Monitoring of renal fluoride excretion in community preventive programmes on oral health

Edited by: T.M. Marthaler

Oral Health Programme
Department of Noncommunicable Disease Surveillance
World Health Organization
Geneva, 1999
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Dr C.E. Ketley, Clinical Dental Sciences, School of Dentistry, University of Liverpool, U.K.

Dr A.G. Kolesnik, Department of Oral Disease Prevention, Central Research Institute for Stomatology, Moscow, Russian Federation

Professor T.M. Marthaler, Centre for Dentistry, University of Zurich, Switzerland

Dr G.N. Pakhomov, Oral Health Programme, Department of Noncommunicable Disease Surveillance, World Health Organization

Dr P.C. Phillips, Borrow Dental Milk Foundation, Portsmouth, U.K.

Dr Wang Weijian, School of Stomatology, Beijing Medical University, People’s Republic of China

Dr A.E. Villa, Instituto de Nutricion y Tecnologia de los Alimentos (INTA), University of Chile, Santiago, Chile

Mrs S.M. Woodward, Borrow Dental Milk Foundation, Portsmouth, U.K.
Preface

The World Health Organization's policy on fluoride has been clearly defined in three World Health Assembly Resolutions (WHA22.30, WHA28.64, WHA31.50) and in a number of reports within the WHO Technical Report Series, various methods and programmes of fluoride use were explored in this series. The first reports of a reduction in dental caries prevalence related to the fluoride content of drinking water appeared in the 1930s. Numerous studies have demonstrated a substantial reduction in the level of dental caries as a result of water fluoridation.

Today, salt fluoridation has been introduced in many countries around the globe. A number of studies have shown that the effectiveness of fluoridated salt in inhibiting caries is of the same order as that of fluoridated water.

Encouraging results have been reported from the WHO Integrated Milk Fluoridation Programme. Large-scale community programmes on milk fluoridation are currently being implemented in Bulgaria, Chile, the People's Republic of China, Peru, the Russian Federation and the U.K. South Africa and Thailand will soon join this Programme.

A decline in the prevalence of dental caries during the past 20 years can also be attributed to the widespread use of fluoridated toothpastes. Regular use, in combination with one of the above mentioned systemic methods of fluoride administration, maintains higher fluoride levels in the mouth, thereby strengthening the dental caries preventive effect.

At the same time, evidence shows that it is not possible to obtain effective fluoride-based prevention without the development of some degree of dental fluorosis (WHO Technical Report Series No.846, 1994, p.14). Therefore, public health administrators should be made aware of the fluoride exposure level in a population before introducing any additional fluoridation/supplementation programme(s) for dental caries.
prevention. Total fluoride exposure of individuals or populations can be monitored by assessing fluoride concentration in plasma, urine or ductal saliva. Although the amount of fluoride in these biological liquids does not directly correspond to the total value of fluoride accumulated in the body, it is indicative of the level of general fluoride exposure. Even though urinary fluoride excretions, as well as concentrations, are more variable than those found in plasma, they are determined by non-invasive methods and consequently are more suitable for monitoring community fluoridation schemes. Professor T.M. Marthaler has pioneered standards for measuring fluoride excretion in populations covered by salt fluoridation programmes. During the last decade, unique field experience has been gained in implementing salt and milk fluoridation when these programmes were monitored by evaluating fluoride excretion in urine. The following example from this experience is given to demonstrate the value of this method not only for demonstrating the efficacy of the programme but also in avoiding potential side-effects. A decision was tentatively made by local health authorities to introduce systemic administration of fluoride in one of the low socio-economic communities with low fluoride levels \((F = 0.26 \text{ ppm in drinking water})\) and a relatively low level of dental caries \((\text{dmf of } 3.4 \text{ at 6 years and DMFT of } 3.0 \text{ at 12 years})\). In addition, fluorosis was rare and measured only at «very mild» or «mild» levels. This observation together with an average fluoride urinary concentration of 1.4 mg/L in pre-school children strongly suggested that an important source of fluoride was present in the pre-selected community. This source was identified as accidental or intentional fluoridated-toothpaste ingestion by pre-school children. Research staff provided corresponding advice and warnings to local health authorities to control this undesirable situation. After considering these observations, a decision was made that the community was not suitable for implementation of an additional fluoridation programme, and a relevant health education programme was recommended with emphasis on the appropriate use of fluoridated toothpaste.
In view of the above the WHO Oral Health Programme initiated a WHO Consultation on a Standard Method for the Determination of Fluoride Intake which took place in Dundee, Scotland from 4-5 July 1997. The participants of the WHO Consultation were scientists directly involved in preventive programme monitoring of fluoride measurements in urine. The main objective of the WHO Consultation was to study available data from literature and various WHO projects and to develop a set of recommendations on the possibility of using excreted fluoride as a marker to indicate optimal use to attain high level dental caries preventive effects, to possibly predict occurrence of dental fluorosis, and to assist public health officials on making decisions to relate to appropriateness of implementing a systemic fluoridation programme with minimum risk of producing occurrence of unsightly fluorosis.

I believe that this publication, as a result of the WHO Consultation, will be of practical value and interest to oral health and health personnel responsible for, or involved in implementing community fluoridation preventive programmes worldwide.

Dr G.N. Pakhomov
Responsible Officer
Oral Health Programme
Department of Noncommunicable Disease Surveillance
World Health Organization
Acknowledgements

The World Health Organization/Oral Health Programme would like to thank the participants of the WHO Consultation on the completion of their work and the publication of this document. We are greatly indebted to Professor T.M. Marthaler for his valuable contribution in the revision and editing of the manuscript. We would like to thank Dr R. Baez who has extensive experience in conducting studies on urinary excretion for assisting this work.

We wish to express our gratitude to the Borrow Dental Milk Foundation, Portsmouth, U.K. for their collaboration and generous support.
1. Introduction

Many studies have demonstrated the effectiveness of fluoride in reducing dental caries, particularly through topical mechanisms. The principal aim of community-based public health programmes for preventing dental caries is therefore to use the most appropriate means of raising fluoride levels in as many mouths as possible as frequently as possible. It is now widely believed that this is most conveniently achieved by means of fluoride toothpaste, used several times a day, either alone or in combination with other sources of fluoride, including drinking-water, milk, salt, and fluoride drops or tablets. However, the possibility that this approach will give rise to undesirable side-effects, including dental fluorosis (unsightly mottling of the teeth), must always be borne in mind, and public health administrators should assess the total fluoride exposure of the population before introducing any additional fluoridation/supplementation programmes for caries prevention. The interested reader can find further details and background information in WHO’s Technical Report TRS 846 Fluorides and Oral Health (WHO, 1994).

This document aims to provide recommendations for:

- determining baseline levels of fluoride and the appropriateness of ongoing caries-prevention programmes;
- the use of fluoride in urine as a marker of fluoride exposure and of the suitability of fluoride supplementation.

1.1 Sources of fluoride intake in humans

The following points are important in any consideration of human exposure to fluorides:

- Most fluoride occurs in the form of chemical compounds, and the availability of free fluoride ions in soils and water is not uniform.
- Although all waters contain fluoride in varying concentrations due to diverse geological conditions, major differences may exist
within a relatively small area and at different depths in boreholes.

- Significant environmental pollution with fluoride may be caused by industrial emissions, the burning of coal, and the use of fertilizers and pesticides.
- The fluoride content of foods and beverages may be substantial and is significantly affected by its concentration in the water used during processing.

### 1.2 Fluoride metabolism and excretion

The fluoride content of the human body is determined by certain physiological relationships:

- Absorption of fluoride from the stomach occurs readily and is inversely related to the pH of gastric contents.
- Fasting plasma levels of fluoride (mol/litre) in healthy young or middle-aged adults are approximately numerically equal to the fluoride concentration of the drinking water (mg/litre) they have habitually ingested over the past few years.
- Approximately 10–25% of the daily fluoride intake is not absorbed. Of the fluoride that is ingested (or occasionally inhaled), about 50% is excreted via the urine during the following 24 hours, and almost all of the remainder will become associated with calcified tissues. (These values are the subject of continuing research and are not relevant to the surveillance envisaged in this document.)

On the basis of these relationships, the most reliable means of monitoring the recent total fluoride exposure of individuals or populations is by determination of fluoride levels in plasma or in urine or ductal saliva, which can be obtained by non-invasive means. Fluoride levels in ductal saliva are 80% of those in plasma. Urinary fluoride concentrations and fluoride excretion are not only dependent on plasma
levels but also influenced by urinary flow and pH and by other minor factors. Essentially, the rate of urinary fluoride excretion is dependent on the amount of bioavailable fluoride ingested, i.e., the fluoride actually absorbed by the organism and distributed in the bloodstream.

From data obtained in relation to ingested fluoride (systemic) — mainly via water, milk and salt and in the form of tablets — it is possible to develop excretion thresholds, i.e., thresholds that should not be exceeded if unsightly dental fluorosis is to be avoided. In projects concerned with fluoride added to milk and to salt, urinary studies will identify instances of insufficient excretion, indicating sub-optimal fluoride levels. Most importantly, studies of urinary fluoride provide a simple means of ensuring that total fluoride intake from all sources does not exceed certain limits, which is especially crucial in young children whose permanent anterior teeth are developing and in whom unsightly fluorosis should be avoided.

1.3 General usefulness of urinary fluoride measurements

Fluoride is a natural constituent of all types of human diet and is present, in varying amounts, in drinking water throughout the world. Because of its value in preventing dental decay, it is being used increasingly for this purpose in most countries. For both these reasons, fluoride intake varies widely across populations; optimal intake provides effective protection against caries without causing mottling of the teeth. Dental fluorosis is the only untoward effect of the use of fluoride in preventive dentistry, but is known to occur spontaneously in regions throughout the world where drinking water contains high levels of fluoride. Since ingested fluoride from all sources — whether deliberately or unintentionally ingested — is excreted primarily in the urine, studies of urinary fluoride levels are ideal for assessing the intake of fluoride by entire populations. More particularly, they also provide a basis for decisions on the use of fluoride for caries prevention, in the light of national or regional conditions.
Monitoring of renal fluoride excretion in community preventive programmes on oral health
2. General design of study

The rate of urinary excretion of fluoride varies throughout the day and night in relation to the time of fluoride ingestion, particularly where fluoridated toothpaste is used and may be swallowed during brushing, and where a fluoride supplement is given once a day, in the form of tablets or fluoridated milk for example. When urine is to be analysed to evaluate fluoride excretion, it is therefore important to cover as much of the 24-hour period as possible. When this is not possible, shorter periods or spot samples may be used, but the limitations of this approach must be recognized.

The recommendations given here for study design deal primarily with surveillance at the community level and field trials.

2.1 Identification of study group

A study of urinary fluoride excretion may be undertaken for any of the following purposes, and it is important that the scale of the study is established before sampling begins:

- **Community surveillance.** Ideally, the study population should be selected randomly, since elements of the diet that affect fluoride intake (e.g., water, salt, dietary habits) may vary with area and with socio-economic status. Where complete randomization is impossible, a careful selection must be made from multiple sites taking into account special or predominant regional dietary habits that may exist.

- **Field demonstration trial.** The study group should consist of individuals selected from the population concerned; additional subjects may be chosen from a reference area with a known low fluoride exposure.

- **Research project.** The selection of individuals will be determined by the nature and design of the study.
2.2 Sampling

Sampling procedures should fulfill the following criteria:

- **Number of subjects.** The study group should comprise a minimum of 30 subjects; however clusters of 45 or 50 children are preferable to compensate for inevitable absences, non-compliance or incomplete samples, beyond this, numbers depend on the type and level of study (see section 2.3).

- **Location.** The study site should be representative of the district, province, or country in terms of fluoride exposure and dietary habits.

- **Age group.** The study population should include all age groups likely to be involved by any planned fluoridation programme, with certain target ages:
  
  - 12 months to 4 or 5 years, which includes the period of highest susceptibility to dental fluorosis; for example 2, 2-3, 3, 3-4, 4, 4-5, or 5-6; these age groups have the highest priority;
  
  - school age, with ranges spanning not more than 2 or 3 years; supervision of urine collection is easiest in this case;
  
  - adolescents;
  
  - young adults, 18-25 years;
  
  - adults 25-60 years.

- **Frequency.** Urinary fluoride excretion studies on the target population should be conducted:
  
  - before the start of the programme;
  
  - 6 months after the start of the programme;
  
  - 24 months after the start of the programme.

- **Time of study.** Extremes of seasonal weather conditions should be avoided.
2.3 Levels of monitoring

The principal aim of urinary fluoride studies is the collection of data that can be collated in a table similar to Table 1.

Whenever possible, 24-hour urine samples should be obtained, which are independent of dietary habits, timing of meals, and periods of maximal fluoride intake. In certain circumstances, however, it may not be feasible to collect urine over an entire 24-hour period or samples may be unreliable. The lifestyle or level of organization and cooperation of the population concerned may favour or suggest alternative ways to obtain 24-hour estimates. Five levels of study are presented in Table 2; clearly, the highest level is the study in which reliable 24-hour urine samples can be analysed, and the lowest covers fluoride analysis in spot sample of urine. Sections 2.3.1 and 2.3.2 elaborate on the advantages and disadvantages of each level of assessment.

In all studies that are not based on 24-hour urine collections, information on the timing of maximum fluoride ingestion is important. Fluoride supplementation other than through drinking water usually results in peaks of fluoride excretion at certain hours. After ingestion of fluoride tablets, fluoridated milk, or food containing fluoridated salt, maximum excretion levels are generally reached within 30-180 minutes; the same is true of toothpaste swallowed during brushing of the teeth. Information on eating habits allows for better planning of any study, and facilitates interpretation of results.

From sections 2.3.1 and 2.3.2 it is clear that, except for simple 24-hour urine collections, there are many factors that have to be considered, all of which will tend to complicate the assessments. Tables 3 and 4 further demonstrate that statistical handling of the data is quite complex and demands both a reasonable working knowledge of statistical techniques and some experience in handling computer spread-sheets (although raw data may be sent to an evaluation centre — an option that is discussed later). Straightforward 24-hour urine collection should therefore be carried out whenever possible.
2.3.1 Methods based on time-controlled urinary collections

F24h

The question of whether or not urinary fluoride excretion in a given population is at the desired level can be answered by straightforward evaluation of 24-hour urine samples; calculations are simple (see Annex, Table A.1). However, it can often be difficult to obtain complete urine collection over the entire 24-hour period: subjects — both children and adults — may on occasion pass urine in the normal way, forgetting that it should be collected. This is the main source of error, and results in underestimation of fluoride excretion. On the other hand, efforts to find subjects who are «dependable» in this respect may introduce bias in the sense that the final study group is not truly representative of the population as a whole.

For particular purposes, such as to establish the time of day at which fluoride elimination is highest in the population of interest, it may be useful to collect urine separately for specific periods (e.g., morning, afternoon, night), or to analyse each micturition separately. Figure 1 illustrates the example of a child who drank fluoridated milk at 08.30. In this subject, excretion levels were high for 8 hours; in other studies, a more rapid return to the «base» or resting level has been observed.

Before 1990 there were few studies based on 24-hour urine collection, but several have been published since then (e.g., Rugg-Gunn et al., 1993, 1998; Pucci, 1997). Rugg-Gunn and colleagues reported the successful use of plastic bottles containing 2.5 ml of 20% chlorhexidine digluconate to minimize bacterial growth in 24-hour urine collections and eliminate the consequent bad odour. Thymol crystals have often been used for this purpose but are only partially effective.

F16h

Collections of urine over intervals of between 12 and 20 hours should cover, as far as possible, three distinct periods in all subjects:
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- «HI»: a supervised sample collected during the period when maximum excretion is expected; if supplementary fluoride is given, in tablet form or in fluoridated milk, collection must begin within the first hour after its ingestion.

- «LOW»: a supervised sample taken during another part of the day and not preceded by high fluoride intake.

- «NOC»: a sample taken during the night (i.e., during the period of sleep); children (or their parents) should bring this sample to school the following morning.

Supervised collection of urine from children is generally possible in school and preschool institutions. Assuming that supplementary fluoride is ingested in the form of fluoridated salt or milk, the three samples should be collected from children according to the following scheme:

- LOW (supervised) — during the morning hours of school;
- HI (supervised) — after the main fluoride intake (in the form of a drink of fluoridated milk or a meal containing fluoridated salt);
- NOC — during the night.

A complete series of three samples should ideally be collected from each subject. Average fluoride excretion levels should generally be similar in the LOW and NOC samples; if they differ widely, efforts should be made to determine why. Table 3 shows results obtained from a complete series of collection from 40 children who each received 5.0 ppm fluoride in milk.

In highly industrialized societies with widely divergent lifestyles, however, it may be difficult to obtain such a complete series of samples because of the strong tendency to encourage individual decision-making, sometimes by the children themselves, regarding cooperation, or because of parents' fears that children are under inappropriate pressure to provide urine samples at predetermined times. In one recent study (Aeschbacher, 1995), 74 parents completed questionnaires and provided informed
consent to the collection of urine. This should have yielded complete sets of three urine sample taken on two different days, i.e., a total of 444 samples \((3 \times 2 \times 74)\). In fact, only 315 valid samples were available for statistical evaluation; 33 samples did not match urinary flow expectations, 11 night samples either began or ended outside the time limits that had been set, and on 85 occasions no urine was obtained. The number of children who provided the full set of six samples was only 47.

In some developing countries, half the children in a community go to school in the morning (from 07.00 to 11.00) and half in the afternoon (from 12.00 to 17.00). It is therefore likely that the first group will provide morning and nocturnal urine samples and the second afternoon and nocturnal samples; in such circumstances, acceptance of these «incomplete» series of samples may be the only way to obtain data.

Whether a subject provides «complete» or «incomplete» series of urine collections has no bearing on the statistical value of that subject in representing the study population. The higher the percentage of subjects who provide incomplete series of samples, the greater is the advantage of including these incomplete series in the evaluation.

Occasionally, it may be more appropriate to local circumstances to collect samples during four periods. Where this is the case there is even greater advantage in using incomplete series in the evaluation, because a higher percentage of subjects will have one or more «unsuccessful» collections.

**F8h**

When it is difficult to obtain three collections covering a total of 12–18 hours, two collections extending over as many hours as possible should be the objective. Urine may be collected at school or at work, preferably under supervision, with total duration of around 8 hours. Two different collections are necessary, one following the main intake of fluoride (HI) and one at another time (LOW or NOC). This may be the only possibility of studies undertaken in poor areas of large cities, where
it may be difficult to secure the cooperation of the population. Nocturnal samples are to be preferred: high nocturnal fluoride excretion is almost invariably indicative of high 24-hour fluoride excretion and thus of high fluoride intake.

2.3.2 Methods based on spot samples

When spot urine samples are collected, there are two important considerations:

- Spot samples must be taken at all times of day. In a project where fluoridated milk is given at noon, for example, use of morning samples alone would obviously yield an underestimate of average 24-hour fluoride excretion.

- Short periods with high fluoride concentrations are common, although both fluoride and plasma levels of fluoride are in fact low during about two-thirds of the 24-hour period. Urine that has accumulated in the bladder over a short period may thus reflect a short-lived peak level. The longer the urine is retained in the bladder, the more «representative» it is of 24-hour results.

$F/\text{creat}$

It is well established that total daily urinary creatinine excretion is relatively constant (approximately 1–1.5 g in adults), and that variations from day to day and within any 24-hour period are small. Thus, if a spot sample of urine is unusually diluted or unusually concentrated, its creatinine content will be unusually low or unusually high. Since standards for 24-hour creatinine excretion are readily available (by gender and for all ages), the fluoride/creatinine ($F/\text{creat}$) ratio can be used as an index of 24-hour urinary fluoride excretion.

$F-\text{conc}$

Note: This method cannot be used as a basis for determining 24-hour fluoride excretion.
In populations in whom, baseline and two-year studies have been carried out and have revealed satisfactory fluoride excretion levels, it may be sufficient for further routine monitoring to be carried out by the F-conc method. When the average fluoride concentration does not change in subsequent studies, it may be assumed that average excretion has also remained unchanged. It is important, however, that spot urine samples are obtained at the same times of day as the samples in the original excretion studies. Concentrations determined at various times of day and night should be considered separately and should be compared only with results obtained for the same times in earlier studies.

The main statistical measure for the 24-hour period is the mean of the average concentration at the different times. The results are easy to interpret since, for subjects aged 8–60 years, urinary fluoride concentration is almost totally independent of age.

### 2.4 Number of subjects

For 24-hour fluoride excretion (method F24h), the number of subjects in each age group and location should not be less than 30. The study should therefore start with 40–50 subjects, since some will provide incomplete data or drop out of the study altogether. Projects based on the F16h or F8h methods should involve somewhat larger numbers.

The number of subject should be substantially greater in studies based on spot urine samples: at least 50–70 samples should be available for each point of time. For the F-conc method, the ease with which the samples are obtained may allow even larger numbers, perhaps in excess of 100.
3. Methods for urinary sampling, and handling and evaluation of results

3.1 Recording of information

Comprehensive recording of all data, both general and personal, on fluoride exposure is essential. General information covers all the factors that bear on the entire study group, community or region; personal data, including information on fluoride exposure and intake at home whenever possible, must be obtained on an individual basis.

3.1.1 General information

The fluoride content of the drinking water must be ascertained; where fluoridated milk is involved, the quantity and time of consumption must be established.

If fluoridated salt is consumed it is essential to determine whether fluoride is added:

- to domestic salt only
- to the salt used by bakeries
- to the salt used in large kitchens (restaurants, workplace canteens, hospitals, etc.)
- to the salt used by the food industry.

Data on the actual fluoride content of salt, which has often been found to be below the required level (usually specified by law or other official regulations and currently in the range 200–250 mg of fluoride per kg), are crucial. Information on salt intake, often estimated from yearly salt «disappearance» (production + imports/exports) or from quantities sold, is also relevant. Moreover, it must be recognized that only a proportion of the salt used in households or in large kitchens is actually ingested; only some 40–70% of the «consumed» amount of salt is in fact ingested, and this proportion can vary widely between different cultures.
3.1.2 **Personal information**

The following details should be recorded for each subject included in the study:

- name and identification number
- age
- gender
- body weight
- use of fluoride supplements (whether prescribed or available over the counter tablets or drops; daily vitamins reinforced with fluoride dose; habitual time of administration, age at which children started receiving fluoride supplements and for how long)
- use of fluoride toothpaste (brand name, fluoride concentration; frequency of use; quantity used, supervised teeth brushing, age at which children started using toothpaste, use of low-fluoride product)
- in salt-flouridation programmes, type of food or meal eaten
- frequency of consumption of high-fluoride mineral waters (where these are available).

If coverage with fluoridated salt or milk is not universal, the use of fluoridated or unfluoridated products must be recorded (and coded) for each individual. Additional information may include the use of other fluoride-containing products for oral care (e.g., mouth rinses, regular topical application by dental professionals), the fluoride concentration in these products, and the frequency of their use.

A simple coding system, such as the following, should be established:

- **Use of fluoridated milk (or salt):**
  - no = 0  yes = 1
• Use of fluoridated toothpaste:
  never = 0  sometimes = 1  once a day = 2
  at least twice a day = 3

• Use of prescribed/over the counter fluoride supplements:
  never = 0  sometimes = 1  daily = 2  (help)

Coding may also be used to distinguish the use of low-fluoride toothpaste from that of «regular» fluoride (1000–1500 ppm) toothpaste.

In this context, it is important to differentiate clearly between «fluoride exposure» and «fluoride intake». Exposure is a general term, indicating availability and/or frequent use of fluoride; intake is more specific, referring to the ingestion of an amount of fluoride that is known approximately (or may even be measured).

3.2 Collection and analysis of samples

When all urine over a 24-hour period is to be collected, preschool children should be supplied with a 1500-ml jar or plastic bottle; for older children and adults a 2000-ml container is preferable. For shorter, time-controlled urine collections during daytime, jars or bottles of 500-ml capacity are appropriate; nocturnal collections may require 1000-ml containers. (Details of the collection procedure and of the labelling of containers can be found in the Annex.)

3.3 Determination of fluoride in urine

Simple and reliable methods for determination of urinary fluoride concentrations are well established, and details of materials and procedures are given in the Annex. However, standardized methodology and strict quality control are essential.

3.4 General rules for tabulation and processing of data

For these recommendations, a standard format has been devised for tables, to illustrate the recording and evaluation of field and laboratory data. This format was developed in a variety of actual working conditions
and has been thoroughly tested; it has been used for Tables A.1 and A.2 (see Annex). Various parts of the Annex provide explanations of part of the computations programmed into the table and of some of the mathematical/statistical details.

3.4.1 Coded recordings of personal data and individual fluoride exposure

In Tables A.1 and A.2, the table titles and column headings, occupying the first nine rows of the tables, are self-explanatory. Column numbers are given in row 10. Data for the first subject are then recorded in row 11, for the second subject in row 12, and so on. (In computer spreadsheet programs, the rows are numbered.) This facilitates the checking of data in studies involving large numbers of subjects; for example, if a study involves 37 subjects, data for the last subject will occupy row 47.

Columns are used in a similarly systematic manner, with columns 1–10 reserved for personal data:

Column 1 Subject number.
Column 2 Gender (male = 1, female = 2).
Column 3 Age (years) at last birthday.
Column 4 Body weight (kg).
Columns 5–10 Coded data on individual fluoride intake or fluoride exposure and other relevant information.

Column 11 is a repeat of column 1 and pertains to the block of columns 11-20.

3.4.2 Standard table for level F24h

In Table A.1, field and laboratory data, i.e., data at the beginning and end of the collection period, urine volume, and fluoride concentration, are entered in columns 12–15:

Column 12 Time (format: hhmm – it is important to emphasize that no commas, fullstrokes or colons are
used) at the start of urine collection, i.e., time at which subject emptied his or her bladder (this urine is not time-controlled and is therefore discarded unless it pertains to and terminates a preceding collection).

Column 13  Time (format: hh:mm – it is important to emphasize that no commas, fullstops or colons are used) of final micturition into the container.

Column 14  Volume (ml) of urine.

Column 15  Fluoride concentration (ppm).

The data that must be entered into the table are given in italics and contained within two frames (covering columns 1-10 and 12-15). Table A.1 has been completed for six subjects to illustrate how it should be used.

Results computed from the field and laboratory data in columns 12-15 are presented in columns 16-24:

Column 16  Duration (format hh.decimals) of the collection; for subject 1, duration is 23.25 hours (23h15 min). Conversion to hours/decimal hours is essential for subsequent arithmetic procedures.

Column 17  Quantity (µg) of fluoride in the urine collection, obtained by multiplying the value in column 14 by that in column 15. For subject 4, the quantity of fluoride is 600 x 0.4 = 240 µg.

Column 18  Correction factor to yield exact 24-hour values, obtained by dividing 24 by the duration (column 16). For subject 4, the factor is 24/24.67 = 0.973.

Column 19  Corrected 24-hour urinary volume (ml), obtained by multiplying the volume collected (column 14) by the correction factor (column 18). For subject 2, who collected 710 ml of urine, the corrected volume is 710 x 0.973 = 691 ml.
<table>
<thead>
<tr>
<th>Column 20</th>
<th>Corrected 24-hour fluoride excretion (µg), obtained by multiplying the quantity of fluoride in the urine actually collected (column 17) by the correction factor (column 18). For subject 2, the corrected fluoride excretion is 256 µg x 0.973 = 249 µg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 21</td>
<td>Urinary flow (ml/hour), obtained by dividing the value in column 19 by 24.</td>
</tr>
<tr>
<td>Column 22</td>
<td>Hourly urinary fluoride excretion, obtained by dividing the value in column 20 by 24. Hourly values are useful for comparison with results of studies limited to certain parts of the 24-hour period.</td>
</tr>
<tr>
<td>Column 23</td>
<td>24-hour urinary volume per kg body weight, obtained by dividing the value in column 19 by that in column 4.</td>
</tr>
<tr>
<td>Column 24</td>
<td>24-hour urinary fluoride excretion per kg body weight, obtained by dividing the value in column 20 by that in column 4.</td>
</tr>
</tbody>
</table>

For subject 4, the data in columns 12-15 were chosen for ease of computation. Repeating the calculation may facilitate an understanding of the results shown in columns 16-24.

Below each column that shows data for individual subjects, basic statistics are displayed automatically. Minimum and maximum values are given and must always be examined to identify «outliers», which may be the result of gross recording or methodological errors (refer to section 3.5). Median values and arithmetic mean values are also given; median values are generally slightly lower than mean values since it is quite common for very high values to be obtained from a few individuals, resulting in a skewed distribution. Values for standard deviation (SD), coefficient of variation, and standard (error) are also presented.
For convenience in studies of circadian variations, 24-hour urine may be collected — and separately analysed — in two (day and night), three, or even four separate periods, in which case Table A.1 cannot be used. These data are not particularly easy to handle; the amount of fluoride in the collection, for instance, must be computed for each time period and not averaged over the periods. Tables are available for use with these data, using the same format as in columns 1-10, 12-15, 22-25, etc. (refer to section 3.7).

3.4.3 Standard table for level F16h, incomplete series

As in Table A.1, columns 1-10 of Table A.2 are reserved for personal data and the codes relating to individual fluoride exposure, and column 11 repeats the subject number. For the first collection period, columns 12-15 are again used for recording field and laboratory data, with the same use of italics and boxes as in Table A.1. In the example given, the first collection took place in the morning, from about 09.00 to 11.00; results for this period are given in columns 16-20.

**Column 16**  Validity code. If the initial time, the final time, the volume, and the fluoride concentration of the collection (columns 11-14) are available, the code 1 — valid — appears in column 15. If one or more of these values are missing, the code in column 15 is 0, indicating an «unsuccessful» collection. It may be the initial or final time of the collection that is missing (or both), the collected urine may be lost, or the fluoride concentration unavailable. It is essential that midnight is recorded as 24.00, not as 00.00, since any value equal to 0 in any of the columns 11-14, renders a collection invalid. Whenever the code in column 15 is 0, an «x» is automatically assigned to columns 16-19.

**Column 17**  Time at initial voiding, only for those children whose urine collection was successful.
Column 18  Duration (format hh.decimals) of the collection.
Column 19  Urinary flow (ml/hour).
Column 20  Urinary fluoride excretion (μg/hour).
Column 21  Subject number (automatically presented).

Data from the second collection period are entered in columns 22-25 (not shown) of Table A.2. Columns 26-31 then correspond exactly to columns 16-21.

For the third collection period — in this example, nocturnal urine — the same pattern of data recording is repeated. As for Table A.1, the row for the third subject (No. 56) shows data that have been adapted for ease of computation.

Results regarding the 24-hour estimates are automatically presented in columns 42-78. The compressed version in Table A.2 shows only the contents of columns 47 to 57 (cn47-cn57).

From columns 48-57 in Table A.2, it is evident that only five children among the nine had complete series of three collections, covering between 12.88 to 18.23 hours (hours.decimals) of the full 24-hour cycle.

Table A.2 is convenient for the original recording of results at the F16h level using three separate collection periods. In its full version (not compressed) comprising 78 columns, it allows:

- determination of subjects who provide three, two, or only one collections;
- identification of gross errors (immediately apparent in the min. and max. rows);
- a decision on whether the definitive evaluation can be restricted to the subjects who provided all three collections, i.e., a complete series.
3.4.4 Standard table for level F16h, complete series

A table of the same format as Table A.2 which is designed to process data from subjects for whom all three collections are valid is also available. It is generally preferable to Table A.2 when at least 80% of the subjects provide complete sets of three valid collections (refer to section 3.5 for more details). Original data (columns 1–9, 11–14, 21–24, and 31–34) for subjects with complete series can be copied from Table A.2 into the table for complete subjects.

3.4.5 Standard table for level F8h and special cases

If only two collections (HI and NOC) are obtained, columns 11–20 and 21–30 are used as in Table A.2, and the field and laboratory data are entered in columns 12–15 and 22–25. However, there is no third collection corresponding to columns 32–40. Formulae for the 24-hour extrapolations correspond to those of Table A.2.

When urine is collected during four periods, Table A.2 must be enlarged; columns 42–50 are then reserved for the fourth collection period, and the columns for 24-hour extrapolations are shifted accordingly. Details for these study levels are not given here.

3.4.6 Standard table for level F/creat

Columns 1-10 contain the personal data and codes for fluoride exposure as explained in section 3.4.1. Column 11 may be used for the fluoride concentration of the first morning urine, column 12 for the creatinine concentration in the same spot sample, and column 13 for the F/creat ratio. Columns 14 and 15 may be used for the estimated total 24-hour fluoride excretion for each subject and the corresponding fluoride excretion/kg body weight.

Data relating a second spot sample, obtained during the late morning for instance, may be recorded in columns 16 and 17, and the computed values in columns 18–20. Columns, 21–25, 26–30, and so on may be used similarly for samples obtained later in the day.
3.4.7 Standard table for level F-conc

Columns 1-10 again follow the standard format for recording personal data. Columns 11, 12, 13, etc. are used for the concentrations at various times of the day (e.g., column 11 for the first sample obtained on waking, column 12 for spot sample obtained between 09.00 and 12.00, and so on).

3.5 Cleaning of data

When all data have been entered into the appropriate table and all parameters have been computed (automatically for the most part), the next step is cleaning of the data. This process begins with inspection of the minimum and maximum values, displayed in all tables in the two rows immediately below that in which the number of subjects is recorded.

In 24-hour studies, the minimum volume of urine collected is inspected to provide information about incomplete collections. If the volume produced is less than 140 ml it is likely that the urinary collection is incomplete (see Table 3). There is no direct method for ascertaining that a child who provided only 150 ml in 24 hours did in fact pass all urine into the collection bottle. However, the total amount of creatinine in the supposed 24-hour collection is a useful indicator, and medical reference books provide standard values for 24-hour creatinine output for all ages.

In level F16h and F8h studies, cleaning of data must be done carefully; additional criteria may be established. For instance, the protocol may require that, for nocturnal urine collection in children, the last urine before sleep must be passed between 19.00 and midnight and the first morning urine between 05.00 and 09.00. A nocturnal collection beginning at 18.30 for example would be excluded according to these limits. Any such criteria for data cleaning must be established during the planning stage and must be clearly stated in the protocol.

The minimum and maximum values shown just below the number of subjects immediately reveal «suspect» data or values that are obviously
outside expected limits. Suggested limits for urinary flow, creatinine concentration, fluoride concentration, and fluoride excretion rate are given in Table 4.

When there are suspect results, the subject should be identified and the original record examined to determine whether there was a typing error or whether something went wrong with the collection or with the laboratory analysis. Only typing mistakes and obvious gross errors should be corrected in the original data table; other suspect data should be left unchanged. No further changes should be made to this table. However, a copy — the «clean data table» — should be made, on which suspect or doubtful data in columns 11–24, 21–24, and so on (i.e., the results that deviate significantly from normal or expected values) can be «cleaned».

Although incomplete voiding of the bladder has no influence on concentrations, time-controlled samples are susceptible to incomplete voiding at the beginning (a) and end (b) of the collection period; this is the principal source of «outliers» with very high (a) or very low (b) urinary flow.

Cleaning of outliers must be done cautiously and restricted to results that are obviously incompatible with the rest of the data. If a decision is made to exclude a particular set of results, only the time, urinary volume, or fluoride concentration are to be deleted; entries in columns 15–19, 25–29, etc. will automatically be changed to an «X».

The effect on the results of such exclusion is easy to check by comparing statistics based on the uncleaned data (in the original data table) with those based on the cleaned data (in the clean data table). Clearly, maximum and minimum values will change when outliers are excluded; averages and medians, by contrast, should change very little. Standard deviation values may decrease appreciable as a result of exclusions.

If more than 80% of the children still have three valid collections in the cleaned Table A.2, data for the children who provided complete collections may be transferred to a table designed for children with all
three collections (available but not shown here); data for the few children providing incomplete collection are thus disregarded (see section 3.4.4). If there is any doubt about whether or not to exclude subjects with incomplete collections, the number of hours covered by the collections may be considered. The reliability of the estimations is largely dependent on the number of hours covered (presented at the bottom of columns 44 and 45); 75 hours were covered by the subjects who provided complete collections. Clearly, addition of another 44 hours of collection by subjects whose collections were incomplete substantially improves the statistical precision.

The advantage of including only the children who have the full set of collections is the simplicity of the statistics. Straightforward statistical evaluations, built into the table, are done on the 24-hour parameters available from each child.

### 3.6 24-hour extrapolations

Extrapolations are based on «external knowledge» of the time of day when highest fluoride excretion rates may be expected. A single peak occurs after consumption of fluoridated milk, generally during the first 3–5 hours after milk intake. Where bread containing fluoridated salt is eaten, two — or even three — peaks would be expected, depending on eating habits. These would generally occur after breakfast and after lunch, and possibly after dinner. Peaks are unlikely in areas where fluoride is derived from fluoridated drinking water, but nocturnal fluoride excretion tends to be lower than daytime excretion.

#### 3.6.1 Extrapolations from F16h data

For extrapolation of 24-hour urinary fluoride excretion on the basis of three collection periods (LOW, HI, and NOC — see section 2.3, «F16h»), the Integral Daily Urinary Fluoride Excretion (IDUFE) model is the standard approach. Use of this model, which is highly suitable for subjects up to school-leaving age or 15 years, requires the following assumptions to be made:
• The LOW period — usually the morning hours, since daytime urine samples are influenced only minimally, if at all, by preceding high fluoride intake — is assumed to last 4 hours.

• The HI period — after ingestion of fluoridated milk or a main meal prepared with fluoridated salt — is assumed to last 8 hours.

• The NOC period — which corresponds to the nocturnal urine and is an almost complete urinary collection over the bed-rest period (typically 9-12 hours) in preschool children — is assumed to last 12 hours.

Example

Results for one particular child («David Morris» — see Figure 2 and Annex) were as follows:

<table>
<thead>
<tr>
<th>Duration</th>
<th>09.05-12.50</th>
<th>14.00-16.02</th>
<th>20.45-08.30</th>
</tr>
</thead>
<tbody>
<tr>
<td>which is:</td>
<td>3 h 45 min</td>
<td>2 h 2 min</td>
<td>11 h 45 min</td>
</tr>
<tr>
<td>µg F/h</td>
<td>20</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>

Extrapolated 24-hour excretion results (in µg F) were obtained as follows, using the IDUFE model:

\[
20 \, \mu g \, F/h \times 4 \, h = 80 \, \mu g \, F \\
30 \, \mu g \, F/h \times 8 \, h = 240 \, \mu g \, F \\
15 \, \mu g \, F/h \times 12 \, h = 180 \, \mu g \, F \\
80 + 240 + 180 = 500 \, \mu g \, F \text{ excreted in 24 hours}
\]

When complete series of collections are available from all three periods (after excluding children on the basis of data «cleaning» or because collections were incomplete), application of the IDUFE model provides an estimate on 24-hour fluoride excretion for each child. The statistical results based on the IDUFE and a second extrapolation pattern are shown at the bottom of columns 48-57 of Table A.2. Below the row of subject 63 there are eight rows with raw statistical results. In the
lowest six rows, 24 hour adjusted values, based on all valid collections, are shown.

The IDUFE model was developed for young children who often receive fluoridated milk at one particular time of day. For reasons of comparability, this model should always be applied to data for children to provide a first approximation of 24-hour parameters.

Figure 3 illustrates the principle of the IDUFE extrapolation on the basis of average hourly fluoride excretion values (Table 3). Since the data for Table 3 were derived from a milk fluoridation project, it is unsurprising that Figure 3 resembles Figure 1.

Other patterns of fluoride intake over the course of the day may be observed, for example, in the case of fluoridated salt. Particular situations may call for specifically adapted extrapolations as is evident in the following cases:

- In many tropical countries, the main meal may be eaten after sunset, when the air temperature is lower.
- Periods of high fluoride excretion may be considerably shorter than 8 hours, as indicated by a number of studies.
- For adults, nocturnal collections cover less than 12 hours - perhaps only 6–9 hours.

The use of different extrapolation patterns is illustrated in Figures 4 and 5. The following baseline values were used:

- average morning fluoride excretion 16.8 µg/h; LOW period; \( N = 37 \)
- average afternoon fluoride excretion 27.6 µg/h; HI period; \( N = 36 \)
- average nocturnal fluoride excretion 13.1 µg/h; NOC period; \( N = 37 \)
These average values were obtained from children who had their main meal, containing fluoridated (250 ppm) salt, shortly after 12.00 (Aeschbacher, 1995).

The IDUFE extrapolation is shown in Figure 4. The assumed duration of the collection periods is: LOW 4 hours, HI 8 hours, NOC 12 hours. The actual average durations are represented by the solid lines, the extrapolated periods by the broken lines. The 24-hour fluoride excretion was:

\[(4 \times 16.8 \, \mu g) + (8 \times 27.6 \, \mu g) + (12 \times 13.1 \, \mu g) = 445 \, \mu g/24 \, h.\]

Another extrapolation pattern, corresponding roughly to the actual lifestyle is illustrated in Figure 5. The period of low daytime fluoride excretion (16.8 \mu g/h) was assumed to be made up of 4 hours in the morning, 1 hour before the (smaller) evening meal, and 1 hour after this same meal, i.e., a total of 6 hours. The subsequent nocturnal period of low excretion (13.1 \mu g/h) was assumed to last for 13 hours. High excretion (27.6 \mu g/h) was assumed to prevail for a total of 5 hours — lasting for 4 hours after the main meal at noon and occurring for 1 hour with the evening meal. Using this model — 6 hours LOW, 5 hours HI, and 13 hours NOC — the 24-hour fluoride excretion was 409 \mu g, which is 8% lower than the 445 \mu g resulting from the IDUFE extrapolation.

In Table A.2, the IDUFE extrapolation model is built into columns 48 and 49. The average 24-hour fluoride excretion for the five subjects providing complete collections was 436 \mu g (see column 49). Column 53 provides the opportunity to use a second extrapolation, which is to be entered in the boxes in the top part of column 53. Extrapolation 2 (copied into column 54) assumes: LOW 7 hours, HI 6 hours, and NOC 11 hours, and yielded from the five children with all three collections an average of 412 \mu g F/24 hours — that is, 24 \mu g or 5.5% less than the IDUFE extrapolation. The results based on all valid collections are shown at the bottom of the table; average values were 378 \mu g F/24 hours (IDUFE) and 362 \mu g F/24 hours (extrapolation 2). A full-size version of Table A.2 allows for a third extrapolation, which does not appear in the compressed version of the table presented in this document.
In order to construct extrapolation models suitable for a particular population, it is essential to know the habitual diurnal pattern of meals. For example, a duration of 12 hours may be assumed for the NOC period, which actually lasted 11.57 hours; eating habits may support an assumption of 5 hours of elevated (HI) fluoride excretion — and thus of 7 hours (the remainder) for the daytime (LOW) excretion. When the extrapolation model is entered in the table (rows 3, 4, and 5 in column 52 for pattern 2 and in column 57 for pattern 3), the results are computed immediately.

Differences between groups of subjects with differing general levels of fluoride excretion remained clear-cut, even when different extrapolation models were used (Marhailer et al., 1992). This is due in part to the fact that, at least in F16h studies, the nocturnal collection and the two daytime collections covered more than half of the full 24-hour cycle.

### 3.6.2 Extrapolations from F8h data

Extrapolation of 24-hour fluoride excretion is clearly less accurate in studies on the F8h level. In an investigation by Steiner et al. (1985; results also published by Marhailer et al., 1995), urine was collected under supervision at school over somewhat less than 3 hours in the morning (LOW) and during 1 hour 20 minutes in the afternoon (HI). In addition, the children passed urine on returning to school after the lunch break, having emptied their bladders before going home for lunch and not having urinated at home; this collection covered approximately 3 hours. In a subsample of 13 children from whom complete urine collections were obtained, average measured fluoride excretion was 516 µg/day. Simple extrapolation of the results obtained for the approximately 7 hours covered by the LOW and HI periods and for the lunch period in between yielded an average 24-hour fluoride excretion of 543 µg. The ratio 543/516 is 1.05, i.e., not far from the ideal value of 1.0. When the ratio was computed for each of the 13 children, the average value was 1.17, with 95% confidence limits at 0.96 and 1.39. While the deviation from the perfect value of 1.0 was neither substantial
nor statistically significant, the reliability of F8h surveys is clearly less than that of F16h studies.

Clearly, the F8h study level will yield useful results if the diurnal pattern of meals is carefully examined in advance of the study and is fairly universally followed by the population from which the study sample is drawn. The researcher can obtain important information about the timing and nature of meals by using questionnaires, and tables for data processing can be similar to Table A.2 and A.3. Nevertheless, more research in this area is needed.

3.6.3 Extrapolations from F/creat data

The F/creat method uses spot sample to provide estimates of 24-hour fluoride excretion. Spot samples obtained 2–4 hours after ingestion of fluoride will yield overestimates, while samples taken at other times will result in underestimates. Thus, for this method too, it is essential to have information on the timing of the main fluoride intake. However, several spot samples may be obtained from the same individual, again following the pattern of nocturnal (first urine on waking in the morning), late morning, and afternoon samples. An average of the three estimates of 24-hour excretion is then calculated for each individual, and these averages are subjected to common statistical analysis.

3.6.4 Extrapolations from F-conc data

For studies based on F-conc, it is important to present the concentration statistics separately for the different periods of the day in which samples were obtained. Changes in the fluoride supply, for instance by ingestion at breakfast of bread made with fluoridated salt, may be detectable only in morning urine samples. Ideally, there should be roughly equal numbers of samples for each time day at which sampling takes place. The samples (morning, noon, afternoon, night, etc.) need not be obtained from the same people, but they should all come from people whose lifestyles and living conditions are similar. For calculation of the
overall average, all available individual concentrations may be pooled. If the number of subjects varies substantially (by more than, say, 10%) from one period to another, the overall average should be calculated as the average of the averages pertaining to different times of the day. The average concentration is to be given in the summary (Table 1).

3.7 WHO support for tabulation and analysis of data

It is obvious that the organization and evaluation of data, except in the case of studies limited to urinary fluoride concentration, are fairly complex. For this reason, the tables in the annex follow a rigid format that has proved most useful in many studies, and it is in the interests of all to make these automated tables freely available to investigators.

Provided funds are available WHO experts can assist researchers in undertaking urinary fluoride studies at F24h, F16h, and F8h levels. Indeed, Tables A.1 and A.2 were developed to automatically provide all statistical results once columns 1-4 (personal data) and columns 12-15, 22-25, etc. (field and laboratory data) have been completed. There are essentially two options for investigators, which are to:

- send their data to an evaluation centre, in clear handwritten or printed form by mail or fax, or by e-mail (the appropriate form for the transmission of the data is presented in Table A.3);
- to adapt Tables A.1 and A.2 for their own use (although it should be noted that this requires using spreadsheet software).

Apart from Tables A.1 and A.2, automated tables are also available for studies on F24h collections subdivided into two, three, or four periods, and for studies on the F8h level. Rules regarding the structure of the data are given in the Annex and Table A.3.
4. **Design of the final report**

In the case of 24-hour urine collections (F24h), the results section of the study report can be fairly brief, since methods and evaluations are straightforward. An example of a detailed presentation of results based on complete 24-hour collections may be found in a paper by Rugg-Gunn *et al.* (1993). When the 24-hour period is subdivided, results should be separately presented for the defined period of the 24-hour cycle (for an example see Obry-Musset *et al.*, 1992).

Reports of the results of F-conc studies may also be short. Only fluoride concentrations will be reported and no extrapolations will be made. In both F24h and F-conc studies, however, the fluoride exposure of the study population should be described in detail.

In all other cases, the report must be more detailed, since the reader must be able to follow the extrapolation procedures. All relevant statistics are given in Table A.1 or the full version of Table A.2.

**4.1 Text**

The *introduction* to the report should state clearly the primary — and secondary where appropriate — purposes of the study, which may range from specific research interests to routine monitoring.

The section on *materials and methods* should comply with the generally accepted recommendations for scientific articles or publications, giving the reasons for and methods of selecting the particular locations(s) of the study and the number and age of the subjects.

A separate section should provide all relevant information on the known fluoride exposure of the subjects and of the population from which they were drawn. If fluoride excretion is high in relation to the known intake, reasons for this should be suggested. Some traditional (mainly African) diets have been shown to be high in fluoride, while the intensive
cultivation in industrialized countries, combined with sophisticated food processing, leads to low dietary fluoride content.

4.2 Results, tables, graphs, discussion

The essential results of any study of this type are the 24-hour urinary fluoride excretion values, summarized when possible in the form illustrated in Table 1. All data for completing a table of this sort may be obtained from the tables given in the Annex.

Urinary data obtained for the purposes of this type of study tend to be skewed, and frequency distributions (presented as histograms or in tabular form) are useful, allowing the maximum amount of information to be extracted from the data.

In F16h and F8h studies, results obtained for the various collection periods should be presented. Urinary findings and details of collection times, as illustrated in Figures 3–5, are important. Reasons for the choice of the other two extrapolation models should be given, and differences between results obtained with these models and those obtained with the IDUFE model should be identified. In studies based on urinary fluoride concentrations (F/creat and F-conc), results obtained at different times of day should be presented separately (as well as being summarized as in Table 1).

The discussion should concentrate on matters relevant to the study design, such as the timing of principal fluoride ingestion in relation to fluoride excretion peaks.

Finally, 24-hour urinary parameters (and, if available, parameters from shorter collection periods) should be compared with the provisional standards given in Table 5.

If the study was designed to provide baseline data, before introduction of a fluoride programme, fluoride excretion data should be related to any available information on dental caries and fluorosis. In follow-up excretion studies, 6 and 24 months after the implementation of
a fluoride programme, conclusions should indicate whether urinary fluoride findings are low, high, or optimal in relation to the standards given in Table 5. In this context, it is important to keep in mind the skeletal storage of fluoride — or fluoride accretion — that is a feature of the growing organism. Fluoride excretion begins to rise immediately after the start of a fluoride programme but will not reach the steady state that reflects the higher intake for another 18–24 months.

4.3 Summary and conclusions

The summary should describe the sources of fluoride exposure and should include the summary table (Table 1). The question of whether or not special precautions should be taken to lower the fluoride intake of children under the age of 5 or 6 years should be addressed, and recommendations may be made for intensifying an existing fluoridation programme in order to achieve maximum effectiveness.
<table>
<thead>
<tr>
<th>Level of study</th>
<th>Urine collected between</th>
<th>Country/region</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Subjects</strong></th>
<th><strong>Location 1</strong></th>
<th><strong>Location 2</strong></th>
<th><strong>Location 3</strong></th>
<th><strong>Location 4</strong></th>
<th><strong>Location 5</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of successful collections</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (or median age, years (range))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration of collection within one 24-hour cycle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average duration, hours (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fluoride concentration, ppm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confidence limits ($P = 0.95$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fluoride excretion/24 hours, $\mu g/h^b$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confidence limits ($P = 0.95$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Urinary flow/24 hours, ml/h$^b$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ In studies on level F16h or F8h. For studies on the level F/creat or F-conc, the number of spot samples is recorded.

$^b$ No measurements (no estimates when fluoride concentrations in spot samples are determined (F-conc). Duration and flow are also unavailable for the level F/creat).
Table 2  Five levels of assessing urinary fluoride as a measure of fluoride intake

<table>
<thead>
<tr>
<th>Level</th>
<th>Description, remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Methods estimating 24-hour fluoride excretion</strong></td>
<td></td>
</tr>
<tr>
<td>F24h</td>
<td>24 hours' continuous collection (the preferred level whenever possible)</td>
</tr>
<tr>
<td>F16h</td>
<td>12-20 hours' time-controlled collection</td>
</tr>
<tr>
<td>F8h</td>
<td>4-10 hours' time-controlled collection</td>
</tr>
<tr>
<td>F/creat</td>
<td>Spot samples of urine, analysis for fluoride and creatinine concentrations, estimation of 24-hour excretion from the fluoride/creatinine ratio</td>
</tr>
<tr>
<td><strong>No estimation of 24-hour fluoride excretion</strong></td>
<td></td>
</tr>
<tr>
<td>F-conc</td>
<td>Spot samples of urine, analysis for fluoride concentration; excretion cannot be estimated, but comparisons with previous data from the same location may indicate changes in fluoride exposure.</td>
</tr>
</tbody>
</table>
Table 3  Summary of urine data from 40 children aged 4–6 years in Staryi Oscol (given 180 ml of milk, containing 5.0 ppm fluoride, at 12.00 daily for 5 days) during the third test period 17–18 February 1994

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time periods</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td><strong>Start of collection periods</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>08.35</td>
<td>12.54</td>
<td>20.58</td>
<td>08.00</td>
</tr>
<tr>
<td>Median</td>
<td>08.40</td>
<td>12.45</td>
<td>21.19</td>
<td>08.10</td>
</tr>
<tr>
<td><strong>Duration of collection period (h min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>2 19</td>
<td>3 24</td>
<td>9 45</td>
<td>2 52</td>
</tr>
<tr>
<td>Median</td>
<td>2 35</td>
<td>3 19</td>
<td>9 34</td>
<td>2 48</td>
</tr>
<tr>
<td><strong>Volume of urine collected (ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>63.1</td>
<td>152.9</td>
<td>178.8</td>
<td>82.8</td>
</tr>
<tr>
<td>Median</td>
<td>75</td>
<td>140</td>
<td>165</td>
<td>80</td>
</tr>
<tr>
<td>SD</td>
<td>32.5</td>
<td>74.1</td>
<td>84.0</td>
<td>50.8</td>
</tr>
<tr>
<td>Minimum</td>
<td>15</td>
<td>35</td>
<td>85</td>
<td>20</td>
</tr>
<tr>
<td>Maximum</td>
<td>200</td>
<td>400</td>
<td>470</td>
<td>300</td>
</tr>
<tr>
<td><strong>Urinary flow rate (ml/hour)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>27.7</td>
<td>47.6</td>
<td>18.3</td>
<td>30.3</td>
</tr>
<tr>
<td>Median</td>
<td>26.0</td>
<td>45.0</td>
<td>17.2</td>
<td>30.1</td>
</tr>
<tr>
<td>SD</td>
<td>13.2</td>
<td>20.0</td>
<td>8.5</td>
<td>17.2</td>
</tr>
<tr>
<td>Minimum</td>
<td>9.2</td>
<td>17.6</td>
<td>9.2</td>
<td>9.3</td>
</tr>
<tr>
<td>Maximum</td>
<td>80.9</td>
<td>88.0</td>
<td>47.3</td>
<td>85.0</td>
</tr>
<tr>
<td><strong>Fluoride concentration (ppm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.61</td>
<td>1.53</td>
<td>0.77</td>
<td>0.58</td>
</tr>
<tr>
<td>Median</td>
<td>0.59</td>
<td>1.61</td>
<td>0.79</td>
<td>0.61</td>
</tr>
<tr>
<td>SD</td>
<td>0.34</td>
<td>0.74</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.21</td>
<td>0.53</td>
<td>0.30</td>
<td>0.24</td>
</tr>
<tr>
<td>Maximum</td>
<td>2.09</td>
<td>4.68</td>
<td>1.59</td>
<td>1.36</td>
</tr>
<tr>
<td><strong>Fluoride excretion rate (µg/h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>15.2</td>
<td>61.1</td>
<td>13.3</td>
<td>15.9</td>
</tr>
<tr>
<td>Median</td>
<td>14.9</td>
<td>59.2</td>
<td>13.6</td>
<td>16.0</td>
</tr>
<tr>
<td>SD</td>
<td>7.2</td>
<td>17.5</td>
<td>6.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Minimum</td>
<td>4.4</td>
<td>33.2</td>
<td>5.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Maximum</td>
<td>40.2</td>
<td>99.9</td>
<td>38.9</td>
<td>35.8</td>
</tr>
<tr>
<td><strong>Integral daily urinary fluoride excretion (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.72</td>
<td></td>
<td>0.43</td>
<td>1.30</td>
</tr>
<tr>
<td>Table 4</td>
<td>Cleaning criteria at various ages and for various levels of fluoride exposure*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Lower limits</strong></td>
<td><strong>Typical values</strong></td>
<td><strong>Upper limits</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Urinary flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age &lt;6 years (ml/24 hours)</td>
<td>140</td>
<td>500</td>
<td>1200</td>
<td></td>
</tr>
<tr>
<td>age ≥6 years (ml/24 hours)</td>
<td>200</td>
<td>1200</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>age &lt;6 years (ml/hour)</td>
<td>5</td>
<td>20</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>age ≥6 years (ml/hour)</td>
<td>9</td>
<td>50</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td><strong>Urinary creatinine concentration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all ages (mg/ml)</td>
<td>0.1</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Level of fluoride supply</strong></td>
<td><strong>Low</strong></td>
<td><strong>“Optimal”</strong></td>
<td><strong>(Very) high</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Urinary fluoride concentration</strong></td>
<td>0.08</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>all ages (ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Urinary fluoride excretion rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age &lt;6 years (mg/24 hours)</td>
<td>0.12</td>
<td>0.5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>age ≥6 years (mg/24 hours)</td>
<td>0.2</td>
<td>1.2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>age &lt;6 years (μg/hour)</td>
<td>2</td>
<td>20</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>age ≥6 years (μg/hour)</td>
<td>3</td>
<td>50</td>
<td>300</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5  Provisional standards for urinary fluoride excretion and concentrations*

<table>
<thead>
<tr>
<th>Fluoride excretion (µg):</th>
<th>in 24-hour collection</th>
<th>24-hour collection</th>
<th>per hour for:</th>
<th>night and morning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Ages 3-5 years:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low F intake</td>
<td>170</td>
<td>290</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>optimal F usage</td>
<td>360</td>
<td>480</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Ages 6-7 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low F intake</td>
<td>190</td>
<td>310</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>optimal F usage</td>
<td>480</td>
<td>600</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Ages 10-14 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low F intake</td>
<td>220</td>
<td>340</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>optimal F usage</td>
<td>600</td>
<td>820</td>
<td>25</td>
<td>34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fluoride concentration (ppm):</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All ages:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low F intake</td>
<td>0.2</td>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>optimal F usage</td>
<td>0.9</td>
<td>1.2</td>
<td>0.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* Data from: Aeschbacher (1995); Baez et al. (1999); Hetzer et al. (1996); Marthaler et al. (1995); Rugg-Gunn et al. (1993, 1998); Zohouri (1997).

Note: Although the relationships between fluoride concentrations in drinking-water, prevalence of dental caries, and frequency/severity of dental fluorosis have been investigated for more than 50 years, there have been relatively few investigations of the relationships between actual fluoride intake and fluorosis. There have been a few recent studies on fluoride and fluorosis, and several projects are currently under way, so that the preliminary standards given in this table are likely to be refined in the near future.
Figure 1  Fluoride excretion per hour as determined from consecutive (spontaneous) micturitions. Such diagrams can be automatically constructed from the corresponding spreadsheet table.
Figure 2  Graphical representation of the example (David Morris) used for illustrating the IDUFE-extrapolation, data in chapter 3.3. Dark horizontal lines: excretion measured during the number of hours as represented in the diagram. Punctuated lines: extrapolated hours completing the 4-8-12-hour IDUFE-model for estimating 24-hour fluoride excretion.
Figure 3  Illustration of the IDUSE-extrapolation using the average fluoride excretion shown in Table 3 (for types of lines see legend to Figure 2).
Figure 4  Fluoride excretion per hour as determined in the period LOW, HI and NOC, based on the averages in each of the three periods shown in Table A.2 extrapolation pattern IDUF (for type of lines see legend to Figure 2).
Figure 5 Illustration based on the same excretion averages as Figure 4; however, an extrapolation pattern adapted to the actual lifestyle of the children (6 hours LO, 5 hours HI, 13 hours NOC) was used, considering that many children had two periods of high excretion, one in the early afternoon and a shorter one after dinner (for type of lines see legend to Figure 2), which is a realistic time-table for meals of Swiss children.
Monitoring of renal fluoride excretion in community preventive programmes on oral health
1. Annex

1.1 Collection and analysis of samples (see Section 3.2)

Most parts of this Annex are taken from pages 82-91 of the WHO publication Milk Fluoridation for the prevention of dental caries (WHO/ORH/MF/DOC96.1 World Health Organization/Borrow Dental Milk Foundation, Geneva 1996). A few adaptations and comments have been made to suit the purpose of these recommendations. The paragraph on pH determination has been omitted.

This Annex may directly be used at the site where urine is collected and in the laboratory. For this reason, it repeats some of the items which are described in the main text.

1.2 Time-controlled urine sampling for complete collections

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

(a) At the beginning of each collection period, the subject is expected to empty his/her bladder completely. If this is the first collection taken from the subject, this urine is discarded. If this micturition terminates a preceding collection period, the urine passed at this time must be passed into (or added to that in) the jar used for the preceding collection.

(b) The name of the subject and the time of this voiding are noted in the box No.1 of the respective period see Figure A.1.

(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 subject is unavailable (for instance a child who left school without the field worker being informed or called by the teacher) or is unable to urinate at this time, his/her collection period is considered to have ended at the last available urination. This time can be read from the label since the time of every micturation is recorded (Figure A.1).

(f) The total volume of urine collected during this 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 initial and final time-point.

This information is recorded for each participant, and then, for the purpose of analysis, a sample of urine (20-50ml) 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 0.02-0.05g 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 in children 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.
1.3 Recording of information

Details of each individual subject: name, number, gender, age, body weight (kg) 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 A.1). 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. 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 quantity of these products.

In Figure A.1, A.2, A.3 and A.4 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:

(a) Note the time the initial voiding of the bladder takes place and enter into Box 1 of Figure A.1. This urine is never collected into the jar used for this collection period.

(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 four 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 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 (0.02-0.05g) 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 A.2).

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

Shown in Figure A.3 is an example of a completed label for a urine
collection undertaken on the 5th and 6th of May 1993 for a
6-year-old boy named «David Morris».

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 collection «A» lasted from 0905 until 1250
(the duration 3 hours and 45 minutes need not be determined; this is
done automatically through the programmed table) on 5 May 1993, and
the collected urinary volume was 122 ml. The afternoon period «B»
which did not immediately follow period «A» lasted from 1400 to 1602
and provided 66 ml. The nocturnal collection lasted from 1945 to 0730,
and a second morning collection (on 6 May 1993) from 0855 until 1305.
Urine passed at 0905, 1400, 1945 on 5 May and 0855 on 6 May was not
collected in the jar. The above periods A, B, C and D are only examples,
and may be varied to suit the local kindergarten or school schedules, and
different times when, for instance, fluoridated milk or the main salted meal is consumed.

1.4 Collection of urine at night

A different jar is used for night collection (Figure A.4) 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 of the other jar.

The nocturnal field data (Figure A.4) are transferred to the label of the pattern shown in Figures A.1 or A.3. The list as shown in Figure A.2 compiles all field data to be entered in the columns 12-14, 22-24 and 32-34 (possibly 42-44) of the automated evaluation Table A.2. As soon as the fluoride concentration is typed in columns 15, 25 and 35 the excretion parameters will be computed for those subjects for which the field data in columns 12-14, 22-24 and 32-34 are available (i.e., for the «valid» collections).

1.5 Determination of fluoride in urine and water

(see Section 3.3)

Rules on how to collect urine are given in the preceding section. Regarding drinking water, a few samples may be sufficient if there is a stable centralized water plant. In the case of widely dispersed settlements, i.e. rural areas, samples of drinking water used by the subjects under study should be collected from each household.

Specific fluoride electrodes are routinely in use for fluoride determinations in urine and drinking water. Basically, the procedure is simple and relies on mixing measured quantities of urine with a buffer (TISAB, total ionic strength buffer) and measuring the potential that is obtained on the fluoride-sensitive surface of the electrode. In this part of the Annex, the materials necessary and procedures are presented in detail.

Most ion meters when calibrated transform this potential to a direct concentration read-out. If this facility is not available and the meter reads
potential (mV) then to determine concentration of fluoride it is necessary to use the potential displayed by the sample (TISAB conditioned) in conjunction with a calibration graph constructed by plotting potential (mV) versus log (fluoride concentration) using standards from 0.1 to 5 ppm F conditioned with TISAB. Generally four calibration points are sufficient.

1.6 Required equipment and solutions

- A direct concentration read-out specific ion meter, such as ORION 720A, 710A, 920A or Model EA220, EA910, SA720, SA270, 290A is recommended.
- Combination Fluoride Electrode such as ORION Model 96-09 or 9609BN.
- Magnetic stirrer and stir bars are recommended for laboratory measurements. The stirrer should not be warmed up; when the samples (the mixture urine + TISAB warms up, the calibration curve becomes inaccurate. Heat transfer should be prevented by use of a piece of cork or board as an insulator between the stirrer surface and the bottom of the test tube.
- Automatic pipette – 5 ml.
- Beaker (polythene) – 25 ml.
- Distilled or de-ionized water to prepare all solutions and standards.
- 1 ppm Fluoride Standard with TISAB – ORION Catalogue No. 040906.
- 10 ppm Fluoride Standard with TISAB – ORION Catalogue No. 040908. Note: For determination of fluoride in urine it is best to prepare serially diluted solutions starting from 100 ppm Fluoride, i.e. Orion Catalogue 940907.
- TISAB II – ORION Catalogue NO. 940909.
• Combination Electrode Filling Solution – ORION Catalogue No. 900001.

1.7 **Analysis Check-List**

- 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.

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

1. Remove the rubber cap covering the electrode tip.
2. Fill the electrode (Model 96-09 or 9609BN) 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°C.

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

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

As the majority of urine samples will have a fluoride concentration within the range 0.3 to 3.3 ppm F, the following four calibration points can be successfully used for determination of fluoride in water, urine and salt: 0.1, 0.5, 1.0 and 5 ppm.

1.9 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 15 ml plastic tube 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.

Note: Electrodes do not last indefinitely. Those in regular daily use may often function satisfactory for one to two years whereas those used intermittently last longer. Indications of breakdown are erratic read-outs taking several minutes to stabilize and slope out of range (normally between 54 and 50 mV per tenfold change in F concentration). If such irregularities are observed, electrode replacement is recommended.

1.10 Processing results

Data processing includes calculating patterns of urine and fluoride excretion and fluoride intake, making statistical analyses of data from the group and identifying any anomalous cases (see 3.5, cleaning of the data).

For determination of fluoride in water, the same procedures can be used. When collecting water samples, the investigator should rinse the bottles several times with the drinking water under study.

Note: In many laboratories carrying out fluoride analyses in the context of caries-preventive uses of fluoride, details of the procedures, the types of TISAB and other methodological details vary to some extent. This is evident from those scientific publications which describe the analytical methods in detail. Some specialized laboratories take only 1.00 ml of urine to be mixed with 1.00 ml of TISAB, to arrive at 2 ml
solution in which the pH is measured. Using small quantities, however, requires meticulous work by well-trained laboratory personnel. If, for instance, 0.04 ml of a strong fluoride solution (say 5 ppm F) is not completely blotted away from the electrode due to less than ideal working conditions, the amount of fluoride in the 0.04 ml will increase the measured concentration in the following urine-TISAB mixture by as much as 0.1 ppm, whereas when working with 5+5 ml, the concentration is raised only by 0.02 ppm. It is a general rule that working with 5+5 ml is five times less sensitive to imprecise handling than working with 1+1 ml.

1.11 Standard table for level F24h (see section 3.4.2)

Table A.2 is mostly self-explanatory. The second and third lines should present as much information on fluoride intake/exposure as possible. Note: midnight must be recorded as 2400, not as 0000 (any value equal to 0 in columns 11-14 renders a collection invalid).

How many decimals are required for the field and laboratory data?

Time: exact to a quarter of an hour in 24 hour collections and to 5 (or 1) minutes in shorter collections. Adhere to the format hhmm regarding the time at initial voiding and final time of collection, with no decimal point.

Volume of urine: exact to 5 ml in 24 hour collections, but 5 or 2 ml in shorter collection periods. Measuring cylinders for up to 250 ml usually have 2 ml divisions, those for up to 500 ml 5 ml divisions.

Fluoride concentrations: two decimals after the decimal point, e.g., 0.65 ppm, are sufficient. In case of low concentrations, 3 decimals may be provided by the laboratory. Do not round figures which are given with 3 decimals, as for instance 0.095 ppm.

The computed values (columns 15 etc) are calculated by the computer using at least 6 decimals (floating point operations). The
format of the depicted values is specified in the programmed part of the tables and does not alter the high precision of the calculations.

1.12 Standard table for level F16, incomplete series (see Section 3.4.3)

The meaning of columns 1-47 of Table A.2 are dealt with in the main text and only a few comments are necessary.

Column 17 copies the time at initial voiding of the bladder (column 12) for the valid collections. The median time at the start of the collections is important when comparing fluoride excretion with a distinct time point of fluoride intake as in Table 3.

Column 18 presents the duration of the collection, expressed in hours and decimals (hh.dec). The underlying formula automatically takes into account the special situation of collections extending over midnight; when the initial time of a collection is a larger figure than the final time, e.g. 2200, or 10 p.m. (initial), and 0700, 7 a.m. (final), 24 hours are automatically added to the final time. This gives the correct duration of 9 hours.

Columns 48 - 62 present the results of the 24 extrapolations based on the three separate collections. The IDUFE-durations or - model (4, 8 and 12 hours) are shown in rows 3, 4 and 5 of column 48 (and repeated for technical reasons in columns 49). The urinary flow in 24 hours of subject 53 is derived from the flow figures in columns 19, 29 and 39 (showing ml urine per hour) in the following way:

\[ 4 \times 107.5 + 8 \times 53.9 + 12 \times 15.3 = 430 + 431.2 + 183.6 = 1044.8 \approx 1045 \text{ (ml)} \]

This figure appears in column 48.

Likewise, the 24-hour fluoride excretion of subject 53 is obtained from the columns 20, 30 and 40 (containing \( \mu \text{gF}/\text{hour} \)) by

\[ 4 \times 17.1 + 8 \times 5.6 + 12 \times 11.0 = 68.4 + 44.8 + 392.4 = 245.2 = 245 \text{ (\( \mu \text{gF} \))} \]

This figure appears in column 49.
Note that the fluoride concentration in the extrapolated 24-hour result is obtained by dividing 245μgF/1045ml, which results in 0.234 ppm (column 50). Columns 51 and 52 correspond to columns 48 and 49, but they present the 24 hour results on a per hour basis. This may be useful for comparison with the respective data in the three collection periods and with data published in a «per hour» format.

Columns 53-57 and 58-62 are reserved for Extrapolation 2 and 3. The lengths of the periods should consider the dietary or fluoride supplementation schedule of the specific situation (chapter 3.6.1). The durations fitted to these patterns are entered in rows 3, 4 and 5 of the heading of columns 53 and 58, marked as a box (the three figures in columns 54 and 59 each are automatically copied from columns 53 and 58, respectively). Row 6 below shows the total length of the three durations (in rows 3, 4 and 5 of columns 53 and 58) which must always be 24 hours. The calculations correspond to those with the IDUFE-model.

Columns 64-66 and 67-69 present the urinary flow and fluoride excretion per kilogram body weight. In columns 70-78, durations, urinary flow and excretions are put together (copied from the respective columns further to left) in order to ease comparisons of the results in the three periods. Below the rows displaying the individual cases, the same statistics are shown as in Table A.1.

The inclusion of subjects who had not provided three valid collections necessitates additional computations, presented in the lowermost six rows of columns 48-62. The mean 24-hour fluoride excretion based on all valid collections is calculated in the following way (for the IDUFE-Model, column 49):

\[4 \times 14.0 + 8 \times 23.2 + 12 \times 11.4 = 56 + 185.6 + 136.8 = 378.4 \approx 378.\]

The figures 14.0, 23.2 and 11.4 are the average excretions, μgF/h, in the morning, afternoon and night, respectively. The resulting 24-excretion of 378 μ F is based on 23 (=7+8+8) collections whereas the average of 436 μg (four rows above in column 49) is based on
15 collections only (five subjects with three collections each). In this example, the subjects with incomplete series of collections obviously had lower excretion than the «complete» subjects.

Nw is a weighted subjects' number where the hours in the extrapolation model provides the weight for the numbers of collections in the morning, afternoon and night. For the IDUFE-model Nw = (4*7 + 8*8 + 12*8) / (4+8+12) = 7.83, the integer of which is 7. The weighted average considers that the nocturnal period with the multiplier 12 (in the IDUFE-model 4:8:12) has the strongest weight in the extrapolation, 12 hours, and the others the weight 8 and 4 hours, respectively. The Nw (its integer) varies in function of the extrapolation pattern. The exact Nw were 7.71 and 7.67 in the case of Extrapolations 2 and 3, respectively, leading also to Nw=7. The standard deviation available from the complete cases are now divided by Nw, which in the IDUFE-model results in a standard error of 49µgF as compared to 58µgF from the five complete cases.

In the example of Table A.2, Student's t-test has 4 degrees of freedom (5-1) because the standard deviation is based on five subjects. The t-statistic (2.778 for the error probability of 0.05) appears automatically in the uppermost cell of column 62 and is used for calculation the confidence limits (241 and 514, column 49, second lowest row). In the lowermost row, the following ratio is shown:

\[
\frac{\text{mean based on all available collections}}{\text{mean based on the «complete» subjects}}
\]

In column 49, the ratio was 378/436=0.866. Since all averages based on all available collections were lower, the ratio was consistently below 1.000.

In the example of Table A.2 the inclusion of eight valid collections from four «incomplete» subjects to the data from five «complete» ones (providing 15 valid collections) results in statistics of the same precision.
as if seven children with complete collections had been available instead of only five.

Note: If cooperation is on a very low level and many initial and final time-points are unavailable or likely to be incorrect, the fluoride concentration in invalid samples (duration or volume of urine unknown) may still be worthwhile; the statistics of all available concentrations (recorded in columns 15, 25 and 35) are automatically presented at the bottom of these columns. These statistics may help to interpret the data.

1.13 24-hour Extrapolations (see Section 3.6)

To what extent does the 24-hour extrapolated excretion depend on the extrapolation pattern? Changing from 4:8:12 (IDUFE) to 7:6:11 (see column 53, cn 53, in Table A2) resulted in 412 µg F/24h, 5.5% less than 436 (from IDUFE extrapolation).

The extrapolation patterns 8:5:11 hours (in the heading of column 59, not shown in the compressed Table A.2) which obviously differs widely from the IDUFE-pattern 4:8:12 resulted in 398 µg/24h excreted fluoride on average, which is 8.7% lower than the 436 µg/24h from the IDUFE extrapolation (column 49). May this be regarded as a minor difference?

The yardstick for meaningful appraisal of differences between the results from different extrapolation patterns is the sampling variation. When approximately 110 subjects are studied, 95%-confidence limits will be approximately at + and - 12 % of the average. In this case, a difference of 8.7% would have some importance. Nevertheless, the statistical variation as expressed by the width of confidence interval from -12% to +12%, would still be three times larger than the variation due to the selection of the extrapolation pattern (8.7%).

In the individual subject, the extrapolation is fairly reliable. If we look at the entire 24-hour cycle, the 16 hours covered are 16/24 = 2/3 = 66 % of the entire period. In classical sampling theory, the «finite
population correction» would apply. Consider the following example. A child may have an average fluoride excretion of 20 \( \mu g/h \) in the 24-hour period. For theoretical 24 one-hour assessments, the standard deviation is assumed to be 6 \( \mu g/h \) (around the average of 20, which is a reasonable estimate based on experience). Accordingly, the standard error of a mean derived from 16 one-hour assessments would be \( 6/\sqrt{16}=6/4=1.5 \). The finite population correction would be \( \sqrt{(1-0.67)} \), 67 \% of the total 24 hour interval being covered, and. \( \sqrt{0.33}=0.57 \). Thus, the sampling error due to collecting urine during 16 periods instead of during 24 periods is \( 1.5*0.57=0.86 \). This is a small error when compared to the 24 hour excretions of this example since the individual 24 hour extrapolations from the five complete individuals, expressed in \( \mu g/h \), were in the range of 10.3-23.2 (Table A.2). It is even much smaller in view of standard deviations between individuals, usually in the range of 4 to 10 \( \mu g/h \), depending on the size of the mean excretion (average 18.2 and the standard deviation 5.4 in the example of the five complete subjects of Table A.2).

In these approximate calculations it is assumed that excretions per hour (theoretical units of one hour each) are fully independent. This is obviously not the case. «Neighbouring» excretions (of one hour in this model) have a strong tendency to be similar. Since excretions during the hours not studied tend to be similar to the level assessed through the collections, the sampling variation is lower than in the case of fully independent 24 units as assumed in classical sampling theory and exemplified above.

1.14 WHO support for tabulation and analysis of data

(See section 3.7)

From Section 3 and the respective Annex and Tables, it is obvious that the absolute minimum requirements for the use of the automated tables (the ones shown in the Annex and others which are available or will be developed) are the initial and final time of each collection (or at least their durations) and the urinary volumes and fluoride
concentrations. While the age of the children is routinely provided, the weight should also be given.

When all data as specified for columns 1-4 (child No., age, gender, body weight), coded data of fluoride intake/exposure (columns 5-10) and the appropriate sets of field and laboratory data (initial and final time of collection, urinary volume, fluoride concentration, columns 12-15, 22-25 etc) are made available, the programmed evaluation will provide all relevant results as shown in the Annex Tables. In addition, the subjects may be grouped according to their fluoride exposure coded in columns 5-10.

There are of course intermediate situations. Some data sets obtained in recent studies and used for testing the programmed tables did not provide gender (a minor disadvantage as there are little if any differences between genders up to the age of 10 years) while others did not list the weight of the subjects (excretion per kg body weight cannot be computed). In another case, only the durations of the collections were available. The computing tables can be adapted to such incomplete data sets. For complete information and rapid processing, it is of course desirable that the complete data are collected in proper format. There are two options for data processing.

**Option 1**

When the field worker has all data ready for evaluation, they should be formatted according to the standards observed throughout these recommendations, i.e., - 10 columns for the personal data (for columns 1-10 in the standard format) sets of 4 columns each containing the field and laboratory data (for columns 12-15, 22-25 etc, with sufficient decimals as specified in Annex Table A.2, they may then be transferred to WHO, preferably on diskette or by e-mail in a format which allows direct insertion of the data into the computing tables. When the data are made available in this way, WHO's service will enter the data into the appropriate table expanded according to the number of subjects. Suggestions for Extrapolation patterns 2 and 3 must be supplied.
with respective explanations. Extrapolation IDUF will always be computed.

In the course of the evaluation of data transmitted to WHO, the data will be examined for outliers and inconsistencies according to chapter 3.5 (cleaning of the data). On the basis of the information on fluoride exposure and intake supplied by the local investigator, the results will be commented on and a draft for the summary prepared. If pertinent information with respect to general fluoride background (chapters 3.1, 3.1.1, 3.1.2) is suitably coded columns 5-10 (with explanations regarding the codes in the accompanying text on fluoride exposure and other relevant items), the children will be grouped accordingly when this is worthwhile. The completed tables and an explanatory text would then be sent back to the investigator or group (via printed results, or by diskette, fax or e-mail).

**Option 2**

A diskette with programmed tables may be sent to the worker. The diskette will also contain information on how to use them in more detail than given in these recommendations. However, use of the tables requires experience in using EXCEL. Care must be taken when adapting the tables to the number of individuals studied. In this case, WHO cannot take any responsibility whatsoever for correct evaluation. The programs as presented here were made for Macintosh computers. Later, they will also be available on PC computers.

Other standard tables are already available. These include level F10h and special cases, 24-hour urinary assessments subdivided into two, three and four separate collections. Additional automated tables may be developed as needed for special situations.
Table A.1, level 24h: data and computing continuous collections, example with 6 cases

<table>
<thead>
<tr>
<th>F-exposure</th>
<th>Six cases for illustration, corresponding to low or moderate fluoride exposure continued (this line also for description of general fluoride intake/exposure). See case 4 with &quot;easy&quot; results illustrating computations</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Personal data, fluoride exposure</th>
<th>Field and laboratory data</th>
<th>Results adjusted to 24 hours</th>
<th>Findings per kg body weight and 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code of individual</td>
<td>Time at collection</td>
<td>Preliminary result</td>
<td>Corrected result</td>
</tr>
<tr>
<td>Subject m=1 Age, weight F-rates</td>
<td>Duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>voiding end of urine conc. urine</td>
<td>24.00 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/l 48 h</td>
<td>ml/24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. tiles 3 kg</td>
<td>µg kg/24 h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| cn1  | cn2 | cn3 | cn4 | cn5 | cn6 | cn7 | cn8 | cn9 | cn10 | cn11 | cn12 | cn13 | cn14 | cn15 | cn16 | cn17 | cn18 | cn19 | cn20 | cn21 | cn22 | cn23 | cn24 |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1 2 5 21 1 0                      | 1 715 630 660 0.52       | 23.25 343 1.032 681 354 28.4 14.8 32.4 16.9 |
| 2 4 4 15 2 0                      | 2 720 800 710 0.36       | 24.67 256 0.973 691 249 28.8 10.4 46.1 16.6 |
| 4 1 4 20 2 0                      | 4 715 715 600 0.4        | 24.00 240 1.000 600 240 25.0 10.0 30.0 12.0 |
| 5 2 5 23 0 0                      | 5 730 700 390 0.39       | 23.50 148 1.021 398 151 16.8 6.3 17.3 6.6 |
| 7 1 4 18 2 0                      | 7 700 730 840 0.54       | 24.50 454 0.980 823 444 34.3 18.5 45.7 24.7 |
| 9 1 4 22 1 1                      | 9 740 750 1070 0.283     | 24.17 303 0.993 1063 301 44.3 12.5 48.3 13.7 |

| Min 4 15.0 | Median 4 20.5 | Min 700 630 390 0.283 | Median 717.5 722.5 685 0.390 | 23.25 148 0.973 398 151 16.8 6.3 17.3 6.6 |
| Max 5 23.0 | Median 4 20.5 | Max 740 800 1070 0.540 | Median 717.5 722.5 685 0.390 | 24.00 240 1.000 600 240 25.0 10.0 30.0 12.0 |

| Mean 4.3 19.8 | Mean 4 15.0 | Mean 721 0.414 | Mean 24.01 291 1.000 |
| SD 0.5 2.9 | SD 0.099 | SD 0.55 103 0.023 |
| Coeff. of variation 32 24 2 36 2 31 35 31 35 33 40 |
| SE 94 0.040 | SE 0.23 42 0.009 | SE 91 41 3.8 1.7 5.0 2.5 |

Four children were 4, two 5 years old.
The accurate mean age is 4.3±0.5±=4.8.
Example: age 5 at last birthday in fact
means an age range from 5.00 to 5.99;
therefore, 0.5 years must be added.

Formulas, cn=column

<p>| cn 17 = cn 14 * cn 15 | t for p=0.05, DF=N=1: 2.570 |
| cn 18 = 24 / cn 16 | Lower limit 476 183 19.8 7.6 23.9 8.7 |
| cn 19 = cn 14 * cn 18 | Upper limit 943 396 39.3 18.5 49.4 21.4 |</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Height</th>
<th>Weight</th>
<th>F exposure</th>
<th>Time at Collect.</th>
<th>F-exposure cont.</th>
<th>Extrapolation</th>
<th>Extrapolation 2 hours</th>
</tr>
</thead>
<tbody>
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<td>25</td>
<td></td>
<td></td>
<td></td>
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<td>240</td>
<td>0.16</td>
<td>a</td>
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<td></td>
<td></td>
<td></td>
<td>900</td>
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<td>60</td>
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<td>914</td>
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<td>925</td>
<td>1100</td>
<td>60</td>
<td>0.72</td>
<td>a</td>
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</tbody>
</table>

**Table A.2: Dataset example and computing table for level F16, 3 collections, incomplete series (compressed, see footnote)**

- **F-exposure**: Six cases for illustration, corresponding to low or moderate fluoride exposure.
- **F-exposure cont.**: This line is also reserved for the summary of fluoride exposure.

**Extrapolation**

<table>
<thead>
<tr>
<th>Hours</th>
<th>Morning</th>
<th>Afternoon</th>
<th>Night</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>12</td>
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<td>12</td>
<td></td>
<td>24</td>
</tr>
</tbody>
</table>

**Extrapolation 2 hours**

<table>
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<tr>
<th>Hours</th>
<th>Morning</th>
<th>Afternoon</th>
<th>Night</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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<td>7</td>
<td></td>
<td></td>
<td>14</td>
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</table>

For additional details and footnotes, please refer to Table A.1.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>F-exposure</th>
<th>F-exposure cont.</th>
<th>Coded items on individual</th>
<th>MORNING Field and laboratory data</th>
<th>AFTERNOON Field and laboratory data</th>
<th>NIGHT Field and laboratory data</th>
</tr>
</thead>
<tbody>
<tr>
<td>cn1</td>
<td>23</td>
<td>1 5 22</td>
<td>F</td>
<td></td>
<td></td>
<td>910 1120</td>
<td>24 0.22</td>
<td>1320 1510</td>
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<tr>
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<td>2 5 24</td>
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<td></td>
<td></td>
<td>910 1100</td>
<td>60 0.43</td>
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<td>2 4 19</td>
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<td>75 0.32</td>
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<td></td>
<td>910 1110</td>
<td>48 0.105</td>
<td>1340 1506</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>1 6 20</td>
<td></td>
<td></td>
<td></td>
<td>905 1110</td>
<td>48 0.105</td>
<td>1340 1520</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>2 4 20</td>
<td></td>
<td></td>
<td></td>
<td>910 1110</td>
<td>45 0.46</td>
<td>1340 1520</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>2 5 26</td>
<td></td>
<td></td>
<td></td>
<td>850 1100</td>
<td>76 0.87</td>
<td>1340 1520</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N</th>
<th>9</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>9</th>
<th>9</th>
<th>9</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min</td>
<td>840</td>
<td>1050</td>
<td>24.0</td>
<td>0.105</td>
<td>1300</td>
<td>1500</td>
<td>30.0</td>
<td>0.103</td>
<td>2045</td>
<td>700</td>
<td>122.0</td>
<td>0.150</td>
</tr>
<tr>
<td>Max</td>
<td>915</td>
<td>1120</td>
<td>76.0</td>
<td>0.870</td>
<td>1340</td>
<td>1520</td>
<td>100.0</td>
<td>1.140</td>
<td>2200</td>
<td>845</td>
<td>260.0</td>
<td>1.200</td>
</tr>
<tr>
<td>Median</td>
<td>910</td>
<td>1105</td>
<td>60.0</td>
<td>0.430</td>
<td>1320</td>
<td>1517</td>
<td>64.5</td>
<td>0.755</td>
<td>2133</td>
<td>820</td>
<td>180.0</td>
<td>0.450</td>
</tr>
<tr>
<td>Mean</td>
<td>53.7</td>
<td>0.441</td>
<td>64.9</td>
<td>0.665</td>
<td>192.4</td>
<td>0.568</td>
<td>0.382</td>
<td>0.337</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Subj No. is mandatory in cn1, optional in further columns

For contents of columns cn2-cn10 see Table A.1

For contents of columns F-exposure and F-exposure cont. Leave blank those cells where the data is missing. Basically, a urinary collection is valid only when all 4 field and laboratory data are available.

Note: data stored in EXCEL should be checked as is possible in EXCEL: The statistics N, Min, Max, Median, mean and SD are useful for checking by the field worker on one hand and for checking of correct transfers at the evaluation center on the other. If the data are sent via an EXCEL table, these statistics should be presented below the rows reserved for the subjects.
**Figure A.1** Design and use of label to be attached to urine collecting jar.

<table>
<thead>
<tr>
<th>NAME ........................................</th>
<th>AGE ....</th>
<th>SEX ......</th>
<th>NO ........</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHOOL ......................................</td>
<td>DATE ........</td>
<td></td>
<td></td>
</tr>
<tr>
<td>..................................................</td>
<td>TIME 1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>..................................................</td>
<td>Vol/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>..................................................</td>
<td>TIME 1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>..................................................</td>
<td>Vol/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>..................................................</td>
<td>TIME 1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>..................................................</td>
<td>Vol/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>..................................................</td>
<td>TIME 1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>..................................................</td>
<td>Vol/ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TIME OF CONSUMPTION OF FLUORIDATED MILK .................................................................
### Figure A.2  Example of record of urine collection.

<table>
<thead>
<tr>
<th>School</th>
<th>Class</th>
<th>Date</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Child details</th>
<th>Weight (kg)</th>
<th>Period A</th>
<th>Period B</th>
<th>Period C</th>
<th>Period D</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Age</td>
<td>Sex</td>
<td>Start</td>
<td>Finish</td>
<td>Start</td>
</tr>
<tr>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
</tr>
<tr>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
</tr>
<tr>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
</tr>
<tr>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
</tr>
<tr>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
<td>Time</td>
<td>Volume</td>
</tr>
</tbody>
</table>

---

66
Figure A.3  Example of a completed label attached to urine collecting jar.

<table>
<thead>
<tr>
<th>TIME</th>
<th>Vol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.05</td>
<td>122</td>
</tr>
<tr>
<td>11.10</td>
<td></td>
</tr>
<tr>
<td>12.50</td>
<td></td>
</tr>
<tr>
<td>14.00</td>
<td>66</td>
</tr>
<tr>
<td>16.02</td>
<td></td>
</tr>
<tr>
<td>19.45</td>
<td>4.16</td>
</tr>
<tr>
<td>07.30</td>
<td></td>
</tr>
<tr>
<td>08.55</td>
<td>103</td>
</tr>
<tr>
<td>11.00</td>
<td></td>
</tr>
<tr>
<td>13.05</td>
<td></td>
</tr>
</tbody>
</table>

TIME OF CONSUMPTION OF FLUORIDATED MILK:

- 09.30 (5/5/93)
- 09.20 (5/5/93)
**Figure A.4  Overnight urine collection label.**

<table>
<thead>
<tr>
<th>NAME ....................................................</th>
<th>NO. ........................................</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVERNIGHT URINE COLLECTION Date. .................</td>
<td></td>
</tr>
</tbody>
</table>

1) **Note the time** at which your child urinated before going to bed.

Do *NOT* collect this urine

2) If your child wishes to urinate during the night he/she should use the jar.
3) When your child gets up in the morning he/she should urinate in the jar. Then close the jar and **note the time**.

4) **PLEASE BRING BACK THE JAR IN THE MORNING**
List of References


