Copper is an essential trace element and is a component of many enzymes. Its absorption from the gastrointestinal tract and retention via enterohepatic circulation depends on load (1). Infants and toddlers consume a relatively large amount of drinking water, and a high concentration of copper in drinking water will give rise to high exposure. Acute and subacute copper intoxication can affect a wide range of organs, such as the liver, kidneys, blood, gastrointestinal tract, and the brain (2). Different clinical outcomes can probably be understood in terms of variation in the binding of different chemical forms of copper. High doses may cause local mucosal lesions in the gastrointestinal tract, which increase the absorption of copper. Chronic intoxication due to copper ingestion is rare, probably because of effective homeostatic mechanisms in the gastrointestinal tract, the excretion of excess intake by bile, and a capacity to link copper to ceruloplasmin (3). Copper is a potent metallothionein-inducing agent, which reduces the nephrotoxic effect of the metal. Infants, however, have immature copper metabolism, and their ceruloplasmin level is low. Nausea, vomiting, abdominal pains, and diarrhea have been reported after daily intakes of copper from drinking water in the range of 2–32 mg (4–7). Some Swedish studies have suggested that copper might cause diarrhea in young children, but a statistical association has not been demonstrated (7–11). Copper is a potent bactericidal agent and might cause diarrhea by changing normal intestinal flora (12).

Most domestic water-pipe lines in the Scandinavian countries are copper, and almost all copper in drinking water originates from pipes. In other parts of Europe, plumbing materials are partially lead. The European Union has recently issued recommendations for a maximum concentration of lead in drinking water of 25 μg/L within the next 5 years, and 10 μg/L within the following 15 years. Accordingly, it is important to know whether copper is a preferable plumbing material with regard to health effects.

In a study from Seattle, Washington, Sharrett et al. (13) calculated mean and median daily drinking-water intakes of copper in standing and running samples. They found an intake of copper among 3- to 9-year-old children of approximately 0.4 mg/day. When copper concentration was measured on running samples of water, the faucet was allowed to run 30–60 sec before each sample was taken.

In a study from the United Kingdom, only 6 of approximately 75,000 samples of public drinking water taken in 1993 showed a copper concentration > 3 mg/L. But in a number of private water supplies, the maximum copper level was high as 26 mg/L (14). The recommended daily intake of copper in the United Kingdom is 1.2 mg for adults and 0.3 mg for children younger than 1 year of age. The recommended dietary intake in the United States is 1.5–3.0 mg for adults and 0.4–0.6 mg for children 0–6 months of age (15). The optimal copper content of infant formula is not known, and different practices for copper supplementation are applied. In a Swedish study, Lönnnerdal and Hernell (16) found a mean concentration of copper in drinking water of only 0.03 mg/L. Based on this finding, they suggested that formulas should contain 0.4–0.6 mg copper/L. However, no information about the number of water samples taken, whether water was flushed before sampling, or about the representativeness of the sampling was presented in their study.

Many factors influence the daily intake of copper from drinking water in children: the eating and drinking habits of the child, parental habits with regard to flushing the pipes before using tap water in the child's food, the quality of the water, and the type and age of the plumbing system in the dwelling. The present study was designed to shed light on some of these factors. More precisely, its aims were a) to estimate the concentrations of copper in consumed drinking water in the homes of...
young children living in two large Swedish cities, b) to estimate the volumes of drinking water consumed per day by these children, c) to estimate the daily intake of copper among the young children, and d) to analyze the possible association between concentrations of copper in single-standingsamples and those in series of consumed samples of drinking water.

Methods

Malmö Municipality, with a population of 237,000, and Uppsala Municipality, with a population of 178,000, were chosen for the study because of the corrosiveness of their water and because high concentrations of copper had been found in samples of drinking water taken following complaints from consumers. Malmö is supplied with water from two sources: a groundwater well (Grevie) supplies the northern and eastern part of the city, and a surface-water source (Vomb) supplies the rest of the area. These two sources are quite different with regard to water composition. Alkalinity is much higher in Grevie than in the lake of Vomb (at 337 mg/L and 178 mg/L, respectively), and sodium and chloride concentrations are more than three times higher in water from Grevie than that from Vomb. Both water sources have a pH of 7.5. Hardness is 17.3 mg Ca/L in Grevie and 12.1 mg Ca/L in Vomb.

In Uppsala, four wells supply the city with drinking water. As well as collecting groundwater, the wells are filled by water from lakes and rivers flowing through banks of sand. Water properties are similar in all four wells: pH is 7.5 and hardness is 15.

Initial questionnaire and unflushed samples of water. The initial study population comprised the 1,556 children in the 9- to 21-month age range who, according to Sweden’s Total Population Register, lived in defined geographic areas in the central parts of Uppsala and Malmö. The families received a mailed questionnaire in January 1993. [Families in which the head of household had foreign citizenship (15.4%) were excluded from the sampling frame due to the possibility of limited communication skills in Swedish.] Forty-three (3.0%) of the children selected had moved away from the study areas before data collection started and were therefore not considered part of the study population. Questions were asked about type of day care, breast-feeding, kind of house or apartment where the family lived, age of the dwelling, and any changes made to plumbing materials used for water pipes within the dwelling. The families were also asked to take and supply an unflushed sample of cold water from the kitchen faucet early one morning. From the 1,513 selected children, questionnaire responses were obtained for 1,259 (83.2%), 1,188 by mail and 71 by phone; and 1,178 (77.9%) samples of unflushed water were delivered.

Of the 1,259 children for whom a questionnaire response was obtained, 703 were selected for an intensive diary study on the basis of the following inclusion criteria: a) the child should not be enrolled in a day-care center or a family day-care home (criterion met by 59.6%); and b) the child should not be breast-fed more than three times per day (criterion met by 93.7%).

Collection of water samples and assessment of consumption. In the case of 573 (81.5%) of the 703 children, parents had agreed in their response to the initial questionnaire to participate in the diary study (conducted as scheduled during the spring of 1993). In March 1993, the parents of the children selected for the intensive diary study were visited by a nurse (specially recruited for the study) who instructed them how to take three samples of water per day on four occasions within a period of 3 months. Parents were asked to fill a bottle with water to be used for their child’s food and then dispense a 15-mL sample of that water into a test tube. They were to take samples from the largest portions of water expected to be consumed by the child during the day and also to spread out their sampling as much as possible during any one day.

Information about drinking-water consumption was collected prospectively on a special record form. All portions of drinking water >15 mL were assessed in milliliters during the days when water was collected for copper concentration analysis, and the hour of consumption was also recorded. Parents were instructed to exclude commercially prepared beverages from the records because their copper content might be unknown. Each test tube was given a unique number, making it possible to merge information about concentration of copper with data on volume of water consumed. Estimation of daily intake of copper was performed by multiplying concentration by amount of water consumed. When the concentration was unknown, this was estimated by means of interpolation and extrapolation from known concentrations on the same day.

At the end of the diary-study period, parents were asked to complete another questionnaire in which they were asked if they usually flushed water before using it for their child’s food. If so, they were then asked to estimate the length of flushing time and the amount of water discarded. Because changes in flushing habits as a consequence

of study participation might potentially bias the results, the questionnaire also included a question about changes in flushing habits during the study period.

At the end of the diary-study period, the 50 children with the highest copper concentrations and the 50 with the lowest in the first three samples taken during the first diary-study week were invited to participate in an in-depth study of chemical and bacteriological compounds in water and the effects of flushing on copper concentration. A member of the staff of the municipal water department in each city visited the homes in question and collected three water samples for analysis: without flushing pipes, after flushing 30 sec, and after the water had run consistently cold. Ninety-one families participated in this in-depth part of the study.

Chemical analyses. Chemical analyses of copper concentrations were performed using a Perkin-Elmer 5000 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT), with flame set according to instructions in Swedish Standard no. 028152. A 0.1-mL diluted solution of HNO₃ was added to each sample before analysis. Twenty test tubes of deionized water contained concentrations below the minimal detection level (0.01 mg/L). Precision of the method was evaluated by sending three samples of the same water to the same laboratory; in total, 70 such triplicates were analyzed blind in the three laboratories. Accuracy was evaluated by analyzing the same water samples at all three laboratories; 6 such triplicates were analyzed blind in each of the laboratories.

Statistics. All information was entered twice in data files, and inconsistencies were corrected. Statistical analyses were performed using the SAS/STAT Version 6 software package, 4th edition (SAS Institute, Cary, NC). Because copper concentration in drinking water and daily intake of copper are not normally distributed, the Wilcoxon test was used to determine the significance of differences between groups of children with various characteristics. These distributions are described by medians and percentiles. For volumes of water consumed by the children, a test of the normality assumption was made using the univariate procedure in the SAS package, and the significance of differences between groups was determined by ANOVA. We used linear regression analysis to estimate the association between the unflushed concentration of copper in drinking water (independent variable) and the consumed concentration (dependent variable). We used logarithmic transformations of copper concentration values.
Table 1. Mean copper concentration and mean daily intake of copper in each 10th percentile (43 children) of 430 children in Malmö and Uppsala.

| Copper concentration in consumed drinking water | Daily intake of copper |
|------------------------------------------------|------------------------|
| Mean (mg/L) | Range (mg/L) | Mean (mg) | Range (mg) |
|----------------|----------------|-----------|-----------|
| 10th | 0.04 | 0.01–0.11 | 0.03 | 0.00–0.07 |
| 20th | 0.16 | 0.01–0.87 | 0.09 | 0.00–0.29 |
| 30th | 0.32 | 0.01–0.90 | 0.15 | 0.00–0.45 |
| 40th | 0.37 | 0.01–3.40 | 0.24 | 0.00–0.64 |
| 50th | 0.61 | 0.01–0.28 | 0.32 | 0.01–0.84 |
| 60th | 0.90 | 0.02–2.60 | 0.46 | 0.03–3.06 |
| 70th | 0.95 | 0.05–4.30 | 0.59 | 0.08–1.36 |
| 80th | 1.20 | 0.07–5.00 | 0.77 | 0.00–1.82 |
| 90th | 1.57 | 0.05–4.20 | 1.07 | 0.13–2.21 |
| 100th | 2.62 | 0.14–5.00 | 2.33 | 0.07–3.20 |

In Malmö, there was a considerable difference in copper concentrations between areas according to water source (Figure 3). The difference between medians for the two Malmö areas was significant at all times of day: $p = 0.0004$ for morning values, $p = 0.0001$ for noon values, and $p = 0.0001$ for evening values. The copper concentrations from the two water sources were stratified with respect to the age of pipes in the houses. After this stratification the differences between the two water sources was significant ($p = 0.0001$) only for the dwellings with pipes installed after 1960.

According to questionnaire responses, the effect of different flushing habits was marginal. The median copper concentration was 0.54 mg/L for respondents who stated that they did not flush at all, 0.65 mg/L for those who flushed for 10 sec or less, 0.52 mg/L for those who flushed for more than 10 sec but for no more than 30 sec, and 0.57 mg/L for those who flushed for more than 30 sec. The in-depth diary study, comprising a total of 275 copper samples from 91 households, in which the effect of flushing on copper concentration was studied much more precisely, showed a median copper concentration of 0.93 mg/L in unflushed drinking water, 0.19 mg/L after flushing for 30 sec, and 0.08 mg/L in flushed drinking water.
after flushing until the water ran consistently cold.

The relationship between median copper concentration and year of building construction is shown in Figure 4. Not only were the medians of concentrations of consumed drinking water in dwellings built between 1940 and 1960 extremely low but also the 75th and 90th percentiles were much lower than the corresponding percentiles for buildings of earlier or later periods. In the results shown in Figure 5, the fact that some residences had been rebuilt and their plumbing systems renewed was taken into account. In such cases, the "building year" used in the calculation was the year when the plumbing system was exchanged. There was a significant rise in copper concentrations in water as the age of pipes decreased (p = 0.0001).

Figure 5 shows different copper concentrations in three types of dwellings. Family houses had significantly lower copper concentrations than apartment buildings (p = 0.0002), and apartment buildings had significantly lower copper concentrations than terraced houses, a continuous row of similar houses joined together in one block (p = 0.02). These differences are significant for the dwellings built after 1960 (p = 0.004), but not for older dwellings.

**Associations between copper concentrations in single-standing samples and samples of consumed drinking water by type of dwelling.** Table 2 shows the results of four separate linear regression analyses, with copper concentration in unflushed samples as the independent variable and consumed value as the dependent variable. Each row represents a separate regression analysis for a particular type of dwelling. Table 2 shows parameter estimates for regressions of logarithmetic-transformed copper values from consumed samples on logarithmic-transformed values of unflushed samples of drinking water from different kinds of dwellings. Rather high linear regression coefficients were found. For groups of children, therefore, it is possible to some extent to estimate the concentration of copper in consumed water from information on concentration of copper in samples of unflushed drinking water.

**Daily intake of drinking water.** Among the 430 children who participated in the intensive diary study, the mean daily intake of drinking water was 0.62 ± 0.21 L [standard deviation (SD)]. In contrast with copper concentrations, consumed volumes of drinking water approximately followed the normal distribution (p = 0.95). The 218 boys in this subpopulation consumed somewhat more water, 0.64 L, than the 212 girls, 0.61 L, but the difference was not significant (p = 0.11). No significant trend in consumed volume of drinking water was found with increasing age (p = 0.76). The mean of drinking-water consumption varied from 0.60 L among 13-14-month-old children to 0.66 L among children 19-20 months of age. No significant difference in the mean daily intake of drinking water was found between Malmö, 0.63 L, and Uppsala, 0.61 L (p = 0.33).

**Daily intake of copper from drinking water.** The median daily intake of copper from drinking water among all 430 children included in the intensive diary study was 0.32 mg. The 10th percentile was 0.03 mg, and the 90th percentile 1.07 mg. The 430 children were ranked according to the mean copper concentrations within each subject and divided into 10 groups with 43 subjects in each. As shown in Table 1, there are large variations (ranges) in daily intake of copper within each of these 10 groups. Figure 6 shows the three groups that include children with the highest values of daily mean copper intake. The subjects were sorted from the lowest to the highest daily mean copper intake. Figure 6 shows the mean and all daily copper intakes for each subject. The within-subject variations expressed by ranges are strikingly large and increase with higher mean values.

No difference was found between boys, with an intake of 0.34 mg/day, and girls, with an intake of 0.32 mg/day (p = 0.44). No age trend was observed concerning daily intake of copper. The intake of copper from drinking water by age was as follows: age 9–12 months, 0.32 mg/day; age 13–16 months, 0.38 mg/day; and age 17–20 months, 0.27 mg/day (p = 0.48).

**Discussion**

**Representativeness, bias, and drop out.** We do not claim that the results of this population-based study are representative of Sweden as a whole. We selected for the study two large cities known to have problems with corrosion of copper pipes and to have received many complaints from their inhabitants about water quality. For this reason, we expected a relatively high proportion of children with high daily intake of copper from drinking water. The study population was rather large, the children having been selected from a register covering the total population, which is updated continuously and covers all Swedish citizens. It is likely that the results are biased by the inclusion criteria and by drop outs. In fact, almost all likely differences between participants and nonparticipants tend to overestimate the daily intake of copper. Accordingly, the results can be presumed not to be fully representative of children 9–21 months of age even in these two cities. However, by contrast with some previous studies, an average of 11 samples of consumed drinking water were analyzed per child, and amounts of water consumed during the day were measured fairly accurately.

We excluded children enrolled in family day-care homes or in day-care centers.

| Type of dwelling | Intercept of regression line | Slope of regression line | 95% CI for the slope | p-Value for the slope |
|------------------|-----------------------------|--------------------------|----------------------|-----------------------|
| Total (n = 391)  | -1.2                        | 0.54                     | 0.43-0.66            | 0.0001                |
| Family house (n = 51) | -1.41                      | 0.45                     | 0.11-0.79            | 0.012                 |
| Terraced house (n = 33) | -0.77                      | 0.07                     | -0.22-0.37           | 0.63                  |
| Apartment building (n = 300) | -1.23                      | 0.6                      | 0.47-0.73            | 0.0001                |

CI, confidence interval.
Otherwise, information about consumption of drinking water would have had to have been collected in at least two different places and records would have had to have been filled in by several persons. The fact that children enrolled in a day-care center or in a family day-care home were not eligible for the study may also have created an upward bias in the estimates of copper concentrations.

The possible effect of flushing pipes before using water for cooking was not discussed with parents, either before or during the study. At the end of the study, however, parents were asked how often and how much they usually flushed water before preparing their child’s food. Also, their participation in the study was concluded before they were given information on the results of analyses of copper concentrations in their domestic drinking water. Information given to parents on copper concentrations was then distributed to the families together with recommendations according to Sweden’s National Food Administration about flushing pipes if the mean water concentration is > 1.00 mg/L.

The overall attrition rate was rather high, 39% (273/703), but acceptable in the light of the large amount of information collected about each child. A relatively low age among participating children (compared with nonparticipants) was expected due to the inclusion criteria. A greater proportion of older children were enrolled in day-care centers. However, because no association was found between age and daily intake of copper, the results were not biased by the large proportion of younger children in the study group.

The rather high proportion of families whose head of household had acquired Swedish citizenship after 1984 may be a source of some concern with regard to the generalizability of the results. Among participating families, the estimated daily intake of copper tended to be higher because of the

Figure 6. The daily intake of copper calculated from consumed drinking water among the three upper percentiles (A, B, C) of 430 children in Malmö and Uppsala, Sweden.
had a low median concentration (0.09 mg/L), and many of these dwellings were equipped with galvanized iron as the main plumbing material rather than copper. The higher copper concentrations in water in buildings built before 1920 might be explained by the replacement of the plumbing system with copper materials. This interpretation is further supported by the fact that very low copper concentrations (< 0.03 mg/L) were found only in buildings built before 1960. Long horizontal copper pipes in terraced houses may explain the higher copper concentrations found in these dwellings. A further explanation might lie in the fact that such buildings were built later than other types.

**Conclusion**

Knowledge of the concentration of copper in drinking water and its variation over time is needed to enable rational decisions concerning the suitable level of copper supplement in infant formula. In most epidemiological studies of copper exposure, only one or a few samples of unflushed or flushed drinking water have been analyzed, and the available exposure information may not reflect the daily intake of copper correctly.

In contrast to most previous research in this field, 11 samples of drinking water were collected from each child in the present study. Great efforts were also made to collect samples of water with exactly the same concentration of copper as in the water actually consumed. Single unflushed samples of drinking water were also collected as part of the initial survey. Analyses were therefore performed of associations between the copper concentration in these unflushed samples and the means of the copper levels (within the individual) in consumed water samples. It is noteworthy that significant correlations were found for family houses and apartment buildings. For groups of children living in these types of houses, it may thus be possible to obtain a crude estimate of the total daily intake of copper from information about a single unflushed sample of drinking water.

The suggestion that a level of 0.4–0.6 mg of copper/L should be used in formula, made in an earlier study (15), is not supported by the results of the present study. Cow's milk formulas given to many young children in Sweden are supplemented by the manufacturers with 0.4–0.6 mg of copper/L. If such formulas are prepared with corrosive water and given to young children as the main source of nourishment, flushing for a few minutes is recommended. The results of the present study clearly show that a majority of young children satisfy their daily need of copper solely from drinking water.

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