Regulatory Toxicology and Pharmacology 34, 137–145 (2001)
doi:10.1006/rtph.2001.1492, available online at http://www.idealibrary.com on IDEAL®

# Determination of an Acute No-Observed-Adverse-Effect Level (NOAEL) for Copper in Water

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Received December 22, 2000

**A prospective, double-blind controlled study was designed to determine the acute no-observed-adverse-effect level (NOAEL) of nausea in an apparently healthy population of 179 individuals who drank copper-containing water as the sulfate salt. Subjects were recruited at three different international sites and given a blind, randomly selected dose (0, 2, 4, 6, or 8 mg Cu/L) in a bolus of 200 ml (final total copper dose was equivalent to 0, 0.4, 0.8, 1.2, and 1.6 mg) once weekly over a consecutive 5-week period. Gastrointestinal (GI) symptoms of nausea, abdominal pain, vomiting, or diarrhea were screened for a period of up to 24 h. Nausea was the most frequently reported effect and was reported within the first 15 min of ingestion. For the combined trisite population ($n = 179$), 8, 9, 14, 25, and 44 subjects responded positively to one or more GI symptoms at 0, 2, 4, 6, and 8 mg Cu/L, respectively. Analysis of the data demonstrated a clear dose response to the combined positive GI effects and to nausea alone. Statistically significant greater reporting of effects occurred at 6 and 8 mg Cu/L. Therefore, an acute NOAEL and lowest-observed-adverse-effect level of 4 and 6 mg Cu/L (0.8 and 1.2 mg Cu), respectively, were determined in drinking water for a combined international human population.** 

*Key Words*: **copper; NOAEL; drinking water; nausea; risk assessment; dose response; acute exposure; upper safe limit.**

## INTRODUCTION

Copper is both an essential element and toxic at high doses. A principal consequence of high copper overload in the gut is the appearance of nausea, cramps, emesis, and diarrhea. It has been reported that excess copper consumed from water, and sometimes following a fasting state, can cause manifestations of acute toxicity (Spitalny *et al.*, 1984).

In humans, copper intake includes intake provided by drinking water, food, and dietary supplements. Drinking water generally is not included when dietary intakes are measured. Public water supplies in the United States and other countries probably do not contribute much to copper intake (Klevay, 1980). Water supplies or contamination of water between reservoir and mouth may provide larger intakes. For example, Sharrett *et al.* (1982) estimated an extra, daily intake of 1.3 to 2.2 mg from tap water from a public utility.

Although exposure to copper results almost exclusively from food and water intake, acute copper toxicity is infrequent in humans and is usually the consequence of consumption of contaminated foodstuffs or beverages, including drinking water, or from accidental or deliberate ingestion of high quantities of copper salts. Acute symptoms include excessive salivation, epigastric pain, nausea, vomiting, and diarrhea. Intravascular hemolytic anemia, acute liver failure, acute tubular renal failure, shock, coma, and death have been observed in severe copper poisoning (U.S. EPA, 1987; National Institute of Public Health and Environmental Protection, 1989). In the United States, the new dietary recommendations will expand the traditional approach that emphasizes traditional recommended dietary allowances to include estimates of deficient levels and upper safe limits for most healthy people (Food and Nutrition Board, 1994).

Anecdotal reports from isolated cases in humans suggest that the consumption of beverages or drinking water contaminated with variable copper concentrations results in epigastric pain, nausea, vomiting, and diarrhea (Spitalny *et al.*, 1984; Wyllie, 1957; Center for Disease Control, 1975; Knobeloch *et al.*, 1994; Buchanan *et al.*, 1994; Kramer *et al.*, 1996; Ross, 1955; Hopper and Adams, 1958; Semple *et al.*, 1960; Le Van and Perry, 1961; McMullen, 1971; Fitzgerald, 1998). However, the data from several of these studies are unreliable and not repeatable. The analysis of these data is further



0273-2300/01 $35.00

complicated by variations in inter- and intraindividual responses to copper, the type of copper salts in the water, and adaptation to prolonged higher exposures of copper. Nevertheless, these very limited data have been used as the basis for the formulation of current guidelines and legislation concerning levels of copper in drinking water of either a maximum contaminant level goal (MCLG) of 1.3 mg Cu/L by the U.S. EPA (U.S. EPA., 1991) or a provisional drinking water guideline of 2 mg Cu/L by The World Health Organization (WHO, 1993).

Pizarro *et al.* (1999) have shown a significant increase in the incidence of gastrointestinal symptoms (nausea, vomiting, and cramps) in adult subjects consuming drinking water with a copper concentration $\geq$3 mg/L. Nausea was the most frequent gastrointestinal symptom. Studies performed by Zacarias *et al.* (2001) have demonstrated that the taste threshold for copper (as copper sulfate or copper chloride) in drinking water is 2.6 mg/L. Administration of a mixture containing varying proportions of soluble ($CuSO_4$) and insoluble (CuO) copper salts, maintaining the Cu concentration at 5 mg/L, has recently suggested that the stomach pH may play an important role in determining gastrointestinal effects (F. Pizarro, unpublished). These preliminary data, plus others available in the literature, have led researchers to postulate that nausea may be an adequate early indicator of gastrointestinal adverse effects. The rationale for this is that if copper reaches an empty stomach (without other compounds present to bind the copper), gastrointestinal symptoms may be elicited by smaller amounts of copper than if there was food present in the stomach. It has also been postulated that excess copper can cause generation of reactive oxygen species. In animal models, excess copper has been shown to generate oxygen radicals that in liver form etheno–DNA adducts and lipid peroxidation (Landolph, 1999). Such redox reactions generating oxygen radicals may also play a role in the gastrointestinal cascade of acute copper toxicity.

Whether copper in the concentrations found in a typical public drinking water supply may elicit acute adverse gastrointestinal symptoms is unclear. Moreover, the spectrum of effects, threshold of appearance (doses and concentrations), and characteristics of the symptoms (sequence of appearance, intensity, duration) are unknown. In addition, the question of whether copper in concentrations found in drinking water (trace amounts in natural waters) may elicit acute adverse gastrointestinal symptoms has been a topic of recent international concern. Copper concentration may increase in areas of hard or acidic waters and in households with extensive copper piping systems (U.S. EPA, 1994).

A clear need, therefore, exists for properly controlled randomized trials and epidemiologic studies to better define the tolerable levels of copper in drinking water. According to the recent data by Pizarro *et al.* (1999) nausea is the most likely symptom to be observed. Nausea is a nonspecific manifestation, strongly influenced by psychological and environmental (cultural) factors. Individuals living in three sites of different cultural backgrounds were studied: Santiago in Chile; Grand Forks, North Dakota, in the United States; and Coleraine in Northern Ireland.

## PURPOSE OF THIS STUDY

The purpose of this study was to determine the threshold for acute gastrointestinal effects associated with drinking water containing a random sequence of copper concentrations (as $CuSO_4 \cdot 5H_2O$), in an experimental, controlled situation. This study was completed through the use of a multicenter approach in order to capture the different areas of expertise among investigators and institutions and to present a sample with a broad cultural representation. A monitoring team ensured that a common standardized protocol was followed; subjects were recruited from populations with similar age and sex distribution with each center contributing equally to the success of the study.

## MATERIAL AND METHODS

*Subjects, sample size, and design.* Three sites were selected to carry out this protocol: the Institute of Nutrition and Food Technology, University of Chile, in Santiago, Chile; the Grand Forks Human Nutrition Research Center, North Dakota, United States; and the Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, Northern Ireland. The study protocols, questionnaires, data sheets to record the results, and operational definitions of the outcome variables were standardized over the three sites based, in large part, on the guidelines to conduct such studies published by the U.S. Department of Health and Human Services, Food and Drug Administration (1997). Ethical approval for testing of human subjects was provided by each site's Institutional Review Board or Ethics Committee. All subjects gave their informed consent before inclusion in the study.

The World Health Organization (WHO, 1993) has identified nausea as the main symptom to evaluate acute effects associated with ingestion of copper-containing waters. Because the prevalence of nausea in the general population is not known, the sample size was calculated on the basis of preliminary data obtained in Santiago, Chile, that revealed a basal prevalence of gastrointestinal (GI) symptoms of 5% (unpublished), to detect significant differences for a $\geq$15% change in gastrointestinal morbidity rate (Zacarias *et al.*, 2001). Using an $\alpha$ error of 0.05 and a $\beta$ error of 0.2 (power = 80%), the number of subjects for each testing dose group was calculated as 47. Our previous collective experiences demonstrated a dropout rate of up to 20%. The number of subjects was, therefore, set at 60 per group, resulting

in a targeted total of 180 recruited subjects for the three sites.

At each site (Coleraine, Santiago, and Grand Forks) a group of 60 adults was recruited by local advertisements. All subjects were informed of study details and signed a written consent. Each subject answered a health and lifestyle questionnaire. Individuals who were heavy drinkers or smokers, were taking prescription drugs, or were pregnant were excluded from the study. The sample was stratified to include approximately 50% of each sex and 50% under and over 40 years of age. All subjects received monetary compensation at the completion of study.

The solutions given were unmasked even though copper in drinking water can have a distinctive metallic taste to some individuals. The research team considered adding either sucrose or aspartame along with citric acid to mask the taste. However, preliminary testing at each site showed that taste masking was highly variable, would be only marginally effective, and would not likely mask the taste completely. Additionally, the use of a taste masking system would have potentially introduced another testing variable through copper binding to components of the taste masking. Therefore, taste masking was not used in this study.

*Solutions.* Distilled, deionized water was prepared daily at each study site. A single, identical lot of copper sulfate pentahydrate (USP) pro analysis grade (98.5–100.5%, dry basis) was obtained (Fisher Scientific, Springfield, NJ, Lot No. 974491) and distributed among the three sites. Concentrations of the test solutions (0, 2, 4, 6, and 8 mg/L of copper as $CuSO_4$) were prepared daily and confirmed by atomic absorption spectrophotometry at each site. The administered bolus amount of water was 200 ml, providing a target dose of 0, 0.4, 0.8, 1.2, and 1.6 mg of elemental copper per trial, respectively. Chemical analysis confirmed that the actual prepared copper solutions were ±3% of the target concentration for the duration of the study for the four copper concentrations at the three sites.

*Protocol.* Subjects fasted overnight and came to the test facility one morning a week for 5 successive weeks. On arrival (time 0), individuals were asked to complete a written questionnaire containing a list of symptoms and signs (Table 1), indicating whether these were present or absent at that time. The list of symptoms included the primary outcome variables, nausea, abdominal pain, vomiting, and diarrhea, as well as several distractor symptoms, backache, sweating, palpitations, heartburn, headache, feeling of anxiety, dizziness, and salivation. When it was confirmed that the subject was asymptomatic, he or she was given one 200-ml test solution of 0, 2, 4, 6, or 8 mg/L; assigned to each subject in a random order; and blinded to the experimental subject and the laboratory supervisor. After 15 min subjects completed the same questionnaire again and were invited into a lounge provided with magazines and a television, where they spent the remainder of the hour. At the end of 1 h of direct observation the individuals again completed the same questionnaire before leaving the study facilities. The following morning, subjects were contacted by telephone and the same questionnaire was completed for any symptoms experienced at 24 h postdosing. At each evaluation time, instructions given and the language used during contact with the subjects were carefully planned and reviewed prior to the beginning of data collection in order to make them comparable at the three sites.

*Response evaluation and analysis of results.* The threshold dose for a symptom was defined as the lowest dose at which a statistically significant increase of the symptom incidence over controls was observed. For the purposes of this study, nausea, vomiting, diarrhea, and abdominal pain were defined as outcome variables. Nausea was defined by the imminent desire to vomit, either mildly or intensely as reported by the study participant. Because each response of nausea, abdominal pain, vomiting, and diarrhea, in this order in any one individual may represent steps in an ascendant scale of response intensity, they cannot be considered independent variables. Thus, a subject who reported one or more of these four response outcomes was interpreted as a single event at a given dose, irrespective of the total number of positive responses.

The outcome variable, GI symptoms, was defined as the occurrence of one or more outcome symptoms (nausea, vomiting, diarrhea, or abdominal pain) after consuming a given copper dose. Outcome data for nausea and GI symptoms were modeled using logistic regression within the generalized estimating equation procedure (Proc Genmod) in SAS/Stat (SAS Institute Inc., Cary, NC). The repeated measures structure of the design was incorporated into the logistic regression models. Included as potential covariates in the models were test location, sex, age group of subject (less than or greater than 40 years of age), and the order in which the dose was administered. Two indicator variables were used to test for differences between the three test locations. Covariates that did not significantly improve the model fit were omitted from the final model. Dose–response estimates and odds ratios were generated using the parameter estimates obtained from the final model for nausea and GI symptoms.

*Quality control, monitoring, and coordination.* Each research institution was responsible for internal quality control and strict adherence to the protocol. Toxicology Excellence for Risk Assessment (TERA), a nonprofit risk assessment research organization, provided study monitoring and coordination, independent of each site's lead investigator, who had an additional oversight role

**TABLE 1**
**Questionnaire**

*Name:*_____ *Age:* _____ *Sex:* _____ *Code number:*_____

*Address:*_____ *Weight*_____ *Height:*_____

How do you feel?

Better than usual? ☐ ☐ ☐
As usual?
Worse than usual?

**Explain:**_____

If you feel anything different than usual please choose from the following list what you have experienced this morning:

| | On arrival | After 15 minutes | After 60 minutes |
|---|---|---|---|
| Backache | | | |
| Sweating | | | |
| Diarrhea | | | |
| Palpitations | | | |
| Heartburn | | | |
| Nausea | | | |
| Headache | | | |
| Vomiting | | | |
| Anxiety | | | |
| Abdominal pain | | | |
| Dizziness | | | |
| Salivation | | | |

Observations:_____

in the progress of this research at each center. TERA also conducted an independent peer review of the proposed study protocol, prior to commencement of the project. The protocols developed and used in this study were based in part on comments received from this independent review that, for the most part, were incorporated into the study design.

## RESULTS

A total of 179 individuals finished the trial (60 in Santiago, 61 in Grand Forks, and 58 in Coleraine). Nausea was the earliest and most prevalent symptom observed (16.7, 36.1, and 29.3% of the subjects in the three sites, respectively, reporting at least one occurrence of nausea) with an average prevalence of nausea among all subjects of 27.3%. Nausea was most frequently reported within the first 15 min after ingestion, and was of a transient nature. Table 2 lists the results for each gastrointestinal outcome reported by location and sex. Distractor symptoms were infrequently reported and were unrelated to copper dose; thus they were not included in the summary analysis.

The distribution of outcomes combined across sites and reported at each concentration tested is shown in Table 3. Of the 49 subjects who reported nausea, 29 reported nausea at only one concentration of copper, 17 reported nausea at two concentrations of copper, and 2 reported nausea at three concentrations tested. Only 1 of the 179 individuals reported nausea after each episode at 2, 4, 6, and 8 mg Cu/L and did not report a positive response at 0 mg/L. Three individuals reported nausea after consuming the water containing no copper. One of these 3 individuals reported no other incidence of nausea; 2 of the 3 reported nausea after consuming 8 mg Cu/L.

Vomiting occurred in only one female at the Grand Forks site and was not considered to be treatment related. Five subjects (2.8%, two women and three men) developed diarrhea at 0, 2, 4, 8, and 8 mg Cu/L,

**TABLE 2**
**Subjects Who Reported One or More Outcome Variable Distributed by Site and Sex**

| | Santiago (Chile) | | | Grand Forks (U.S.A.) | | | Coleraine (Northern Ireland) | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gastrointestinal symptoms[a] | Male | Female | Total | Male | Female | Total | Male | Female | Total | Male | Female | Total |
| Nausea only | 3 | 3 | 6 | 7 | 15 | 22 | 6 | 10 | 16 | 16 | 28 | 44 |
| Nausea and vomiting | 2 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 5 |
| Nausea and diarrhea | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Nausea and abdominal pain | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 3 | 3 |
| Vomiting only | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Diarrhea only | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 2 | 1 | 3 |
| Diarrhea and abdominal pain | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |
| Abdominal pain only | 3 | 1 | 4 | 3 | 2 | 5 | 6 | 2 | 8 | 12 | 5 | 17 |
| Total GI symptoms[b] | 7 | 8 | 15 | 10 | 17 | 27 | 13 | 11 | 24 | 30 | 36 | 66 |
| Total other symptoms | 3 | 2 | 5 | 12 | 16 | 28 | 26 | 27 | 53 | 41 | 45 | 86 |

[a] Subjects may have reported different symptoms at different doses tested.
[b] The total number of subjects reporting any GI symptoms may not necessarily equal the sum of the reports of individual symptoms.

respectively. Diarrhea occurred between 1 and 24 h after administration of the copper. While nausea and vomiting appeared clearly related, diarrhea was not associated with the other two symptoms (only one of the five individuals with diarrhea presented with nausea; this occurred after consuming 8 mg Cu/L).

The final reduced models obtained from the repeated measures logistic regression analysis for nausea and GI symptoms included gender, test site, and copper dose as significant outcome predictors (Table 4). Order of dose administration and age did not significantly affect the experimental outcomes ($P > 0.05$). As copper dose increased, female subjects reported significantly more occurrences of nausea and GI symptoms than male subjects (odds relative to males, 2.66, $P < 0.004$; and 1.68, $P < 0.05$, respectively). Subjects tested in Santiago reported significantly fewer occurrences of nausea and GI symptoms than did subjects in Grand Forks, while subjects in Coleraine were similar to those in Grand Forks. Copper concentration was the strongest predictor of both nausea and GI symptoms, with incidence significantly increasing $P < 0.0001$) as the dose administered increased. The relative odds of reporting nausea were 0.66, 3.53, 7.67, and 17.15, while the relative odds of reporting any GI symptom were 0.87, 1.83, 3.54, and 7.29 for 2, 4, 6, and 8 mg Cu/L, respectively. Incidence of abdominal pain and diarrhea were not related to copper concentration ($P > 0.05$).

The predicted frequencies of occurrences of nausea and GI symptoms at the concentrations tested are given for men, women, and all subjects in Table 5. Although there was a gender difference in the frequency of responses, the original intent of this study was to estimate the minimum dose effect across all subjects, regardless of age, sex, and cultural background. A two-staged polynomial regression model

**TABLE 3**
**Subjects Who Reported One or More Outcome Variables at Each of the Copper Concentrations Tested**

| | Copper (mg per liter) in drinking water | | | | |
|---|---|---|---|---|---|
| Gastrointestinal symptoms | 0 ($n = 179$) | 2 ($n = 179$) | 4 ($n = 179$) | 6 ($n = 179$) | 8 ($n = 179$) |
| Nausea only | 3 | 2 | 9 | 17 | 33 |
| Nausea and vomiting | 0 | 0 | 1 | 1 | 3 |
| Nausea and diarrhea | 0 | 0 | 0 | 0 | 1 |
| Nausea and abdominal pain | 0 | 0 | 0 | 2 | 1 |
| Vomiting only | 0 | 0 | 0 | 1 | 0 |
| Diarrhea only | 1 | 1 | 0 | 0 | 1 |
| Diarrhea and abdominal pain | 0 | 0 | 1 | 0 | 0 |
| Abdominal pain only | 4 | 4 | 3 | 4 | 5 |
| Total GI symptoms | 8 | 7 | 14 | 24 | 44 |
| Total other symptoms | 31 | 41 | 44 | 46 | 34 |

**TABLE 4**
**Results of Repeated Measures Logistic Regression Analysis of Nausea and Gastrointestinal Symptoms**

| | Odds ratios[a] (95% CI) | P< |
|---|---|---|
| **Nausea[b]** | | |
| Gender: Female vs male | 2.66 (1.38–5.15) | 0.004 |
| Test site: Santiago vs others | 0.35 (0.16–0.76) | 0.008 |
| Dose | | |
| 2 mg Cu/L | 0.66 (0.11–4.10) | 0.6 |
| 4 mg Cu/L | 3.53 (0.92–13.50) | 0.07 |
| 6 mg Cu/L | 7.67 (2.14–27.49) | 0.002 |
| 8 mg Cu/L | 17.15 (5.32–55.34) | 0.0001 |
| **GI symptoms[c,d]** | | |
| Gender: Female vs male | 1.68 (1.00–2.84) | 0.05 |
| Test site: Santiago vs others | 0.38 (0.21–0.70) | 0.002 |
| Dose | | |
| 2 mg Cu/L | 0.87 (0.30–2.53) | 0.8 |
| 4 mg Cu/L | 1.83 (0.75–4.45) | 0.2 |
| 6 mg Cu/L | 3.54 (1.62–7.77) | 0.002 |
| 8 mg Cu/L | 7.29 (3.33–15.94) | 0.0001 |

[a] Odds ratio with 95% confidence intervals (CI). Odds ratios for dose are relative to 0 mg Cu/L.
[b] Overall effect of dose: $\chi^2 = 66.22$, $P < 0.0001$.
[c] Occurrence of one or more outcome symptoms (nausea, vomiting, diarrhea, or abdominal pain).
[d] Overall effect of dose: $\chi^2 = 52.26$, $P < 0.0001$.

was used to generate a single dose–response curve for nausea (Fig. 1) and GI symptoms. The minimum low-observed-adverse-effect level (LOAEL) for nausea and GI symptoms across all subjects was 6 mg Cu/L and the no-observed-adverse-effect level (NOAEL) was 4 mg Cu/L for acute exposure to copper.

**TABLE 5**
**Predicted Frequency of Nausea and Gastrointestinal Sympotms in Individuals That Ingested Graded Concentration of Copper in Deionized Water**

| Cu, mg/L | Men | Women | All subjects |
|---|---|---|---|
| | **Nausea** | | |
| 0 | 0.01 (0.002, 0.04)[a] | 0.02 (0.01, 0.07) | 0.02 (0.005, 0.05) |
| 2 | 0.01 (0.002, 0.02) | 0.02 (0.003, 0.07) | 0.01 (0.003, 0.05) |
| 4 | 0.03 (0.02, 0.07) | 0.08 (0.04, 0.16) | 0.06 (0.03, 0.11) |
| 6 | 0.07 (0.04, 0.12) | 0.16 (0.09, 0.25)* | 0.11 (0.06, 0.19)* |
| 8 | 0.14 (0.08, 0.23)* | 0.29 (0.20, 0.41)* | 0.21 (0.14, 0.32)* |
| | **GI symptoms[b]** | | |
| 0 | 0.03 (0.01, 0.08) | 0.05 (0.03, 0.11) | 0.04 (0.02, 0.09) |
| 2 | 0.03 (0.01, 0.07) | 0.05 (0.02, 0.10) | 0.04 (0.02, 0.08) |
| 4 | 0.06 (0.03, 0.10) | 0.10 (0.05, 0.17) | 0.08 (0.04, 0.14) |
| 6 | 0.11 (0.07, 0.17) | 0.17 (0.10, 0.26) | 0.14 (0.09, 0.22)* |
| 8 | 0.20 (0.14, 0.29)* | 0.29 (0.20, 0.40)* | 0.24 (0.17, 0.34)* |

[a] Predicted from logistic regression model (95% confidence interval).
[b] Occurrence of one or more outcome symptoms (nausea, vomiting, diarrhea, or abdominal pain).
* Significantly different from 0 mg Cu/L ($P < 0.05$).

The 4 mg Cu/L NOAELand 6 mg Cu/L LOAEL for nausea and GI symptoms was determined from the logistic regression analysis presented in Table 4. For both nausea and total GI symptoms the 6 mg Cu/L concentration was significantly increased ($P < 0.002$). At 4 mg Cu/L, there was no statistically significant increase in symptoms for either nausea ($P < 0.7$) or total gastrointestinal symptoms ($P < 0.2$). An apparent threshold for both copper-induced nausea and total GI symptoms occurred between 4 and 6 mg Cu/L (0.8 and 1.2 mg Cu).

Factors that may have influenced the results included site, sex, and age. However, there was no consistent, statistically significant differences in the total responses for these factors (Tables 4 and 5) even though the answers to the questionnaire in Santiago may have indicated that there may be cultural differences in defining the GI symptoms listed. Calculation of the threshold of appearance of GI symptoms from the pooled data from three sites showed that for adult females this value was 8 mg Cu/L. Fewer subjects in Santiago reported nausea than at the other two sites. However, five of the six episodes of vomiting were reported at this site; cultural differences may account, in part, for these observations.

## DISCUSSION

The Western diet so closely associated with ischemic heart disease, osteoporosis, and other diseases of industrialization frequently is low in copper in comparison to dietary reference values. For example, 61% of 849 daily diets analyzed in Belgium, Canada, the United Kingdom, and the United States contained less than 1.51 mg of copper (Klevay *et al.*, 1993). In the United States the estimated safe and adequate daily dietary intake (ESADDI) of copper for adults is 1.5 to 3.0 mg per day (Natural Research Council, 1989). Approximately one-third of the daily diets contain less than 1.01 mg, an amount proved insufficient for more than 30 men and women using biochemical and physiological criteria in depletion studies (Klevay and Medeiros, 1996).

Dietary intake has great variability and copper absorption is tightly regulated; unless foods consumed are contaminated with copper, they do not represent a common cause of acute toxicity. In the context of dietary access to copper in Western society, supplementation with minerals has become an issue of concern because self-administration of readily available products has become a frequent practice. There is little information about oral tolerance of copper supplementation using different regimens. A formula diet supplemented with cupric sulfate to a total of 8 mg of copper daily for 24 days was without mention of toxicity (Turnlund *et al.*, 1989). Researchers at the USDA have supplemented diets of conventional foods similarly to 3 mg/day for 4 months; this dose was well tolerated and extensive biochemistry was normal. In an earlier pilot study,

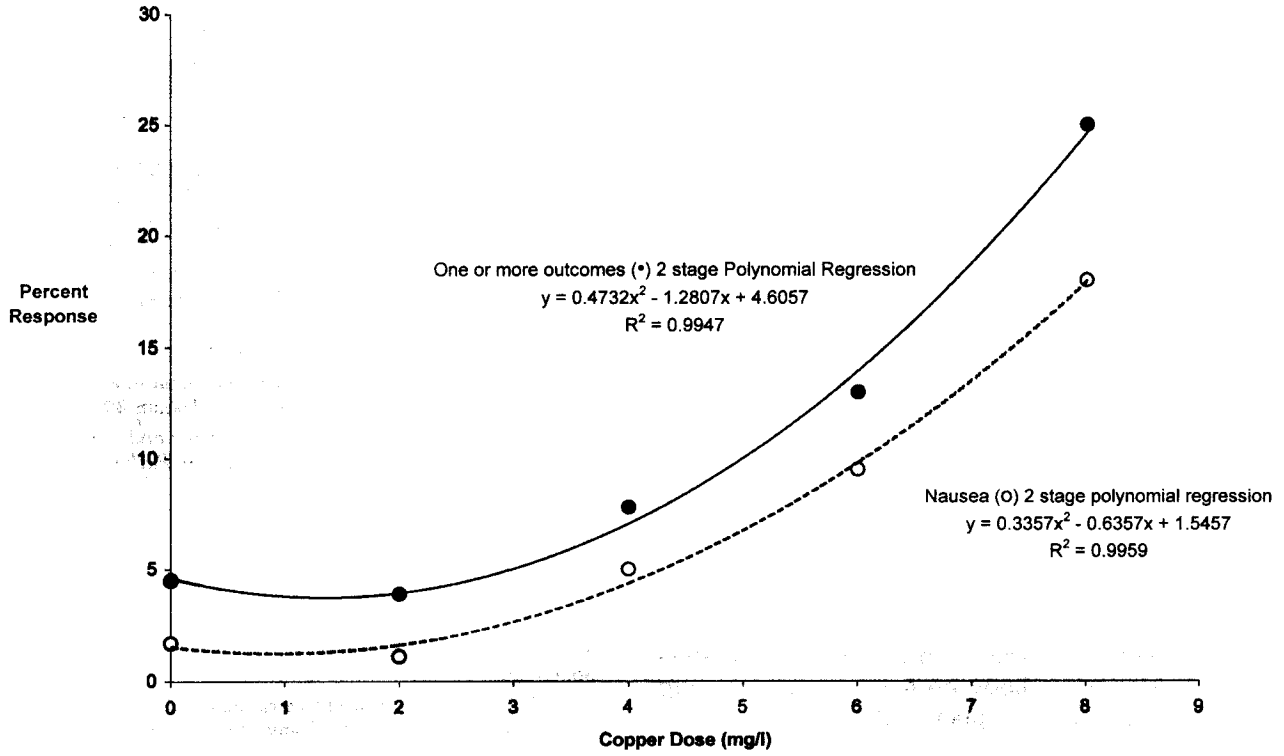


**FIG. 1.** Subjects that reported one or more adverse gastrointestinal outcomes (●) or nausea alone (○) as a function of copper concentration.

these scientists gave adults 4.0 mg copper for 39 days (Klevay *et al.*, 1984) or 5.2 mg of copper for 30 days (Klevay *et al.*, 1986) without significant complaints of gastric symptoms. Klevay has completed a 100-day supplementation trial of 2 mg copper daily (as sulfate) with minimal inconvenience and no evidence of biochemical change (unpublished results). Also, Kehoe *et al.* did not observe intolerance from daily supplementation of adults with 3 mg of copper as the sulfate or 3 and 6 mg of copper as an amino acid chelate (Kehoe *et al.*, 2000).

In the only other controlled human study reported to date, Pizarro *et al.* (1999) determined the acute gastrointestinal effects in 60 adult Chilean females who were each given 0, 1, 3, or 5 mg Cu/L in a public (tap) drinking water source. This was the sole drinking water source for these individuals for a 2-week period. Mild gastrointestinal disturbances (nausea, abdominal pain, vomiting, and diarrhea) were recorded at least once in 35% of the subjects. Nausea, abdominal pain, and vomiting were significantly related to copper concentrations with a recorded incidence of 5, 2, 17, and 15% in the individuals consuming 0, 1, 3, or 5 mg Cu/L, respectively, suggesting that copper concentrations greater than 3 mg Cu/L can be associated with these gastrointestinal symptoms. This is consistent with the data reported here that suggests that consumption of water greater than 4 mg Cu/L will significantly increase the chance of experiencing these same mild gastrointestinal symptoms. Differences in experimental protocol (source of water, frequency of consumption, subject size, experimental design) may account for the slight difference in the apparent respective acute NOAELs for copper.

Results of this study show that when apparently healthy individuals ingest physiological amounts of water containing up to 8 mg Cu/L under an experimental, controlled situation, the threshold for appearance of adverse acute gastrointestinal effect is 6 mg Cu/L. These experimental conditions were designed to describe the dose–effect phenomenon in a carefully controlled laboratory setting, but they do not necessarily represent the habits of most people. The experimental design maximized the potential for an effect by having subjects fast. This was based on the hypothesis that copper on an empty stomach would elicit symptoms more quickly owing to easier solubilization, interaction with the receptors in the stomach wall, and/or the use of distilled-deionized water.

At concentrations of up to 8 mg Cu/L (as copper sulfate), 35.8% of individuals reported nausea, vomiting, diarrhea, and/or abdominal pain. Within the concentration range tested, nausea was clearly the first and most frequent symptom reported, while vomiting and diarrhea were infrequent findings. The nausea appeared shortly after ingesting the water, primarily within the first reporting interval of 15 min. This is consistent with results in laboratory animal studies (Wang and Borison, 1952).

Among those individuals who reported nausea at or below 8 mg Cu/L, more than half of the individuals also reported feeling nauseous at a lower level, but only 1 subject in 179 reported nausea at all four copper concentrations. Positive responses showed no pattern in relation to the copper concentrations in the dosing sequence used throughout study.

Although the number of subjects who reported an outcome variable was similar for the three sites, the total number of positive responses was fewest in Santiago and greatest in Northern Ireland. This tentatively suggests a cultural difference in the importance given by the study participants to the experienced symptoms. Standardized explanations were presented to the study participants at all three sites to insure reporting of all symptoms, even those considered to be mild and unimportant. The fact that both outcomes and distracters were similarly reported suggests that the finding may be related to local cultural patterns rather than to real differences in susceptibility to experiencing or feeling nausea.

As shown in Figure 1, there is an increase in the slope of the curve for nausea between 4 and 8 mg Cu/L suggesting a threshold level. Upper confidence levels show that the first 3 and 5% of the population would respond at 2.5–3 and 3.5–4 mg/L, respectively, which supports the provisional guideline value of 2 mg Cu/L set by the WHO (1993) as a safe value. The determination of an upper safe limit and the gastric tolerance observed in this study will also help to define copper doses in supplements as well.

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