creatinine ratio was significantly higher for the group taking fluoridated milk.

Table 4.5 Mean ratios obtained from urine analyses after 2 years' participation in the fluoridated milk study. Data are expressed as mM ratios ±1 SD

<table>
<thead>
<tr>
<th>Number of urine samples</th>
<th>Calcium/creatinine ratio</th>
<th>Phosphate/creatinine ratio</th>
<th>Hydroxyproline/creatinine ratio</th>
<th>Fluoride/creatinine ratio (x 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test group</td>
<td>57</td>
<td>0.28 ±0.20</td>
<td>3.50 ±1.14</td>
<td>0.12 ±0.02</td>
</tr>
<tr>
<td>Control group</td>
<td>53</td>
<td>0.33 ±0.28</td>
<td>3.39 ±1.05</td>
<td>0.13 ±0.09</td>
</tr>
<tr>
<td>Difference between means</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

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4.2.4 Discussion

After five years, this double-blind clinical study resulted in 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 4.3, 46 of these teeth had appeared in the mouths of these 4½–5½-yr-olds when they were seen originally. 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 4.4). Prior to the fourth year, earlier DMFT reductions, while being in favour of fluoridated, rather than non-fluoridated subjects, did not attain statistical significance.
However, although the percentage DMFT difference between mean values in 1978 appeared large (31.3%), the actual difference was unchanged compared to that found in 1979 (0.36), despite the mean test score doubling. With respect to DMFS indices, the differences between test and control were small. In addition, there was little, if any, benefit for primary teeth in terms of mean dmft or dmfs values between test and control subjects from 1976–79 (Table 4.2).

As stated, this trial sought to interfere as little as possible with normal school milk distribution and ingestion routines. Children therefore sucked the test or control milk through straws, although drinking and rinsing may well have given increased topical benefit, and the only procedural interference was a request to teachers that milk should be distributed to children at least 15 min before the mid-morning teaching interval to avoid a potential wash-out effect of other food or drink shortly thereafter. Little classroom disruption apparently occurred, and throughout the study no school expressed a desire to have a class withdrawn from the scheme on the grounds of inconvenience.

The greatest movement took place in the fourth year when 23 subjects left in the test group and 12 departed from the control. However, not all were milk abstainers. 37 children moving to schools which were not participating in the project over the five years. In 1981, one child who was absent during the three days set aside for the clinical re-examination in 1980 was re-examined, hence the increase of one subject in the 1981 test group. Unfortunately, due to distribution problems, it was not possible to deliver milk to a child who moved away from these four schools. None the less, as these children grew older they showed a decreased interest in taking milk, at least outside the home.

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 ($\pm SD 3.18$), while that for the controls was 3.47 ($\pm SD 2.87$), a non-significant difference. Likewise, the mean test dmfs value of 13.02 ($\pm SD 12.0$) was again higher than that of the controls (9.58$\pm SD 10.2$), but not significantly so. Thus, both primary indices indicate that the inclusion of these subjects throughout the whole study would not have altered the result obtained.
In the associated laboratory investigations, the indirect methods used for detection of sub-clinical rickets failed to substantiate any grounds for concern that the daily addition of 1.5 mg fluoride to school children's diet in the West of Scotland might promote a resurgence of rickets, and parallel studies using vitamin-D deficient rats failed to demonstrate an effect of fluoride on the intermediary metabolism of vitamin-D. However, most screening for rickets in large populations is done on the basis of radiological changes or serum biochemistry, in particular, alkaline phosphatase (Cooke et al, 1973), and local experience at Glasgow Royal Infirmary had suggested that the relevant abnormalities in teenage children may occur in as many as 20% of cases (Gardner, 1980), with an even higher prevalence in the children of Asian immigrants. In these studies, however, calcium and hydroxyproline excretion were not measured so that a direct comparison could not be made with the subjects in the present series.

In comparison to the previous fluoridated milk studies referred to earlier, Rusoff et al (1962) had only 16 children who were originally 6 years old at the outset remaining in the test and control groups after 3½ years of their trial, where subjects consumed 285 ml milk providing 3.5 ppm F⁻ per school day. However, during the vacation periods, parents of those in the test group were supplied with a sodium fluoride solution to allow continued fluoride dosage of milk at home. Here, for these few subjects, a 76% DMFT reduction was claimed. Ziegler's 1964 study, and that of Wirz (1964), gave permanent first molar caries reductions of 14.8%–31.4% by 6 years of age for children who began consuming milk with 1 ppm F⁻ between the ages of 9 and 44 months.

In the Yokohama trial of Imamura (1959), 2/2.5 mg NaF was added to milk or soup provided at school. Here the overall caries reduction after five years was 33.8% in favour of the F⁻ group for those who began school in 1952, while for the 1953 intake there was a 28.8% reduction. When only permanent first molars were considered, the decrease ranged from 14.4% to 19.6% after the same time-period.

Compiled in Table 4.6 are data from several water fluoridation studies which commenced when children were 5 years of age, and it will be seen that after 3, 4 and 5 years of water fluoridation, the DMFT reductions quoted were not dissimilar to those found in this study, that of Imamura (1959) and Ziegler (1964). There would thus appear to be a similar topical benefit provided by the fluoride ion whether it is delivered via a public water supply throughout 365 days of the year, or in school milk for approximately 200 days per annum. However, as
greater advantage was obtained by those teeth which erupted during the study, further benefit might accrue should fluoridated milk consumption (with or without vitamin D supplementation) commence earlier in life than proved possible in this purely primary school-based investigation.

Finally, with regard to the vitamin-D deficient problems of areas like the West of Scotland, it would seem that a balance between fluoride and vitamin-D ingestion may only be important at daily intakes of fluoride above 10 mg.

**Table 4.6 Permanent tooth DMF reductions obtained (DMFT Δ%↓) in various artificial fluoridation studies after 3, 4 and 5 years of water adjustment where children were aged 5 years at onset of fluoridation. Present 3, 4 and 5 year milk data are shown for comparison.**

<table>
<thead>
<tr>
<th>Location (ref #)</th>
<th>Water F− duration</th>
<th>DMFT Δ%↓</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hastings (NZ) (1)</td>
<td>3 yr</td>
<td>17.7</td>
</tr>
<tr>
<td>Newburgh (USA) (2)</td>
<td>3 yr</td>
<td>27.6</td>
</tr>
<tr>
<td>Evanston (USA) (3)</td>
<td>3 yr</td>
<td>38.6</td>
</tr>
<tr>
<td>Grand Rapids (USA) (4)</td>
<td>3 yr</td>
<td>26.5</td>
</tr>
<tr>
<td>Grand Rapids (USA) (4)</td>
<td>4 yr</td>
<td>36.4</td>
</tr>
<tr>
<td>Cork (Ireland) (5)</td>
<td>4 yr</td>
<td>28.4</td>
</tr>
<tr>
<td>Grand Rapids (USA) (4)</td>
<td>5 yr</td>
<td>27.7</td>
</tr>
<tr>
<td>Newburgh (USA) (6)</td>
<td>5 yr</td>
<td>31.0</td>
</tr>
<tr>
<td>Athens (USA) (7)</td>
<td>5 yr</td>
<td>32.6</td>
</tr>
<tr>
<td>Newark (USA) (8)</td>
<td>5 yr</td>
<td>30.6</td>
</tr>
<tr>
<td>Milk (present data)</td>
<td>3 yr</td>
<td>18.4</td>
</tr>
<tr>
<td>Milk (present data)</td>
<td>4 yr</td>
<td>33.8</td>
</tr>
<tr>
<td>Milk (present data)</td>
<td>5 yr</td>
<td>35.5</td>
</tr>
</tbody>
</table>


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### 4.3 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 at-
tended 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.

4.3.1 Materials and methods

Prior to commencement of milk fluoridation, the F\(^{-}\) content of the Fót drinking water was determined via an ion selective electrode (Radelkis-type 108) 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, 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 daily 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 (Bánhóczy et al, 1981). 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 criteria (1977) and recorded on forms suitable for computer evaluation. No radiographic investigations were performed, and dmft, dmf\(_\text{s}\), 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.

4.3.2 Results

After three years, data for 936 children were analysed, although for follow-up purposes only 269 were available, 112 of these having participated continuously over that timescale, with 157 being involved for two years, at which time their ages were between 5–9 years. At five years, 165 subjects aged 7–12 years were contactable. Of these, 72 had been involved continuously, while 93 had participated for four years. By ten years, 162 institutionalized youngsters aged 7–14 years were available for follow-up analysis.

The number and ratio of caries-free children at the five year evaluation is shown in Table 4.7. In the group then 7–10 years of age (T.), only a small difference in the ratio of children with caries-free primary teeth was noted. However, a clinically, and statistically highly significant (P<0.001) difference in the ratio of subjects with caries-

---

**Table 4.7 Number and ratio of caries-free children in the test (T) and control (C) groups after 4 (T₁/C₁) and 5 (T₂/C₂) years of milk fluoridation**

<table>
<thead>
<tr>
<th>Caries-free children</th>
<th>Age (years)</th>
<th>At examination</th>
<th>At start of milk F⁻</th>
<th>Total number of children</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>9–12</td>
<td>5–8</td>
<td>83</td>
<td>—</td>
<td>8</td>
<td>9.5</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>C₁</td>
<td>9–11</td>
<td>—</td>
<td>64</td>
<td>—</td>
<td>1</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₂</td>
<td>7–10</td>
<td>2–5</td>
<td>69</td>
<td>20</td>
<td>59.4</td>
<td>41</td>
<td>P&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>C₂</td>
<td>7–10</td>
<td>—</td>
<td>81</td>
<td>17</td>
<td>20.9</td>
<td>14</td>
<td>17.2</td>
<td></td>
</tr>
</tbody>
</table>

N.S., not significant

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free permanent teeth was observed, as compared to the control. None the less, no differences were seen in those aged 9–12 years (T₁), who had been drinking milk for only four years.

After ten years of milk fluoridation, only permanent tooth status was evaluated. The greatest differences between test and control groups were found for both 7 and 14 year olds. In the 12 and 14 year old controls, there were no caries-free children, whereas in the corresponding test groups, nearly 20% of subjects were caries-free. In both groups, with increasing age, the number of caries-free children decreased and a difference in excess of 10% was found in all age groups, apart from 13 year olds.

With respect to the caries reductions achieved in the primary dentitions, these were evaluated after five years (Group T₁), and four years (Group T₂) of milk fluoridation (Table 4.8). 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 40.1% lower in the T₁ group, than in the control group. However, the differences between the T₂ and control group (9–12 year old) primary teeth values showed neither statistical, nor meaningful clinical variations.

Table 4.8 Comparison of dmft and dmfs mean values in the test (T) and control (C) groups after 4 (T₁/C₁) and 5 (T₂/C₂) years of milk fluoridation

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Group</th>
<th>At examination</th>
<th>At start of milk F-</th>
<th>N</th>
<th>dmft</th>
<th>Sigm</th>
<th>dmfs</th>
<th>Sigm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁</td>
<td>9-12</td>
<td>5-8</td>
<td>83</td>
<td>1.42</td>
<td>N.S.</td>
<td>2.67</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>C₁</td>
<td>9-12</td>
<td>-</td>
<td>64</td>
<td>1.59</td>
<td>2.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>7-10</td>
<td>2-5</td>
<td>69</td>
<td>2.40</td>
<td>R&lt;0.001</td>
<td>3.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C₂</td>
<td>7-10</td>
<td>-</td>
<td>81</td>
<td>4.01</td>
<td>R&lt;0.001</td>
<td>6.40</td>
<td></td>
</tr>
</tbody>
</table>

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The caries reductions in first permanent molars were assessed after three and five years. Statistical analyses of these data showed significant negative correlation between DMF means and lengths of fluoridated milk consumption in subjects aged 5–6 years at time of evaluation. The caries reduction after three years was 74%, and statistically significant (P<0.001). In 7–9 year olds, the permanent first
molar caries reductions were less, and statistically non-significant. Data in Table 4.9 show that, after five years (Group T\_1) and four years (Group T\_2) of milk fluoridation, a statistically significant difference existed between first permanent molar caries for Group T\_1 compared to its control Group C\_1 (P<0.001), as well as between Groups T\_2 and C\_2. In Group T\_2, the combined DMFT mean values were 24.5%, and the DMFS means 35.7% lower than those of the C\_2 Group. The T\_2 Group showed a clinically greater caries reduction, the DMFT mean being 54.0%, and the DMFS mean 53.2% lower than the corresponding values of the control group (C\_2). The youngest age group (7 year) had the greatest DMFT reduction of 85.3%.

Changes in total DMFT and DMFS mean values were measured at the fifth and tenth year assessments. As shown in Table 4.10, after five

<table>
<thead>
<tr>
<th>Group</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Total 9-12 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMFT Sig</td>
<td>DMFT Sig</td>
<td>DMFT Sig</td>
<td>DMFT Sig</td>
<td>DMFT Sig</td>
</tr>
<tr>
<td>T_1</td>
<td>2.00</td>
<td>2.50</td>
<td>2.70</td>
<td>2.22</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td>N.S.</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>C_1</td>
<td>2.43</td>
<td>3.44</td>
<td>3.67</td>
<td>3.43</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td>DMFS Sig</td>
<td>DMFS Sig</td>
<td>DMFS Sig</td>
<td>DMFS Sig</td>
<td>DMFS Sig</td>
</tr>
<tr>
<td>T_2</td>
<td>2.00</td>
<td>2.60</td>
<td>2.60</td>
<td>3.05</td>
<td>2.79</td>
</tr>
<tr>
<td></td>
<td>N.S.</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>C_2</td>
<td>3.33</td>
<td>5.37</td>
<td>4.50</td>
<td>4.00</td>
<td>4.34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>Total 7-10 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMFT Sig</td>
<td>DMFT Sig</td>
<td>DMFT Sig</td>
<td>DMFT Sig</td>
<td>DMFT Sig</td>
</tr>
<tr>
<td>T_2</td>
<td>0.26</td>
<td>0.63</td>
<td>1.94</td>
<td>2.42</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.01</td>
<td>N.S.</td>
<td>N.S.</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>C_2</td>
<td>1.77</td>
<td>1.78</td>
<td>3.00</td>
<td>4.75</td>
<td>2.60</td>
</tr>
<tr>
<td></td>
<td>DMFS Sig</td>
<td>DMFS Sig</td>
<td>DMFS Sig</td>
<td>DMFS Sig</td>
<td>DMFS Sig</td>
</tr>
<tr>
<td>T_2</td>
<td>0.26</td>
<td>0.63</td>
<td>2.06</td>
<td>2.57</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>N.S.</td>
<td>N.S.</td>
<td>P&lt;0.05</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>C_2</td>
<td>1.90</td>
<td>1.96</td>
<td>3.94</td>
<td>6.63</td>
<td>3.27</td>
</tr>
</tbody>
</table>

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Table 4.10  Comparison of DMFT and DMFS mean values between test (T) and control (C) groups, after 4 (T₁/C₁) and 5 (T₂/C₂) years of milk fluoridation

<table>
<thead>
<tr>
<th>Age in years</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Total 9-12 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>iDMFT</td>
<td>Sig</td>
<td>iDMFT</td>
<td>Sig</td>
<td>iDMFT</td>
</tr>
<tr>
<td>T₁</td>
<td>2.14</td>
<td></td>
<td>2.73</td>
<td></td>
<td>2.89</td>
</tr>
<tr>
<td>C₁</td>
<td>3.00</td>
<td></td>
<td>4.75</td>
<td></td>
<td>5.44</td>
</tr>
<tr>
<td></td>
<td>iDMFS</td>
<td>Sig</td>
<td>iDMFS</td>
<td>Sig</td>
<td>iDMFS</td>
</tr>
<tr>
<td>T₁</td>
<td>2.14</td>
<td></td>
<td>3.08</td>
<td></td>
<td>4.15</td>
</tr>
<tr>
<td>C₁</td>
<td>3.94</td>
<td></td>
<td>6.63</td>
<td></td>
<td>6.44</td>
</tr>
<tr>
<td></td>
<td>iDMFT</td>
<td>Sig</td>
<td>iDMFT</td>
<td>Sig</td>
<td>iDMFT</td>
</tr>
<tr>
<td>Group</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>Total 7-10 yr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₂</td>
<td>0.26</td>
<td></td>
<td>0.68</td>
<td></td>
<td>1.94</td>
</tr>
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<td></td>
<td>1.78</td>
<td></td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>iDMFS</td>
<td>Sig</td>
<td>iDMFS</td>
<td>Sig</td>
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<tr>
<td>T₂</td>
<td>0.26</td>
<td></td>
<td>0.68</td>
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</tr>
<tr>
<td>C₂</td>
<td>1.90</td>
<td></td>
<td>2.00</td>
<td></td>
<td>3.94</td>
</tr>
</tbody>
</table>

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years, the T₂ and C₂ combined group data were highly significantly different, with DMFT values varying by 60.0%, and DMFS by 66.6%. 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 (T₁) and control (C₁) groups, statistically significant differences were found for the total mean DMFT value of the 9–12 year group (a 36.8% reduction), but for the overall DMFS mean, the 29.5% reduction was not significant. Again, for 9 year olds, no data achieved significance. With respect to the ten year results, as shown in Table 4.11, only at ages 12–14 years were differences between test and control DMFT mean values significant (P<0.05), the greatest being demonstrated in the 14 year olds (P<0.001). However, for the equivalent DMFS data, only at 12 and 14
years of age were significant differences found. With both indices, it was evident that those fluoridated longest, benefited most, the 14 year olds' DMFT and DMFS mean values being approximately three times lower than the control group's means. However, when the distribution of ages was assessed in two groupings of 7–10 years, and 11–14 years, the trends in mean DMFT and DMFS were apparent (Table 4.12). Here, significant statistical differences between the test and control groups were noted in the younger children (P<0.05). Finally, when the overall mean caries increment was calculated between test and control groups, there was a 36.8% DMFT, and a 40.0% DMFS reduction favouring subjects in the fluoridated milk group.

<table>
<thead>
<tr>
<th>Table 4.11 DMFT and DMFS mean values in the test (T) and control (C) groups after 10 years of milk fluoridation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>DMFT</td>
</tr>
<tr>
<td>T</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>Sig</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>DMFS</td>
</tr>
<tr>
<td>T</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>Sig</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Table 4.12 DMFT and DMFS mean values in the age groups 7–10 and 11–14 years, after 10 years of milk fluoridation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
</tr>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Test</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Sig</td>
</tr>
</tbody>
</table>

Reproduced with permission of the publisher from Gyurkovics et al. (1992).
4.3.3 Discussion

The above clinical field study investigated the caries-inhibiting effectiveness of fluoridated fresh milk, after several years' regular consumption. However, since such milk must be consumed within 30 min after the addition of fluoride, organizational difficulties could influence the practicality of implementing such a method. Nonetheless, 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. The beneficial effect of milk fluoridation on the primary dentition has been recognized in studies where implementation began at an early age, even before eruption of the primary teeth (Wirz, 1964; Ziegler, 1964). In this Hungarian study, statistically and clinically significant differences between the test and control groups' dmft values were found for the younger age groups (2–3 years) whose primary teeth had already erupted at the beginning of the programme. Hence the effect obtained can only have resulted from a topical mode of action. The longitudinal analysis of first permanent molar DMFT and DMFS mean values of 165 children followed-up for four and five years showed evidence of a declining caries trend (Table 4.9).

As mean DMFT values were still around 5.0 in Hungary, the caries decrease experienced in the study might be judged significant. In the group of 93 children followed-up for four years, the higher overall mean values may be attributable to the later starting age at which children commenced the milk fluoridation programme.

According to Stephen (1977), the erupting first molars are eminently suited, in 5½–6½-year-olds, for the clinical testing of potential caries-inhibiting techniques and programmes. Therefore, the caries data relating specifically to these teeth were analysed separately. Here, reductions of more than 85% were found for first permanent molars unerupted at baseline in the youngest (7-year-old) cohort. This, again, emphasizes the importance of commencing such a programme at an early age, and its continuation thereafter.

Concerning the overall DMFT and DMFS mean value changes, after ten years, in spite of the two year technical interruption, statistically significant caries reductions were achieved. These findings are in contrast to the study of Künzel (1980), when temporary interruption of drinking water fluoridation resulted in a lack of anti-caries efficacy.
Although, after ten years, the caries reductions in permanent teeth (DMFT = 36.8%; DMFS = 40.0%) were lower than those achieved at five years (DMFT = 60.0%; DMFS = 66.6%), by the end of the study the mean DMFT value of 3.0 for 11–14-year-olds receiving fluoridated milk, was in the range envisaged by WHO for the year 2000. On the contrary, for control subjects, the DMFT mean values were considerably in excess of 5.0, and would be classified as “high” and “very high” by WHO.

Finally, the correlation between DMF mean values and duration of residence within the institute was calculated for each subject, and all who came into care within one or two years of the conclusion of the study had 6–10 DMF teeth. However, for “siblings” who participated in the programme from kindergarten age, much lower DMFT mean values (0–2) were evident, emphasizing the importance of commencing such “therapy” at an early age, and of ensuring its continuation over a prolonged timescale to promote a greater level of caries protection.

4.4 Bulgaria

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

From the outset, it was never intended that this would be a controlled longitudinal clinical trial, but rather a field demonstration study to show whether, under real-life conditions, milk fluoridation could be an effective and practical means of reducing caries incidence in youngsters.

4.4.1 Materials and methods

The project was sited in the southern Bulgarian town of Asenovgrad. A dairy in nearby Plovdiv produced fluoridated milk by simply adding the appropriate quantity of sodium fluoride to fresh milk prior to its packaging into plastic containers. The dairy was responsible for providing milk to all kindergartens and schools in Plovdiv. In addition it also served 16 kindergartens and seven schools in Asenovgrad, i.e. approximately half the institutions there, to which fluoridated milk was supplied throughout the study period. For the other 50% of Asenovgrad subjects, fluoride-free milk was provided from another source.
Approximately 3,000 Asenovgrad children aged 3–10 years received fluoridated milk in kindergartens and schools during the observation period, but not at home. Daily fluoride analyses were completed in the Plovdiv dairy using an Orion specific ion electrode. The average age of children in the 1988 base-line group 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 study 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% on total dmft or DMFT scores as per the WHO criteria of Eklund, Moller & Leclercq (1993). Dental caries was recorded according to the criteria defined by the World Health Organization (1987), but 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 (4.13–4.16).

4.4.2 Results

In those kindergartens and schools where fluoridated milk was delivered on a regular basis, the estimated consumption corresponded to a daily intake of 200 ml milk, containing 1 mg F⁻ (approx 5 ppm F as NaF) for a period of 180–200 days per year. Results in Table 4.13 relate to 6½-year-old children after three years’ participation in the project. Here, the reduction in deciduous caries prevalence in “fluoridated” Asenovgrad was approximately 40% (P<0.001) as compared to 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 to a 14% non-significant DMFT increase in Panaguriche.

In Table 4.14, 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 non-significantly by 25%.

Although the project was scheduled originally for only a three year period, in order to obtain additional experience — particularly with regard to establishing a reliable infrastructure in a community project of this nature — it was decided to extend the study to five years. Unfortunately, however, it was not possible to maintain Panaguriche as the reference site and, as stated above, for comparative purposes subjects from the nearby town of Karlovo were enrolled for the last two years.
### Table 4.14 Dental caries prevalence (dmft and DMFT) in children aged 7½-years in Asenovgrad (F- milk) and Asenovgrad (Non-F- milk) at base-line (1988) and at the end of the study (1991)

<table>
<thead>
<tr>
<th></th>
<th>1988 Mean (SD)</th>
<th>1991 Mean (SD)</th>
<th>Percentage difference</th>
<th>P-value (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asenovgrad (F- milk)</td>
<td>6.7 (3.7)</td>
<td>3.8 (2.8)*</td>
<td>-44%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>n=47</td>
<td>n=135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMFT</td>
<td>1.2 (1.3)</td>
<td>0.2 (0.7)**</td>
<td>-83%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>n=47</td>
<td>n=135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asenovgrad (Non-F- milk)</td>
<td>6.7 (3.7)</td>
<td>8.4 (4.0)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=47</td>
<td>n=101</td>
<td>+20%</td>
<td>N.S.</td>
</tr>
<tr>
<td>DMFT</td>
<td>1.2 (1.3)</td>
<td>1.6 (1.3)****</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=47</td>
<td>n=101</td>
<td>+25%</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* versus *** P<0.001; ** versus **** P<0.001

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In Table 4.15, the results obtained relating to caries in the primary dentitions of 6½ and 8½-year-olds 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 obviously been drinking fluoridated milk for only three years, in contrast to the 8½-year-olds who had

### Table 4.15 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 to Karlovo (new Non-F- milk)

<table>
<thead>
<tr>
<th>Age of children in 1993</th>
<th>Years of participation</th>
<th>dmft mean (SD)</th>
<th>Percentage difference</th>
<th>P-value (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asenovgrad (F- milk)</td>
<td>3</td>
<td>3.2 (3.1)</td>
<td>-52%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>n= 139</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karlovo (Non-F- milk)</td>
<td>0</td>
<td>6.8 (4.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=114</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asenovgrad (F- milk)</td>
<td>5</td>
<td>3.6 (2.6)</td>
<td>-40%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>n=178</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karlovo (Non-F- milk)</td>
<td>0</td>
<td>6.0 (3.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=176</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Table 4.16 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 to Karlovo (new Non-F milk)

<table>
<thead>
<tr>
<th>Age of children in 1993</th>
<th>Years of participation</th>
<th>dfmft mean (SD)</th>
<th>Percentage difference</th>
<th>P-value (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asenovgrad</td>
<td>6½</td>
<td>3</td>
<td>0.1 (0.4)</td>
<td>-89%</td>
</tr>
<tr>
<td>(F- milk)</td>
<td></td>
<td>n = 125*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karlovo</td>
<td>6½</td>
<td>0</td>
<td>0.9 (1.3)</td>
<td></td>
</tr>
<tr>
<td>(Non-F- milk)</td>
<td></td>
<td>n = 104*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asenovgrad</td>
<td>8½</td>
<td>5</td>
<td>0.5 (0.9)</td>
<td>-79%</td>
</tr>
<tr>
<td>(F- milk)</td>
<td></td>
<td>n = 178</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karlovo</td>
<td>8½</td>
<td>0</td>
<td>2.4 (1.8)</td>
<td></td>
</tr>
<tr>
<td>(Non-F- milk)</td>
<td></td>
<td>n = 176</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Not all the children in whom dfmft scores were assessed (see Table 15) had erupted first permanent molars present at the time of examination.

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received fluoridated milk during the full project period of five years. In primary dentition, the dfmft difference between the new reference area (Karlovo) and the test area (Asenovgrad) was 52% (P<0.001) for 6½-year-olds, albeit their 1993 dfmft value (6.8) was greater than that of similarly-aged subjects in Panaguriche in 1991 (5.2). However, the equivalent 8½-year-olds’ figures showed Asenovgrad children to have a mean dfmft value 40% lower (P<0.001) than those in Karlovo. The corresponding figures for permanent dentition, i.e. 89% (P<0.001) and 79% (P<0.001) respectively, are indicated in Table 4.16.

4.4.3 Discussion

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

In addition, two other studies, one employing flavoured fluoridated milk and the other reconstituted powdered cow's milk, have also given positive results. In the former, Legett et al. (1987) utilized two groups of elementary U.S. school children i.e. a two year cohort and a three year cohort. Here, for 170 school days per year, subjects consumed 236 ml chocolate-flavoured, sweetened, low fat milk containing 1.0 mg of NaF. From the original 706 total of grade 4 children, final data were available for 187 in the 2 year group and for 157 in the 3 year
group. In the former subjects, DMFT and DMFS reductions of 77.2% and 77.6% respectively were recorded (P<0.05 in both cases), while in the latter the corresponding values were non-significant at only 2.2% and 21.8%. With respect to the superiority of the two year data, the authors cited particular compliance difficulties which were experienced by the 3-year-old subjects, as well as an unavoidable ten month gap in product delivery as major contributing factors. In contrast, continuous milk availability was ensured for the 2-year-old children, and uptake of the beverage was actively encouraged with small tokens of recognition being given to participants.

In Israel, Zahlaka et al. (1987) published results relating to 120 children from Bethlehem, aged 4–7 years at outset. Here, test group subjects received 100 ml of reconstituted powdered cow's milk, supplemented with 1 mg fluoride as NaF. The control group received no drink at school. After 3 years, children who consumed fluoridated milk on school days, had lower incremental caries scores for the deciduous dentition (mean deft difference = 1.3) and first permanent molars (mean DMFT = 0.2), as compared with those in the control group (mean deft and DMFT differences = 3.5 and 0.55 respectively), both comparisons being significantly different (P<0.01). The designs of previous studies have, however, differed significantly with regard to the age of children at outset (1–10 years), as well as with respect to their duration (2–5 years). The greatest caries-reductions have been obtained in those investigations where, at base-line, children were 4 years of age or younger, and the study length of three years or more (Imamura, 1959; Ziegler, 1964; Stephen et al., 1981; Bánóczy et al., 1985b; Zahlaka et al., 1987). Reductions in caries incidence have varied from 15% to 60% in the primary dentition, and from 30% to 85% in the permanent dentition. Due to the age of subjects at the end of these studies, the permanent dentition caries-reductions quoted relate mainly to first permanent molars. 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 large-scale Bulgarian investigation was to determine whether a similar effect could be obtained in a "real life" situation, and the results of that cross-sectional study would appear to fall at the upper end of previously reported limits.
4.5 Conclusions

On the basis of data reviewed in this Chapter, it can be concluded:

the caries-preventive effect of fluoridated milk was greater the earlier in the child’s life the consumption commenced the caries-reducing effect of fluoridated milk appears to be comparable to that of other community-based fluoride vehicles.

However, there would still seem to be a series of unanswered questions, and additional studies should be performed to determine:

i) the age at which it is best to start drinking fluoridated milk;

ii) for how many years it should continue;

iii) the frequency of consumption;

iv) the optimum concentration of fluoride to be added;

v) the anti-caries effect of milk and milk products alone.
5
Legislation and community based aspects of the implementation of a milk fluoridation programme


5.1 Introduction
A proposal to produce, distribute or market fluoridated milk will, in most countries, require a careful review of legislation in general, and of food legislation in particular. It will almost certainly involve the proponents of a scheme in discussions and negotiations with regulatory bodies, both national and local. Thus, the purpose of this chapter is to review the legal and organizational aspects of four current milk fluoridation demonstration studies in the United Kingdom, China, Chile and Russia. Communities in all four countries are operating these schemes targeted towards children, as part of an international demonstration programme coordinated by the World Health Organization and funded by the Borrow Dental Milk Foundation.

The aims of this text are fourfold. To:

(i) provide a brief overview of the four schemes,

(ii) summarize the relevant national legislation,

(iii) consider local regulation,

(iv) consider community aspects of the programmes, including health promotion.

The paper concludes with a set of guidelines, developed in the light of experience to date, for any who may wish to establish a new fluoridated milk programme.
5.2 The milk fluoridation demonstration programmes

5.2.1 The United Kingdom

In the United Kingdom, 189 ml of milk is provided daily at school to many children attending nursery and primary schools as part of a national programme, jointly funded by the European Union, central and local government (Jones et al., 1992; Lennon et al., 1995). In the town of St Helens, this daily “school milk” has provided the vehicle for 0.5 mg of fluoride for 3000 children aged 3–7 years. Since 1996 the scheme has been extended to a further 3000 children living in the community of the Wirral in Merseyside.

5.2.2 Chile

The project in Chile also involves the addition of fluoride to a well-established programme involving the distribution of powdered milk and a milk-cereal mix. Here the products are distributed to parents by the local health/welfare centres under the National Complementary Feeding Programme, which provides free powdered milk and other dairy products throughout Chile, reaching about 80% of children in the 0–6 year age group. The fluoridated milk demonstration programme is being undertaken in the municipality of Codegua, a rural, low socio-economic area situated 90 km south of Santiago. Another special feature of this exercise is the use of disodium monofluorophosphate (Villa et al., 1989), rather than the more usually employed sodium fluoride.

5.2.3 China

The Beijing fluoridated milk programme was established ab initio and involves the distribution of fluoridated milk to 4000 children aged 3–6 years attending kindergartens in the Haidian district.

5.2.4 Russia

In Russia, three separate milk fluoridation schemes were established in Voronezh, Smolensk and Maykop, involving 15,000 kindergarten children, mainly aged from 3–6 years.

5.3 National legislation

5.3.1 The United Kingdom

Within the United Kingdom, the addition or removal of any substance to or from milk, other than a reduction in its fat content, has impor-
tant legal implications. Thus fluoridated milk becomes a “milk-based product” and, as such, becomes subject to the “Milk-based Drink Regulations”, with special requirements for labelling which have to be agreed between the dairy and the local environmental health office of the local government area.

Milk provided to children in full-time education is eligible for a 60% subsidy from the European Union’s agricultural budget, while milk provided to children attending nursery schools is funded fully by central government under the Welfare Food Regulations (Jones et al, 1992).

5.3.2 China

The National Food Hygiene Law (1982) of China allows, in general, for certain food fortifiers including vitamins, minerals, trace elements and amino acids. Fluoride is included as one of the fourteen necessary trace elements and, in 1984, the National Standards Bureau issued documents (GB 809-84 and GB 480-9-84) which established permitted levels of fluoride in foods. In 1994, the Ministry of Public Health Inspection and Supervision issued a document (Number 75, 1994) “... on approval of a Community Study Project on Milk Fluoridation for Caries Prevention”. The Department requested that “... the results of the study project should be reported promptly for the sake of providing evidence to improve the National Hygiene Standards of Using Nutrient Fortifiers”.

5.3.3 Chile

In Chile, the National Complementary Feeding Programme has been in existence for around 40 years. From 0–2 years of age, children are provided with 2 kg of milk powder per month, and from 2–6 years old, with 1 kg of a milk-cereal product containing 40% milk. Both products are distributed to parents via the local community health centre.

5.3.4 Russia

Prior to proceeding in Russia, it was necessary to obtain the approval of both the Ministry of Health Care, and the Ministry of Agriculture. Thereafter, a legal document was written in cooperation with the Institute for General Nutrition of the Russian Academy of Medical Science, the Research Institute for Childrens’ Nutrition, and the Milk Industry. The document, which incorporated technical instructions for producing fluoridated pasteurized milk for children, labelling, and
methodological instructions for fluoride determination, was subsequently submitted to, and approved by, the State Committee for the Food and Processing Industry, and the Federal Sanitary Control Committee.

5.4 Local regulation

Local regulation of a fluoridated milk demonstration programme usually involves tripartite cooperation between a university-based research group, local government and/or a local health authority, and a dairy. In Beijing this tripartite agreement is particularly well-documented and provides a useful model for others e.g.

Party A – The Department of Preventive Dentistry, School of Stomatology, Beijing Medical University, will be responsible for:

1. Designing the study protocol including the working plan, planned activities and schedules.

2. Conducting base-line surveys of the oral health status of preschool children; of fluoride levels in drinking water, milk and urine and monitoring these on a regular basis.

3. Advising and cooperating with the dairy in the production technology of fluoridated milk, and in the training of technicians for testing fluoride levels in milk.

4. Conducting oral health education and promotion for the parents of the children, the teachers and directors of kindergartens, as well as for local government officers.

Party B – The Haidian District Government will be responsible for:

1. Promoting oral health integration within the overall children’s development project of the district.

2. Organizing, managing and coordinating other sectors involved in the F-milk project at the community level.

3. Selecting the kindergartens and age groups of pre-school children, in cooperation with the main investigator of the project.

4. Holding regular meetings for parents, teachers and directors of kindergartens, to promote their understanding, support and active participation in the F-milk project.
Party C – The Beijing Western Suburb Dairy will be responsible for:

1. Providing all necessary equipment and staff required to produce F-milk daily.
2. Defining and operating the technical procedures for producing F-milk under supervision.
3. Providing an agreed amount of F-milk of good quality, and transporting it to the target kindergartens daily.
4. Limiting F-milk distribution to the target kindergartens, and ensuring it is not sold commercially.
5. As a daily routine, testing the fluoride level in the F-milk before distribution.

Although the allocation of these responsibilities varies slightly from scheme to scheme, the overall distribution is remarkably similar in all four countries, and can certainly be recommended as a starting point for any further projects.

5.5 Health promotion

A further consistent feature in all four demonstration programmes is the integration of the fluoridated milk provision into a wider oral health promotion programme. In both St Helens and Beijing, for example, this has involved briefing sessions for school governors and head teachers; workshops for nursery and school teachers; the preparation of workbooks and other materials for the children; explanatory letters for parents and, in St Helens, the design of attractive and relevant logos — and dental health messages — for the milk cartons. In addition, opportunities are taken via distinguished visitors and special events to involve television, radio and newspapers, and to build a socially supportive atmosphere.

5.6 Guidelines for the implementation of a milk fluoridation scheme

As discussed earlier in this chapter, it is of vital importance that legislative and regulatory issues are addressed when contemplating the implementation of a scheme in which fluoridated milk is distributed as a means of improving dental health. It is of equal importance, if milk is to be distributed through an existing programme, to obtain the cooperation and authorization of all bodies involved at a community level.
Over-and-above, there are a number of points which have to be considered when deciding whether milk fluoridation is appropriate for any given community. These relate to the dental health status indicators for fluoride intervention, fluoride availability from different sources, and existing milk distribution systems.

In the first instance, the oral health status within the community (and particularly that of the children) must be determined. Available data can be used to assess the DMFT caries prevalence in 12-year-olds, the World Health Organization defining the following levels:

i) Very low: 0.0–1.1
ii) Low: 1.2–2.6
iii) Moderate: 2.7–4.4
iv) High: 4.5–6.5
v) Very high: >6.6

These data should be taken from the most recent survey available, taking account of any secular trends. The values should subsequently be verified by a baseline clinical examination of a representative sample of children in the actual community where the decision would be made to introduce a milk fluoridation scheme. The milk fluoridation projects which constitute the International Milk Fluoridation Programme have been implemented in communities with moderate and high caries levels, and are still under evaluation. In localities where such decay levels exist, and where fluoride intake by children is suboptimal, there is a clear indication for fluoride intervention.

The second consideration regarding the availability of fluoride must relate to the level of fluoride in the local drinking water. This should be measured to ascertain whether it has a constant value or whether it is subject to seasonal variation. Thereafter, the recommendations of the WHO Expert Committee (1994) should be used to determine the appropriate fluoride supplementation dose to be delivered in the form of fluoridated milk. When considering fluoride availability, it is also necessary to note other potential fluoride sources to which children in the community may be exposed, e.g. fluoridated dentifrice, which younger children may ingest, or selective dietary components such as fish or black tea which could be popular in some communities. When considering toothpaste, it is appropriate to ascertain:

(i) whether toothbrushing and toothpaste usage is the main means of oral hygiene;
(ii) whether the toothpaste is fluoridated;
(iii) the fluoride content of the toothpaste if it is fluoridated.

Where a decision has been taken to implement a milk fluoridation scheme, it is recommended that the standard method for urine sampling and analysis should be made. This technique, which is also used for monitoring ongoing schemes, is detailed later in this chapter.

As control of the fluoridated milk distribution to children is best achieved through an established milk distribution system, e.g. school milk or, for younger children, allocation through clinics, the next consideration relates to the existence of such systems. In order to determine how effective these channels would be for reaching the children in the community it is necessary to ascertain:

(i) the availability of nursery schools, kindergartens and schools which distribute milk as a routine;
(ii) the percentage of eligible children who attend the nursery schools/kindergartens and schools;
(iii) the frequency with which they attend;
(iv) the uptake of school milk by those who attend.

A similar situation exists with younger children dependent upon milk allocation via clinics.

Having ascertained that the uptake of school or clinic milk is high, the next step is to identify the dairy which supplies such milk, and enter into discussion regarding its participation in the scheme.

In order to ensure that children would have daily delivery of fluoridated milk and have the correct allocation, (which may be age-dependent), it is desirable to identify persons at the schools who would supervise its distribution and consumption.

In order to assist any person contemplating the introduction of a milk fluoridation scheme, and wishing to judge its feasibility, the World Health Organization's Consultative Group on Milk Fluoridation has compiled the following list of questions addressing the points covered above:

1. Is cows' milk a traditional part of baby and infant nutrition?
2. Is cows' milk available on a daily basis in the community?
3. Is cows' milk affordable for all families in the country?
4. What type of milk is available: fresh, condensed, powdered?

5. Is milk processed in a local dairy or imported from outside the community?

6. Is milk sold primarily in supermarkets, smaller shops or special dairy-product shops, or delivered at home, or through other distribution channels?

7. Is milk provided in cartons, plastic bags, bottles or canisters (in bulk)?

8. Is it possible to identify local persons who can supervise a milk fluoridation project (i.e. ensure daily delivery of milk to kindergartens; provide oral health education; ensure that the children consume the milk, etc)?

9. What percentage of children aged 3–6 attend kindergartens?

10. Do you think it would be feasible to provide milk each day to children in kindergartens?

11. Is there any legal obstacle for adding fluoride to milk?

12. Would it be easy to obtain official permission to produce fluoridated milk?

13. What is the migration rate in the community (i.e. percentage of the population leaving the community per year)?

14. What is the present price of 1 litre of milk?

15. What was the price of milk three years ago?

16. What is the dmft level at 3 years of age?

17. What is the DMFT level at 12 years of age?

18. Is toothbrushing with toothpaste used as the main means of oral hygiene?

19. What percentage of toothpaste is fluoridated?

20. Is there any fluoride in the local water supply. If so, what is the concentration (average and seasonal variation, if any)?

It is through this sort of situation analysis that a picture will gradually emerge as to how appropriate it would be to consider fluoridated milk as a caries-preventive method for any particular community. For those contemplating such a programme, any of the authors would be pleased to provide advice.
5.7 Ongoing monitoring and evaluation of schemes

Once a scheme is in operation, it is important to subject it to periodic assessment and to ensure participant compliance by conducting programmed studies of dental health and fluoride metabolism.

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. For this purpose, the dmfs/DMFS index or the dmft/DMFT indices may be employed. A typical schedule for an evaluation programme is shown in Figure 5.1. The continuous monitoring of a scheme also includes a periodic check that children are ingesting fluoride (from all sources) at the appropriate level. This is achieved by studying the urinary fluoride excretion of a representative sample of subjects. As detailed in this

Figure 5.1 Tentative schedule for oral examination of children participating in the milk fluoridation project and from the control areas in Bulgaria

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▲ Test group. Randomized selection of children for clinical examination (n=300).
Clear evidence related to the average number of days F-milk consumption per year should be available

○ Control group. Randomized selection of children for clinical examination (n=200).
chapter, the recommended technique for this process, which may be used in a school or kindergarten situation, is to employ a "supervised, time-controlled urine excretion study". This provides an accurate and reliable assessment of 24 hour fluoride excretion from which the amount of ingested fluoride may be evaluated. In communities where this type of study is not feasible e.g. with children who consume fluoridated milk or milk products at home, an alternative method involving a study of fluoride/creatinine excretion (Stephen et al, 1981; Kertész et al, 1989) may be employed. This latter method has been used by Villa (1994). It is recommended that fluoride excretion studies be conducted at the following times with respect to a demonstration scheme: at the outset, 6 months, 1 year, 2 years, 3 years and 5 years.

5.8 Standard method for urine sampling and analysis for the determination of fluoride intake

The rate of urinary fluoride excretion varies throughout the course of the day (and night) in relation to the time of fluoride ingestion, particularly where fluoride supplementation is given in the form of fluoridated milk, or tablets, or where fluoridated toothpaste may be swallowed during tooth cleaning. Thus it is imperative that urine sampling to evaluate fluoride excretion (and hence fluoride intake), should be undertaken in such a manner that it covers as much of the 24 hours of the day as possible, and continues for a period of at least two consecutive days.

In children who drink fluoridated milk as a routine, urinary fluoride excretion rapidly follows ingestion. A pattern of excretion observed in children who drank 0.2 litre of fluoridated milk per day containing 0.5 mg of fluoride as a single dose is illustrated in Figure 5.2.

It follows, from this excretion pattern that the fluoride content of a spot urine sample taken without regard to ingestion time will, at best, tell little about the amount of fluoride ingested, and at worst may be misleading. It is recommended, therefore, that "time-controlled urine samples" be obtained in order to measure the fluoride excreted per unit time. This is best performed at schools, kindergartens and other childrens' institutions where access to toilets is controlled and supervision is at hand.
5.8.1 General design of study

- Minimum number of children with successful collections: 30–35.
- Age recommended: 4–5, 4–6, 5–6 or 5–7 years.
- The study should include children who drink fluoridated milk, and those from a control area where fluoridated milk is not available.
- Sample should be obtained at (i) the start of the project, (ii) 6 months after the start and (iii) 18–24 months later.

5.8.2 Time-controlled urine sampling

The procedure which allows the total urine generated during a specific period of time to be collected is:

(a) At first each subject is expected to empty his/her bladder completely. This urine is discarded because it is not time-controlled.
(b) The name of the subject and the time of this voiding is noted.
(c) When the subject arrives for the next urination, he/she is given a jar into which to urinate.
(d) The time of this second urination is noted. The jar is closed and put in the coolest place at hand. The next, and any subsequent, urination in the same pre-set collection period should be handled in an identical manner.

(e) When the end of the supervised pre-set collection period approaches, the child is asked to urinate into the jar. The time when this takes place marks the end of his/her personal period. If the child is unable to urinate at this time, his/her collection period is considered to have ended at the last available urination. This time is noted on the label.

(f) The total volume of urine collected during the period is measured and noted on the label.

The basic information available at this point of time is:

(a) Time point of initial voiding of the bladder.
(b) Time point of last urine collection into the bottle.
(c) Total volume of urine collected between the initial and final time-point.

This information is recorded for each participant, and then, for the purpose of analysis, a sample of urine (20–50 ml) taken from each jar is placed in a small tube. If the analysis is not conducted immediately (i.e. within hours) it will be necessary to add preservative (a small crystal of thymol) and keep samples cool (refrigerated or preferably frozen at -18 °C) in the meantime. The tubes should be labelled with a reference number which identifies the individual from whence the sample came, and the timed period to which it refers.

It should be possible to make a night collection and two supervised day collections. Typically a night collection would be obtained under parental supervision and cover 8–10 hours. The two daytime collections would be made under supervision of teachers and one or two members of the project team. They should last about 3–5 hours each. In this way a total of 14–18 hours of the entire 24 hour cycle will be covered.

5.8.3 Recording of information

Details of each individual subject: name, number, age, sex and the urine volume for each specific period, should be recorded both in tabular form and on the labels of the collecting jars which have been designed for this purpose (see Figure 5.3). Labels should be attached
to urine collecting jars with adhesive tape, top and bottom, so that they may be easily removed and retained as a record. Information should be gathered with regard to the level of fluoride in drinking water and mineral waters (if consumed). In addition, the use of fluoridated toothpaste (and any other fluoridated product, e.g. rinses) should be ascertained. This is particularly important in children under seven years of age, some of whom tend to swallow a substantial percentage of these products.

In Figure 5.3, A, B, C and D refer to different collection periods morning, afternoon etc., as appropriate. The subjects are supervised during the urine collection periods. The information regarding urine volume versus time is recorded thus:

![Figure 5.3 Design and use of label to be attached to urine collecting jar – supervised daytime collection](image)

<table>
<thead>
<tr>
<th>NAME</th>
<th>AGE</th>
<th>SEX</th>
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**TIME OF CONSUMPTION OF FLUORIDATED MILK**
(a) Note the time the initial voiding of the bladder takes place and enter into Box 1. Do NOT collect this urine.

(b) Collect all the urine produced thereafter and note the time of each urination in boxes 2, 3 and 4. For collecting periods of typically 4 hours duration e.g. 09.00–13.00, it may not be necessary to use all the boxes.

(c) When the end of the collection period is reached, encourage the child to urinate into the jar. If he/she cannot do this, then the last urination time marks the end of their personal collection period.

(d) When the collection period is terminated, measure the accumulated volume of urine and record it in the box under the last recorded time.

(e) Set aside a sample (ca 30 ml) for fluoride analysis using a screw top tube* carrying the appropriate collection period letter A, B, C or D and the child’s identification number.

* The tube should be made of a plastic material which does not interact with fluoride e.g. polythene.

(f) If the sample is to be kept for more than 12 hours before analysis, add a few small crystals of thymol to act as a preservative and store it in a refrigerator. If the sample is to be kept for long periods (weeks or months) it should be stored at -18 °C.

(g) Tabulate the information recorded on the labels using the form illustrated (Figure 5.4).

(h) Carefully remove all labels from the jars and retain as a reserve record.

Shown in Figure 5.5 is an example of a completed label for a urine collection undertaken from May 5/6th 1993 for a 6-year-old boy named “David Morris”.
Figure 5.4 Example of record of urine collection

<table>
<thead>
<tr>
<th>Child details</th>
<th>Period A</th>
<th>Period B</th>
<th>Period C</th>
<th>Period D</th>
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Samples A and B are collected under supervision at school on the morning and afternoon of the first day. C is the night sample collected in a separate jar under parental supervision.

David Morris' morning period "A" was 3 h 45 min duration producing 122 ml of urine. The afternoon period "B", which did not immediately follow period "A", lasted for 2 h 02 min, producing 66 ml of urine. The night period "C", of 11 h 45 min produced 416 ml. D is a repeat morning sample on 6 May. These are accumulated volumes up to and including the last urination during the collection period. Urine passed at 09.05, 14.00, 19.45 on 5 May and 08.55 on 6 May was not collected in the jar. Note: The above periods (A,B,C,D) are only examples, and may be varied to suit the local kindergarten or school schedules, and different times when fluoridated milk is consumed.
5.8.4 Collection of urine at night

A different jar is used for night collection (Figure 5.6) which is made under parental supervision. When it is brought in the following morning the volume of urine is measured and recorded in the “C” period (in this example) on the label on the other jar.

5.8.5 Determination of fluoride in urine samples

Required equipment and solutions

- A direct concentration read-out specific ion meter, such as ORION 720A, 920A or Model EA220, EA910, SA720, SA270 is recommended.
- Combination Fluoride Electrode such as ORION Model 96-09.
- Magnetic stirrer and stir bars are recommended for laboratory measurements.
- Automatic pipette – 5 ml.
- Beaker (polythene) – 25 ml.
- Diagnostic pH strips are recommended for urine testing.
- Distilled or de-ionized water to prepare all solutions and standards.
- 1 ppm Fluoride Standard with TISAB – ORION Catalogue No. 040906.
- TISAB II – ORION Catalogue No. 940909.
- Combination Electrode Filling Solution – ORION Catalogue No. 900001.
- Hydrochloric Acid – 0.1 M.

**Analysis Check-List**

- Analysis includes determination of pH and its correction in some cases.
- Electrode preparation.
- Checking electrode operation.
- Direct calibration of measuring system (ISE-meter, combination electrode and solution).
- Preparation of mixtures for analysis and determination of fluoride concentration.

**pH determination in urine samples**

Urinary pH is determined by dipping of a diagnostic pH strip into the urine sample for several seconds. pH is determined by comparing the colour of the strip with the colour scale. If urinary pH is more than 8, the urine sample is acidified by one drop of 0.1 M hydrochloric acid.

**Combination fluoride electrode preparation and checking electrode operation**

1. Remove the rubber cap covering the electrode tip.
2. Fill the electrode (model 96-09) chamber with special filling solution, Catalogue No. 900001 and ensure a proper flow-rate (according to the electrode instruction manual).
3. Connect the electrode to the meter.

4. Place 10 ml of more dilute standard 1 ppm F\(^-\) with TISAB into a 25 ml beaker.

5. Rinse the electrode with distilled water, blot dry and place into the beaker, stir thoroughly, wait for a stable reading and record the electrode potential in millivolts.

6. Place 10 ml of more concentrated standard 10 ppm F\(^-\) with TISAB into another 25 ml beaker.

7. Rinse the electrode with distilled water, blot dry and place in the solution prepared in step 6 above, stir thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.

8. The difference between the first and second potential readings (slope of the electrode) should be in the range of 54–60 mV/decade, when the solution temperature is 25 \(^\circ\)C.

9. Rinse the electrode with distilled water and blot dry.

Now you are ready to proceed with direct calibration and measurements.

**Direct calibration and determination of fluoride concentration in urine samples**

1. Measure 5 ml of standard 1 ppm F\(^-\) with TISAB, 5 ml of de-ionized water and 5 ml of TISAB II into a 25 ml beaker and stir thoroughly. (The beaker will contain fluoride standard 0.333 ppm with TISAB).

2. Rinse the electrode with de-ionized water, blot dry and place in the solution prepared in step 1 above. Stir thoroughly, wait for a stable reading, then calibrate the meter to display the value of the standard (0.333 ppm F\(^-\)) as described in the meter instruction manual.

3. Measure 5 ml of standard 10 ppm F\(^-\) with TISAB, 5 ml of de-ionized water and 5 ml of TISAB II into a 25 ml beaker and stir thoroughly. (The beaker will contain fluoride standard 3.33 ppm with TISAB).

4. Rinse the electrode with de-ionized water, blot dry and place in the solution prepared in step 3 above. Stir thoroughly, wait for a stable reading, then calibrate the meter to display the value of the standard (3.33 ppm F\(^-\)), as described in the meter instruction manual.
5. Measure 5 ml of the urine sample and 5 ml of TISAB II into a 25 ml beaker, stir thoroughly.

6. Rinse the electrode with de-ionized water, blot dry and place in the solution prepared in step 5 above. Stir thoroughly, wait for a stable reading. The concentration will be displayed on the meter.

It is very important to keep the same temperature of standards and samples during measurements (+25 °C is recommended). It is also important to retain a constant stirring speed. Two parallel determinations are made and the average value is calculated and used for further processing.

5.8.6 Processing results

Data processing includes calculating parameters of urine and fluoride excretion and fluoride intake, making statistical analyses of data from the group and identifying any anomalous cases.

**Calculation of necessary parameters for individual children**

From primary data for each period of urine collection the following parameters are calculated:

- Duration (Dur) of the period — in h — by subtracting the time of period beginning from the time of its ending;

- Urinary flow rate (UFR) — in ml/h — by dividing the volume of collected urine (VCU) by the period duration;

- Fluoride excretion rate (FER) — in µg/h — by multiplying UFR by an average value of fluoride concentration (FC) in ppm.

From data for all periods, the following “Integral daily urinary fluoride excretion” and “Daily fluoride intake” may be assessed as follows:

- Integral daily urinary fluoride excretion (IDUFE) — in mg — by summation of the corresponding values of 3 periods — A (or D if for some reason period A is excluded), B and C according to the equation:

\[
\text{IDUFE} = \text{FER}(A) \times 4h \times \text{FER}(B) \times 8h \times \text{FER}(C) \times 12h / 1000
\]

where: FER(A) – fluoride excretion rate during period A,
FER(B) – fluoride excretion rate during period B,
FER(C) – fluoride excretion rate during period C,
4h, 8h and 12h – numbers of hours determining mean accepted
duration of periods A, B and C correspondingly, 1000 – coefficient of transforming μg into mg;

— Daily fluoride intake (DFI) — in mg — by multiplying corresponding IUFE by coefficient 2.00 for children up to 18 years of age or coefficient 1.66 for adults from 19–55 years.

Identification of anomalous cases

From this information it should be easy to identify any anomalous cases (if there are any) considering the following limits:

Flow-rates: since voiding of the bladder is the main problem in assessing output per time, eliminate samples which indicate a flow of less than 9 ml/h or more than 420 ml/h;

Fluoride concentrations: these should be in the range of 0.1–2.0 ppm in the absence of special sources of fluoride, but may reach 4 ppm in the hour(s) following intake of fluoridated milk or other supplemented fluoride;

Fluoride excretion rates: these should be in the range of 3–200 μg F/h.

On completion of a urinary fluoride monitoring study the following information would be available for (a) each individual child and (b) groups of children.

(a) Each individual child

1. Identification number.
2. Age (years, months).
3. Sex (male or female).
4. Time of fluoride supplementation (if given).
5. Period of urine collection (times and duration – h).
7. Urinary flow rate (ml/h).
8. Fluoride concentration (ppm).
10. Integral daily urinary fluoride excretion (mg).
11. Daily fluoride intake (mg).

(b) Statistical data for groups of children

For groups of children, the information on necessary parameters for individual children should be supplemented with:
— the number of children;
— the averages and medians of the start and duration of each collection time (5):

**Table 5.1 Table for reporting group data on urine (WHO, 1998).**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Start of collection periods (h. min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration of collection periods (h. min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
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</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Volume of collected urine (ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
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<tr>
<td>Median</td>
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<tr>
<td>SD</td>
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</tr>
<tr>
<td>Minimum</td>
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<td></td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Urinary flow rate (ml/h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Median</td>
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<tr>
<td>SD</td>
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</tr>
<tr>
<td>Minimum</td>
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<td></td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Fluoride concentration (ppm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
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<tr>
<td>Median</td>
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<td>SD</td>
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<td>Minimum</td>
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<td></td>
<td></td>
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<tr>
<td>Maximum</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
the averages, medians, standard deviations, minima and maxima of: Volumes of collected urine (6), urinary flow-rate (7), fluoride concentration (8) and fluoride excretion rate (9) for each collection period, as well as of integral daily urinary fluoride excretion (10) and daily fluoride intake (11) for each child.

The format for reporting the group data on urine is shown on Figure 5.7.

5.8.7 Utilization of results

On receipt of the reliable data of daily fluoride intake, it is not difficult to take the appropriate decision as to whether it is necessary to use supplemental fluoride to reduce caries by comparing these data with ranges of optimal fluoride intake for corresponding ages cited in Table 5.1 below.

Table 5.1 “Conservative” ranges of optimal fluoride intake (mg) introduced by T.M. Marthaler (J. Biol. Buccal, 1982, 20: 121-127).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Proportional to weight</th>
<th>Proportional to energy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Under 1</td>
<td>0.16</td>
<td>0.33</td>
</tr>
<tr>
<td>1–3</td>
<td>0.30</td>
<td>0.60</td>
</tr>
<tr>
<td>4–6</td>
<td>0.45</td>
<td>0.90</td>
</tr>
<tr>
<td>7–9</td>
<td>0.63</td>
<td>1.25</td>
</tr>
<tr>
<td>10–12 girls</td>
<td>0.85</td>
<td>1.70</td>
</tr>
<tr>
<td>10–12 boys</td>
<td>0.62</td>
<td>1.65</td>
</tr>
<tr>
<td>13–15 girls</td>
<td>1.11</td>
<td>2.23</td>
</tr>
<tr>
<td>16–19 females</td>
<td>1.21</td>
<td>2.43</td>
</tr>
<tr>
<td>Adult females</td>
<td>1.23</td>
<td>2.45</td>
</tr>
<tr>
<td>Adult male</td>
<td>1.45</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Reproduced with permission of the publisher from Marthaler (1982).
Conclusions

Milk is an essential component of the human diet throughout life, both as a source of micro- and macronutrients, as well as being a carrier for undesirable contaminants e.g. drugs and extraneous chemical pollutants.

The manufacture of fluoridated milk in various forms (pasteurized, sterilized, UHT and powdered) using sodium fluoride or sodium monofluorophosphate as fluoridating agents as a potential caries-inhibiting vehicle, involves simple production techniques.

All of the products have been shown to be stable with relatively high fluoride availabilities remaining throughout their complete shelf-lives.

Laboratory methods for monitoring product quality in terms of fluoride content using ion selective electrode techniques are rapid, reliable and easy to perform.

The caries-protective effect of fluoridated milk on experimental animal caries under programmed feeding conditions, showed a moderate caries-preventive effect which did not depend on its fat content. Fluoride concentrations of 5–15 ppm F as CaF₂, NaF, Na₂-monofluorophosphate or Na₂-silicofluoride caused a significant caries reduction of 40–50% and did not depend on the compound or concentration of the fluoride used. The bioavailability of fluoride was not reduced by milk, and a low accumulation of fluoride was measured in the enamel. The composition of dental plaque was not influenced by the fluoride dosage, but milk probably effected an increase in Actinomyces. It was concluded that fluoridated milk keeps a permanently low level of ionized fluoride within the oral cavity, promoting remineralization. It is likely that this topical mechanism contributes to the caries-preventive effect of fluoridated milk. On the other hand, fluoride uptake from milk into the developing enamel and dentine of sheep incisors supports also a systemic effect, and points to the finding that ionization levels of fluoride in milk are not relevant to subsequent fluoride absorption and physiological availability. Finally, human fluoridated milk enamel biopsy and amniotic fluid studies have confirmed fluoride’s dual mode of action i.e. topical and systemic.
On the basis of human dental caries-inhibiting studies it is evident that the preventive effect of fluoridated milk was greater the earlier in the child’s life the consumption commenced. Furthermore, the caries-reducing effect of fluoridated milk appears to be comparable to that of other community-based fluoride vehicles.

Based on experience gained in Bulgaria, Chile, China, Russia and the United Kingdom, legislation-related guidelines have been identified and may be of assistance for any who may wish to establish a new community-based milk fluoridation programme.

In addition, details of the urinary fluoride monitoring procedure, now recognised as mandatory with respect to safety and compliance assessment of any fluoridation programme, are presented.

None the less, there would still seem to be a series of unanswered questions, and additional studies should be performed to determine:

i) the age at which it is best to start drinking fluoridated milk;

ii) for how many years it should continue;

iii) the frequency of consumption;

iv) the optimum concentration of fluoride to be added;

v) the anti-caries effect of milk and milk products alone.
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