

Determination of the Taste Threshold of Copper in Water

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Abstract

Copper effects on human health represent a relevant issue in modern nutrition. One of the difficulties in assessing the early, acute effects of copper ingested via drinking water is that the taste of copper may influence the response and the capacity to taste copper in different waters is unknown. The purpose of the study was to determine the taste threshold of copper in different types of water, using soluble and insoluble salts (copper sulfate and copper chloride). Copper-containing solutions (range 1.0–8.0 mg/l Cu) were prepared in tap water, distilled deionized water and uncarbonated mineral water. Sixty-one healthy volunteers (17–50 years of age), with no previous training for sensory evaluation, participated in the study. A modified triangle test was used to define the taste threshold value. The threshold was defined as the lowest copper concentration detected by 50% of the subjects assessed. To evaluate the olfactory input in the threshold value obtained, 15 of 61 subjects underwent a second set of triangle tests with the nose open and clamped, using distilled water with copper sulfate at a concentration corresponding to the individual's threshold. The taste threshold in tap water was 2.6 mg/l Cu for both copper sulfate and copper chloride. The corresponding values for distilled deionized water were 2.4 and 2.5 mg/l Cu for copper sulfate and copper chloride, respectively. In uncarbonated mineral water the threshold values were slightly higher, 3.5 and 3.8 mg/l Cu for copper sulfate and for copper chloride, respectively, which are significantly higher than those observed in tap and distilled waters ($P < 0.01$, Kruskal–Wallis test). The taste threshold did not change significantly when the nose was clamped. In conclusion, the median values for copper taste threshold were low, ranging between 2.4 and 3.8 mg/l Cu, depending on the type of water.

Introduction

Copper is an essential nutrient for humans, necessary for enzymes and proteins indispensable for life. However, it is also toxic when ingested in large amounts. Most of the cases that suffer toxic effects are due to accidental or deliberate ingestion of many milligrams or grams of copper salts. Studies by Cohen *et al.* and Béguin-Bruhin *et al.* using distilled water and mineral spring water suggested that copper concentrations >3 mg/l Cu would produce changes in taste and color, a concentration at which toxic effects are not observed (Cohen *et al.*, 1960; Béguin-Bruhin *et al.*, 1983). For this reason it was generally assumed that changes in the color and taste of drinking waters are relevant and efficient in protecting humans from toxic effects. In recent years though, interest in the early adverse effects of copper has led to the question of to what extent color or taste in drinking water may be sufficient to protect from early toxic effects. In fact, there are several anecdotal reports of individuals that suffered gastrointestinal symptoms (mainly nausea, vomiting, diarrhea and abdominal cramps) after ingestion of copper-contaminated waters.

Tap water usually contains a few milligrams of copper per

liter. Copper concentration in drinking water varies according to the natural mineral content, pH, hardness of the water, anion concentrations, oxygen concentration, temperature and characteristics of the plumbing system. The principal source of copper in water is corrosion of copper pipes (Olivares and Uauy, 1996a,b; WHO, 1996, 1998; Environmental Protection Agency, 1997). Although most drinking waters contain <3 mg/l Cu, values as high as 22–28 mg/l Cu have been reported, especially if acidic water was stagnant in copper tubes (WHO, 1993; Bent and Bohn, 1995).

Studies carried out by us in order to characterize the early adverse effects of copper contained in water showed that the first symptom to be observed is mild and transient nausea, which appears at 4 mg/l Cu (delivered as copper sulfate) in the most sensitive individuals (M. Olivares *et al.*, submitted for publication); about two thirds of the individuals tested repeated the positive response when rechallenged with the same dose. These results posed the question of to what extent a mild non-specific symptom such as nausea may be influenced by the capacity to taste the 'bad', metallic taste

reported for copper by the same individuals. To shed light on this aspect we carried out this study with the objective of determining the taste threshold of copper in different waters using some of the copper salts commonly found in drinking waters.

Materials and methods

Subjects and design

Apparently healthy volunteers 18–60 years of age were invited to participate in this study. They received oral and written explanations of the protocol and answered questionnaires aimed at applying exclusion criteria. The volunteers were to be free of chronic health problems, especially oral and nasal conditions that may have interfered with tasting or olfactory capacities, and to have no previous experience with sensory evaluations. Those that fulfilled these requirements and volunteered signed a written informed consent before beginning the study. The Institutional Review Board approved the protocol at INTA, University of Chile.

Taste evaluations were performed early in the morning, in a blind fashion, at least 2 h after a light breakfast. Because it was assumed that perceiving copper taste at low concentrations could be difficult, the subjects were instructed to keep the sample bolus in their mouth for at least 1 min, spit it out, rinse their mouth with a sucrose solution (0.005 M) (which at this concentration gave a pleasant neutral sensation and eliminated bitter and other off tastes) and wait for at least 60 s before tasting the next sample (Amerine *et al.*, 1965; Witting de Pena, 1980; Meilgaard *et al.*, 1991). Individuals gave their judgments after tasting all the samples assigned for that session. At the end of the evaluation salty crackers were served with coffee or tea in order to eliminate any persistent or unpleasant taste.

To avoid any persistent taste influencing the following tasting events, in each session the volunteers received one single copper concentration, ranging from 1.0 to 8.0 mg/l Cu, as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ or CuCl_2 . Panelists were tested three times per week, beginning at 1 mg/l Cu and incrementing at 1 mg/session until a positive report defined the threshold value for that person. A positive report was defined as correct identification of the copper-containing sample (see below).

Screening test

This was performed with the purpose of defining individual taste threshold zones (Amerine *et al.*, 1965; Witting de Pena, 1980; Meilgaard *et al.*, 1991). Briefly, it consisted of presenting the subject with eight cups, each one containing 20 ml of solution, encoded with random three digit numbers. The copper concentrations were presented in ascending order from 0 to 8 mg/l Cu at room temperature (18–23°C) in a blind fashion. When the subject perceived a distinct taste sensation, he or she recorded and described the

particular taste. This value and those near it represented the threshold zone for that individual. This test was applied prior to the first set of trials only, using copper sulfate as the copper salt. Once the threshold zone was defined, in the next session the subject underwent a modified triangle test.

Modified triangle test

The triangle test is a three-product test in which the subject's task is to identify the different one (Meilgaard *et al.*, 1991). To decrease the possibility that the result was due to random guessing in this study we modified the method, presenting five samples to each subject: four identical ones and one containing the test solution. The panelist was instructed to identify the odd sample and indicate the perceived taste. Five cups of 20 ml each were presented at room temperature (18–23°C); the cup itself did not impart a taste or odor to the sample. The samples were coded with a three digit number and the set of five cups was presented to the subject in a random order (ABBBB, BABBB, BBABB, BBBAB and BBBBA, where A was the cup containing the test solution and B the placebos).

Triangle test with nose 'clamped'

With the purpose of evaluating the effect of olfactory input on taste threshold and the capacity to confirm the taste threshold when the nose was 'clamped', a subgroup of 15 individuals underwent a second series of triangle tests, one with the nose open and the other with the nose clamped, during which they received copper sulfate solution prepared in distilled water at the concentration corresponding to his/her previously defined taste threshold.

Sample size calculation, expression and analysis of results

To obtain statistical significance when differences between groups were at 20%, using an α of 5% and β of 80%, the sample size was calculated as 60. Results were expressed as the median threshold value, defined as the lowest copper concentration detected by 50% of the subjects evaluated. Statistical analysis included non-parametric tests and the Kruskal–Wallis ANOVA median test (Colton, 1974), analyzing by copper salt and by water type.

Test waters

Tap water (TW)

This was obtained from taps located in our laboratory, which fulfil Chilean quality norms (Empresa Metropolitana de Obras Sanitarias, 1996). Its chemical composition was 169 mg/l Ca, 46 mg/l Na, 2 mg/l K, 12 mg/l Mg, 68 mg/l Cl, 280 mg/l SO_4 . The pH was 7.4. These values are also in agreement with recent data published by Lagos and co-workers (Lagos *et al.*, 1999).

Uncarbonated mineral water (UCMW)

This was purchased in the local market (Cachantun; Chile). Its composition before the addition of copper was 216 mg/l

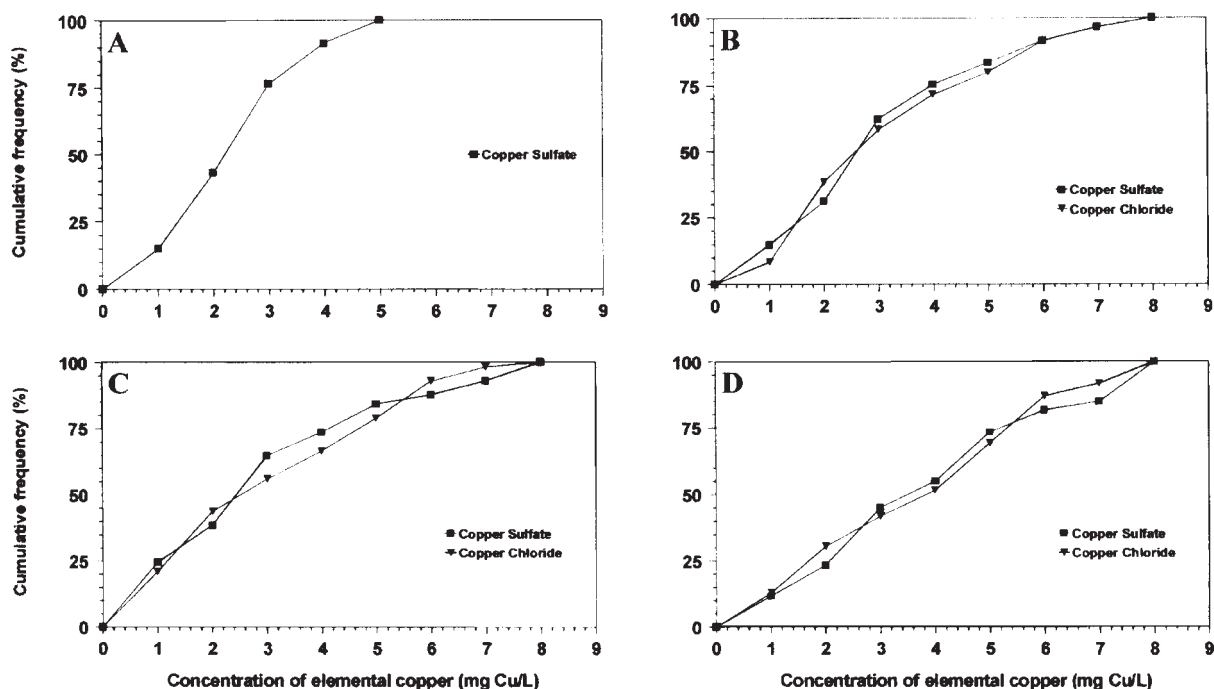


Figure 1 (A) Cumulative frequency of positive reports obtained in the preliminary evaluations using copper sulfate delivered in tap water (taste threshold zones). (B) The cumulative frequencies of positive reports obtained for copper sulfate or copper chloride delivered in tap water (taste threshold curve for tap water). (C) The taste threshold curve for copper sulfate and copper chloride delivered in distilled deionized water. (D) The taste threshold curve for copper sulfate and copper chloride delivered in uncarbonated mineral water.

Na, 4 mg/l K, 76 mg/l Ca, 10 mg/l Mg, 28 mg/l Cl, 85 mg/l SO_4 , and 25 mg/l SiO_3 . The pH was 6.3.

Distilled deionized water

This was purchased as a single batch from Corning (Mega-Pure System MP-6^a). The pH was 5.8.

Copper salts

Because the most common soluble copper compounds in water are copper chloride, copper nitrate and copper sulfate (Environmental Protection Agency, 1998), for this study we chose $\text{Cu SO}_4 \cdot 5\text{H}_2\text{O}$ and CuCl_2 (pro-analysis grade), both purchased from Merck (Darmstadt, Germany).

Solutions

These were prepared daily, at room temperature, using a 100 ml stock solution prepared with distilled deionized water containing 0.5 mg/ml Cu. This solution was added to each test water in amounts such as to obtain the test concentrations 1–8 mg/l Cu. The final copper concentration in the test solutions offered to the volunteers was verified by atomic absorption spectrometry (Perkin Elmer 2280). Results were within 5% of the expected concentration.

Results

Screening test

The results of this test helped define the perception zone of

each subject (Figure 1A). Of the 60 panelists, 43 and 77% perceived the copper taste at concentrations of 2 and 3 mg/l Cu, respectively.

Modified triangle test

Cumulative frequencies of threshold values for copper sulfate and copper chloride in tap water are shown in Figure 1B. Of the individuals, 31 and 38% were able to identify the copper taste at a concentration of 2 mg/l Cu as copper sulfate and copper chloride, respectively. Corresponding values for 3 mg/l Cu were 62 and 58%, respectively. Fifty percent of the subjects reported taste perception at a concentration of 2.6 mg/l Cu for both copper salts.

In DDW, tasting of 2 mg/l Cu (as copper sulfate or chloride, respectively) was reported by 39 and 44% of the subjects (Figure 1C). The corresponding figures for 3 mg/l Cu were 65 and 56%, respectively. Fifty percent of the subjects perceived taste at concentrations of 2.4 and 2.5 mg/l Cu, respectively.

In UCMW, tasting of 2 mg/l Cu (as copper sulfate or chloride, respectively) was reported by 23 and 31% of the subjects (Figure 1D). The corresponding figures for 3 mg/l Cu were 45 and 42%, respectively. Fifty percent of the subjects perceived taste at concentrations of 3.5 and 3.8 mg/l Cu, respectively.

ANOVA analysis of the combined data for both salts and the three types of waters showed that the results obtained

Table 1 Copper concentration that defined the individual taste threshold and confirmation/no confirmation of the response on a second (nose open) and third trial (nose clamped), repeated at the same threshold concentration

Subject code	Threshold concentration (mg/l Cu)	Second evaluation	
		Nose 'open'	Nose 'clamped'
1	4	no	no
2	3	no	yes
3	2	yes	no
4	5	no	yes
5	1	no	no
6	8	yes	no
7	1	yes	yes
8	8	no	yes
9	2	yes	yes
10	6	yes	yes
11	3	no	no
12	2	yes	yes
13	1	no	no
14	3	no	no
15	1	yes	yes
Total		7/15 (n.s.)	8/15

with TW and DDW were similar. In contrast, UCMW gave significantly higher threshold values ($P < 0.01$). For all three waters and the two copper salts the vast majority of the subjects reported a bad, unpleasant or disgusting metallic, acid, bitter or salty taste.

Effect of 'clamping' the nose

When the trials were repeated offering the individuals the copper concentration at which they first reported tasting, 7/15 individuals confirmed their threshold when their nose was open while 8/15 individuals confirmed the threshold when their nose was clamped. When the second set of trials performed in a subgroup of 15 individuals are compared, in 10 of the 15 individuals tested the response was the same, either confirming or not confirming the threshold concentration, with the nose open or clamped. χ^2 analysis showed no significant differences in taste thresholds reported with the nose open or clamped (Table 1).

Discussion

The results of this study help establish the taste threshold for copper in three types of water and with two copper salts, some of them frequently present in daily life. The values 2.5–2.6 mg/l Cu for DDW and TW and 3.7 mg/l Cu for UCMW show that water quality does influence the capacity to taste copper sulfate and copper chloride. We would like to speculate that the higher threshold observed for UCMW may be attributed to copper chelation by polyvalent organic anions (WHO, 1998). The thresholds found are in a range of concentrations slightly below those at which the earliest

adverse effects due to acute copper exposure appear, therefore raising the possibility that the unpleasant taste of copper in the water may be a factor influencing the appearance of mild symptoms. However, because in these studies the individuals did not swallow the solution, it was not possible to evaluate the actual relationship between taste reporting and the appearance of symptoms. None of the subjects experienced symptoms.

The fact that drinking waters usually have <3 mg of copper coincides with the fact that in most places people do not taste copper in water. Taking into account that nausea and other gastrointestinal symptoms may appear at as low as 4 mg/l Cu (as copper sulfate) (M. Olivares *et al.*, submitted for publication), the perception of taste in tap water may be an important indicator to sensitive individuals preventing them from ingesting a specific water, but it must be taken into account that ~40% of people will not taste copper content at concentrations <4 mg/l Cu and therefore will not have the chance to become aware of any risk. This means that color and taste of a specific drinking water are not necessarily good indicators to alert people to avoid drinking a specific water.

It is interesting that very little differences were observed between the salts used, sulfate or chloride. Total sulfates in drinking water are usually high (250 mg/l in Santiago) (Empresa Metropolitana de Obras Sanitarias, 1996) and they are not tasted, therefore, it is unlikely that sulfate instead of copper could be responsible for the taste reported. Béguin-Bruhin *et al.* postulated that tasting copper in water would depend on its chemical species and its solubility. In his study the taste of copper was described as metallic, bitter, chemical and astringent. In our study the subjects also described a variety of tastes, adding acid, bitter and salty to those previously described (Béguin-Bruhin *et al.*, 1983).

In studies of some heavy metal ions, such as Ag^+ , Hg^{2+} and Pb^{2+} , and compounds like ferrous sulfate, tasting and olfactory capacities appear clearly related (Moncrieff, 1967; Hettinger *et al.*, 1990; Meilgaard *et al.*, 1991). In our study repeat testing at the copper concentration that defined individuals tasting thresholds showed that only half of the subjects confirmed their thresholds. Having the nose open or clamped did not significantly change the threshold concentrations, either as individuals or as a group.

Since the methodologies used in this study were based on those described by Cohen *et al.* and Béguin-Bruhin *et al.* (Cohen *et al.*, 1960; Béguin-Bruhin *et al.*, 1983), it is interesting to compare our results with those obtained by them. The threshold for copper in distilled water obtained by us (2.4–2.5 mg/l Cu for copper sulfate and copper chloride) is lower than those reported by Cohen *et al.* and Béguin-Bruhin *et al.* (6.6 and 3 mg/l Cu, respectively). For spring water their values using copper chloride were 1 and 13 mg/l Cu, while our mean values were 3.5 and 3.8 mg/l Cu, depending on the salt used. Unfortunately, tap water was

not evaluated in these earlier studies. It is difficult to speculate about possible explanations for the differences obtained. Several factors may be involved, such as differences in the chemistry of the waters used, cultural characteristics of the study subjects or adaptation to basal concentrations of copper in drinking water in the locations of the studies, which in the case of Santiago are clearly low (<0.01 mg/l).

In conclusion, the tasting thresholds of copper sulfate and copper chloride are between 2.5 and 3.7 mg/l Cu in TW, DDW and UCMW. It is highest when copper is delivered in UCMW, probably because part of the copper dose is chelated by organic anions present in higher amounts in this type of water. The copper salt used, sulfate or chloride, had little influence on the capacity to taste copper. These findings will be relevant information to help better understand the effects of acute copper exposure.

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