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Evaluation of Copper Speciation and Water Quality Factors That Affect Aqueous Copper Tasting Response

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Abstract

This study determined taste thresholds for copper as its speciation was varied among free cupric ion, complexed cupric ion, and precipitated cupric particles. The impact of copper chemistry on taste is important as copper is added to many beverages and can be present in drinking water as a natural mineral or due to corrosion of copper plumbing. A one-of-five test was used to define thresholds with solutions containing 0.025–8 mg/l Cu (from copper sulfate) in distilled or mineralized water of varying pH. The mineralized water was designed to mimic the composition of a typical tap water. Group thresholds for copper in either distilled–deionized water or mineralized water were not significantly different and ranged from 0.4 to 0.8 mg/l Cu. A difference from control test was used to assess the impact of soluble and particulate copper on taste. Soluble copper species, including free cupric ion and complexed copper species, were readily tasted, while particulate copper was poorly tasted.

Key words: copper, speciation, taste, threshold, water

Introduction

Copper is an essential nutrient for which the World Health Organization (WHO, 1998) recommends a daily intake of 30 μg/kg body weight. Copper in drinking water can be an important source of dietary copper for humans (Zacarias *et al.*, 2001). A major source of copper in drinking water is corrosion of copper pipes, which can impart a taste to the water (Edwards *et al.*, 1996; Dietrich *et al.*, 2004, 2005). There are few literature citations on the taste of copper and the role of copper speciation on its taste in water. The taste of copper has been described as bitter, astringent, sour, salty, or metallic (Zacarias *et al.*, 2001; Lawless *et al.*, 2005). Copper in water may at times exceed health-based standards, resulting in increased potential for flavor changes and health concerns (Edwards *et al.*, 1996; Dietrich *et al.*, 2004, 2005).

Drinking water standards have been established to prevent adverse health effects resulting from ingestion of too much copper. WHO (1998) recommends a limit of 2 mg/l Cu to prevent adverse health effects from copper exposure. WHO guidelines also state that a long-term intake of copper between 1.5 and 3 mg/l has no adverse health effects but levels greater than 5 mg/l in water can impart an undesirable bitter taste. The US Environmental Protection Agency (USEPA) developed a health-based action level of 1.3 mg/l Cu in drinking water (USEPA, 1991) and an aesthetic-based standard of 1 mg/l Cu. Copper above this aesthetic standard level can stain plumbing fixtures and laundry as well as contribute to metallic- or bitter-tasting water (USEPA, 1997). USEPA databases from 2003 identified 471 drinking water systems in violation of the copper health-based action level of 1.3 mg/l Cu. Recent problems with pinhole leaks (or nonuniform corrosion) in copper pipes have raised awareness and concerns about an increase in copper levels in drinking water (Edwards *et al.*, 2004).

Copper chemistry

Typical drinking water pH and mineral content allow for the presence of free, complexed, and particulate copper, each of which may play a role in the taste. Copper, like many metals, interacts in water to form free metal cations, a variety of soluble complexes, and insoluble particles or precipitates, depending on the mineral content of the water. Free copper, which is the cupric ion (Cu^{2+}), is soluble and the preferential form at low pH levels (typically below pH 6) and when there is a lack of anionic ligands. In pure water, soluble copper hydroxo complexes form at low and high pH values. The metal precipitates most frequently as copper hydroxide [K_{sp} $Cu(OH)_2 = 10^{-19.32}$] at intermediate pH levels (typically pH 6.5–12). Precipitation is dependent on copper concentration, presence of other anions and cations, temperature, and time to thermodynamic equilibrium (Jensen, 2003). Figure 1 demonstrates the distribution of individual hydroxo Cu(II) complexes in pure water as a function of pH and identifies where copper hydroxide precipitate will form. Figure 2 demonstrates how the concentrations of individual hydroxo Cu(II) complexes vary when the total copper concentration is fixed at the USEPA aesthetic standard of 1 mg/l. While hydroxo complexes are always present in water, individual or combinations of anions can bind to cupric ion to form complexes based upon stability constants. Copper will form complexes with common anions, including SO_4^{2-}, OH^-, PO_4^{3-}, HCO_3^-, NO_3^-, and CO_3^{2-}. Precipitates of these complexes form when the solubility product is exceeded. A common multianionic precipitate is malachite [$Cu_2(OH)_2(CO_3)$], which is a blue–green cupric-hydroxide-carbonate precipitate.

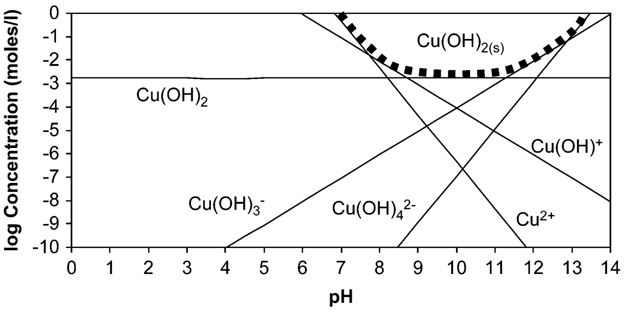


Figure 1 The pC–pH diagram [(−log concentration)–(−log[H^+]) diagram] for cupric solubility in pure water demonstrating at which pH values and concentrations hydroxo complexes [$Cu(OH)_{n]aq)}$] form and copper hydroxide [$Cu(OH)_{2(s)}$] precipitates; inside dotted line shows zone where copper hydroxide solid will form.

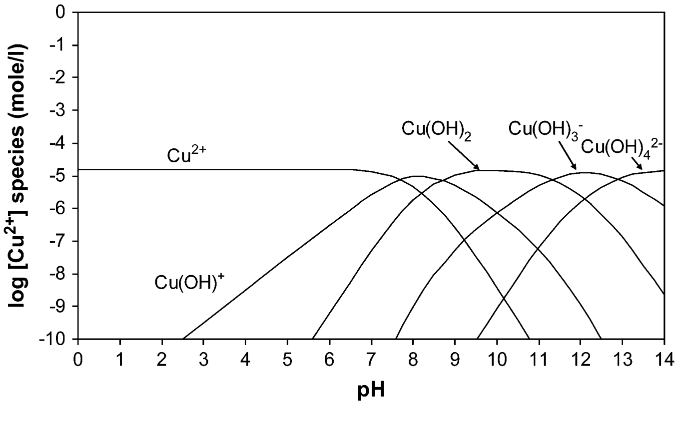


Figure 2 Theoretical copper speciation for hydroxo complexes in pure water for a total copper concentration of 1 mg/l, which is the value for the USEPA aesthetic-based standard.

It is well established that copper speciation affects toxicity and bioavailability in aquatic organisms (from algae to fish). Free copper (II) ion and monohydroxo Cu(II) are considered highly toxic, while other anionic complexes, especially carbonato complexes, are less toxic to aquatic organisms. Particulate copper is not toxic unless it is solubilized in water or the fluids within an organism. Lethal aqueous concentrations at which 50% of the organisms die vary among aquatic species from 0.005 to 1 mg/l depending on the organism and its life stage (Hodson *et al.*, 1979; USEPA, 1985). Copper is much less toxic to mammals, as reflected in the health-based standards previously discussed. Although the role of speciation of copper is known to be important in aquatic toxicity, its role in human sensory response is not well established.

The taste of copper

Aesthetic-based standards for copper in drinking water, ranging from 1 to 5 mg/l Cu, are in a similar range to the health-based recommendations of 1.3–2 mg/l. Only a few previous studies, summarized in Table 1, addressed the taste threshold of copper with limited focus on the speciation of copper in water. Global locations of these studies are reported in the following discussion because mineral content of tap and natural waters are primarily influenced by local geography and may have been vastly different in these studies; however, authors did not report detailed water quality data.

One research study in the United States found taste thresholds of 6.6 and 13 mg/l Cu in distilled water and spring water, respectively (Cohen *et al.*, 1960). Threshold results represented the concentration at which 50% of the panelists tasted copper, and 95% confidence intervals were used in the statistical analysis. Soluble copper was maintained by adjusting pH to 6.0. The triangle test method was used, and three sets of copper concentrations were administered per person per session with a test range of 1.6–16.8 mg/l. This technique did not specifically address copper taste adaptation of the 15–20 panelists, some of whom were smokers.

Lower thresholds, 2.4–3.2 mg/l Cu and 0.8–1 mg/l Cu in distilled and mineralized waters, respectively, were reported in a study conducted in Belgium (Beguin-Bruhin *et al.*, 1983). The importance of copper solubility to taste perception was identified, and solution pH was adjusted to control copper solubility. A one-of-five test format was used to decrease the likelihood of guessing correctly. Only one concentration was given per session because panelists exhibited a decreased sensitivity to the copper stimulus when given multiple copper-containing samples in one session (adaptation). To control effects from aftertastes, 1-min wait periods were mandated between samples. A weak sucrose solution instead of distilled water was used as the control and rinse water due to the unpleasant taste of distilled water. The copper range was 0.1–20 mg/l in pH 5.9 or 6.5 water, but interval concentrations for

Table 1 Copper taste thresholds from previous studies

Research study	Threshold in distilled water, mg/l Cu	Water pH	Range tested, mg/l Cu	Sensory method	Comment
Cohen *et al.* (1960)	6.6	6.0	1.6–16.8	Triangle test	Did not address adaptation
Zacarias *et al.* (2001)	2.5	7.4	1–8	1 of 5	Wait period between samples
Beguin-Bruhin *et al.* (1983)	2.4–3.2	5.9–6.5	0.1–20	1 of 5	Wait period between samples; sucrose rinse

copper were not provided. The low pH conditions would reduce likelihood of particulate formation until high copper levels (several milligrams per liter) were reached. Confidence intervals (95%) and a correction for guessing were applied to threshold calculations. This study concluded that only soluble copper provided a taste sensation.

Zacarias *et al.* (2001) found thresholds of 2.6 mg/l Cu for tap water, 2.5 mg/l Cu for distilled water, and 3.5 mg/l in mineral water (pH 7.4). This study, performed in Chile, also used the one-of-five protocol, and only one concentration was given per session to address adaptation. The copper range was from 1 to 8 mg/l Cu, with 1 mg/l concentration steps. One-minute wait periods were mandated between samples to minimize aftertaste effects. Copper chloride and sulfate salts were used, and no significant difference in threshold values was found for the two salts. Threshold results represent the concentration at which 50% of the panelists could taste copper. However, neither confidence intervals nor guessing correction techniques were used. The effect of pH on soluble and particulate copper species was not addressed. Nose clamping was used to determine the retronasal effect on copper tasting; no significant effect was shown by clamping the nose. Lawless *et al.* (2004) showed that nasal occlusion did not significantly reduce panelist's metallic, bitter, and astringent ratings of copper in water.

These previous studies show a wide deviation in threshold values for copper with conflicting results in distilled and other water sources. In addition, these studies did not provide detailed water quality data and therefore could not thoroughly evaluate the distinct effects of copper speciation in taste threshold determination. The goal of this research was to specifically evaluate the role of free, soluble, and particulate copper in taste and do so at concentrations below and near health-based standards. The pH and presence of anions were used to control copper speciation. The specific objectives were 1) to determine the taste threshold of free and complexed soluble copper and 2) to evaluate the role of particulate copper in taste sensation.

Materials and methods

Panel description and initial training

Thirty-six healthy adults, with no previous copper taste threshold experience, participated in four studies. The panel consisted of 15 males and 21 females ranging from 22 to 54 years of age and reporting no chronic health problems. The sensory protocol was approved by the Institutional Review Board at Virginia Tech; all panelists signed informed consent forms.

All panel members underwent an initial training session to familiarize them with the taste of copper and the sensory test methods. Panelists were instructed to swallow the samples as many panelists reported tasting low concentrations on the back of the tongue and throat. Preliminary testing with five panelists indicated that an aftertaste was prevalent with copper. Therefore, only one tasting session was administered per day, and only one copper concentration was tasted per session. Zacarias *et al.* (2001) and Beguin-Bruhin *et al.* (1983) also reported aftertaste and administered only one copper concentration per session.

Copper stimuli

A 100-mg/l copper stock solution was prepared from copper (II) sulfate pentahydrate (catalog number BP346, Fisher Scientific, Pittsburgh, PA, USA) and diluted to obtain concentrations in the range of 0.025–8 mg/l Cu. All samples were prepared fresh daily to avoid increased precipitation with time. All copper solutions were maintained and presented to panelists at room temperature, within 22–24°C.

Fourteen concentrations (0.025, 0.05, 0.1, 0.5, 1, 1.3, 2, 2.5, 3, 4, 5, 6, 7, and 8 mg/l total Cu) were used for threshold testing. Concentration intervals used for this research were not uniform but were selected to emphasize health and aesthetic-based standards. Soluble and particulate copper concentrations in the test water samples were manipulated by controlling the pH. Actual concentrations were verified by flame or furnace atomic absorption spectrometry (Perkin-Elmer 5100 PC, Norwalk, CT, USA). Filtration through a 0.45 μm filter was used to separate dissolved from particulate copper. Free copper ion concentrations were measured using a cupric electrode (accumet cupric combination electrode, catalog number 13-620-547, Fisher Scientific).

Test water sample preparation

Distilled–deionized water was generated from a Barnstead Nanopure system that was fed distilled water and subsequently deionized and carbon filtered. This system produced water with a chemical resistivity of 18 MΩ/cm and pH 5.5.

A mineralized water at pH 7.4 was designed to simulate a typical drinking water from the eastern United States. The chemical composition of this water sample was 21 mg/l Na^+, 10.0 mg/l Cl^-, 1.5 mg/l NO_3^--N, 41 mg/l SO_4^{2-}, 8 mg/l Mg^{2+}, 4 mg/l K^+, 12 mg/l Ca^{2+}, 34 mg/l HCO_3^-, and 2.6 mg/l SiO_3^{2-}. The pH of the mineralized water was adjusted with small amounts of 1 M HCl or 1 M NaOH to alter copper solubility. Copper stock solution was added to achieve concentrations of 0–8 mg/l.

Taste thresholds were measured at pH 5.5, 6.5, and 7.4; the distribution of soluble and particulate copper in water at these pH values is shown in Figure 3. Copper was soluble at all concentrations in pH 5.5 distilled water. At higher pH values, the amount of soluble copper was a function of both pH and the total copper concentration, with a maximum of 4 mg/l soluble copper at pH 6.5 and a maximum of 1.3 mg/l soluble copper at pH 7.4. The nonsoluble, or particulate, copper resulted in formation of a fine precipitate.

The amount of free copper ion also varied with pH. Free copper ion, or Cu^{2+}, is copper that is not a particulate or complexed with an anion. Figure 4 demonstrates that at pH 5.5 in distilled water all the copper was present as free copper ion. At pH 6.5, the maximum amount of free copper ion was 3 mg/l, while at pH 7.4, the maximum amount was only 0.3 mg/l.

Evaluation of pH effects on sensory response

To test the effect of pH alone, panelists participated in a similarity test with distilled–deionized water adjusted to pH 7 or 9 with NaOH. The triangle test was used with $n = 53$, $\alpha = 0.3$, $\beta = 0.01$, and portion of distinguishers (p_d) = 30%. Values of α and β were chosen to achieve power and minimize Type II errors. Panelists were told to choose the odd sample. Out of 53 respondents, only 15 correctly chose the odd sample. The results demonstrated that pH alone did not affect panelist's perceptions of water (Meilgaard *et al.*, 1999). Therefore, any differences in sensory perception for these experiments were not related to pH changes and could be linked to copper speciation and subsequent interactions.

Experiment 1: effects of copper speciation on copper thresholds

The first experiment evaluated which copper concentrations consumers could taste in water. Copper taste threshold results from previous studies ranged from 1 to 13 mg/l but did not thoroughly investigate copper speciation (Cohen *et al.*, 1960; Beguin-Bruhin *et al.*, 1983; Zacarias *et al.*, 2001). Preliminary research in our laboratory demonstrated that humans could readily taste copper at much lower concentrations than these published thresholds. Copper chemistry and the effect of water quality were investigated in our research experiment by using pH adjustment and presence of anions to varying formations of free, soluble complexed, or particulate copper.

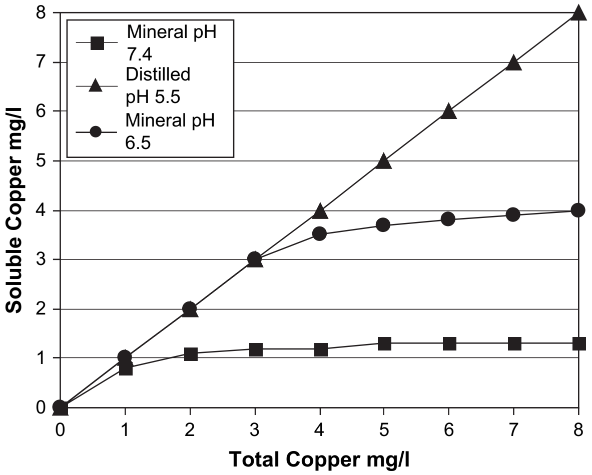


Figure 3 Soluble copper as a function of pH as measured by filtration through a 0.45-µm filter and atomic absorption.

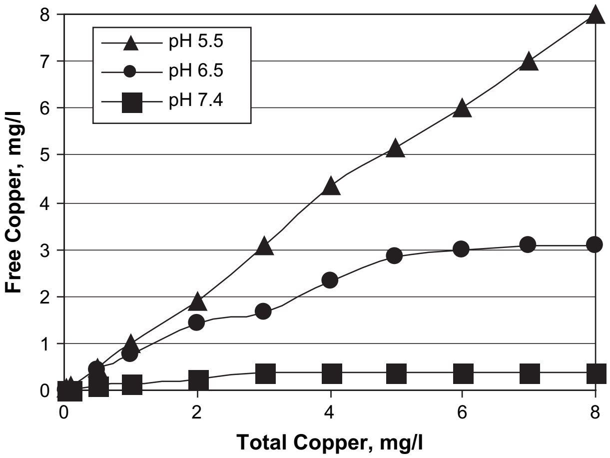


Figure 4 Measurement of free copper ion, Cu^{2+}, in distilled and mineralized water by ion-specific electrode.

Test water sample preparation

As described in Materials and Methods, the test waters were distilled–deionized water compared to a mineralized water at pH 7.4 designed to simulate a typical drinking water from the eastern United States. The distilled–deionized water was generated from a Barnstead Nanopure system. Copper was added to produce concentrations of 0–8 mg/l Cu.

Sensory procedure

All test waters were presented at room temperature (22–24°C) in a controlled atmosphere with minimal noise or odor influence. For each test, the control, rinse water, and the copper solution were the same pH and mineral content. An ascending concentration forced choice test was used to determine human taste thresholds (ASTM, 1991, 1997;

Lawless and Heymann, 1999; van Aardt *et al.*, 2001). To decrease the possibility of guessing correctly, the protocol was modified to five samples (four controls and one copper sample). Beguin-Bruhin *et al.* (1983) and Zacarias *et al.* (2001) also used the one-of-five method.

Five 3-oz white plastic sample cups were coded with three-digit random codes, filled with 20 ml sample and presented to panelists in randomized order. One of the five samples contained an aqueous solution with copper added; the others contained the same aqueous solution with no copper. A session began by panelists rinsing with copper-free test water, then tasting the first sample, waiting at least 20 s, and then tasting the next sample. Panelists were instructed to taste the samples from left to right and to taste each sample only once; panelists were told to choose the ''odd'' sample. Panelists were asked to use their own descriptors to describe the taste of copper; a list of descriptors was not provided. Only one set of five samples was evaluated per day; panelists were exposed to increasing concentration steps within the testing range on subsequent days. A positive report was defined when a panelist correctly identified three correct samples in a row.

Thresholds were calculated by both geometric mean and logistic regression methods. The geometric mean is based on where the subject fails to detect the sensation of interest. The taste threshold for an individual panelist was calculated as the geometric mean of the last incorrect copper concentration and the first correct copper concentration when the panelist correctly detected three copper concentrations in a row. For geometric mean calculations, a value of 10 mg/l Cu was applied as the upper copper concentration if a panelist had an individual threshold >8 mg/l Cu. The group threshold was calculated as the geometric mean of individual geometric mean values. The logistic regression method (ASTM 1432-91) uses binary data to predict where a certain proportion of the group will correctly identify the copper taste. For this research, the threshold is based on using 50% as the proportion that should be able to detect copper. Logistic regression group threshold concentrations were calculated using the Abbott's formula [equation (1)] with 50% as the criterion and a probability of guessing by chance of 20% (one-of-five), leading to the probability of 0.60 to define the group threshold.

$$0.5 = \frac{x - 0.20}{1 - 0.20} \rightarrow x = 0.60. \qquad (1)$$

Results and discussion

The experimental design allowed >8 mg/l Cu to be present as soluble free copper in the pH 5.5 distilled–deionized water but only ≤0.3 mg/l free copper and ≤1.3 mg/l soluble (free and complexed) copper in pH 7.4 mineralized water (Figures 3 and 4). Panelists described the taste of copper mostly as metallic, but bitter and bloody were also used as descriptors. The majority (~70%) of 36 panelists that participated in threshold testing had individual geometric mean thresholds <1 mg/l Cu in either distilled–deionized pH 5.5 or pH 7.4 mineralized water. Interestingly, at concentrations <1 mg/l, most copper are in the soluble form (Figure 3).

Analyses of the individual threshold results in these two water samples provided insight on the effects of chemistry on copper tasting. A Wilcoxon nonparametric paired test indicated that the 36 individual geometric mean values for copper taste thresholds for the pH 5.5 distilled–deionized and 7.4 mineralized water were not significantly different ($P = 0.357$). The similar individual threshold values in these two water samples with very different copper speciation indicated that both free copper and soluble complexed copper were tasted. The role of particulate copper was not well evaluated in this experiment because most panelists could taste copper at levels below 1.3 mg/l where copper was present in the soluble form at pH 7.4.

The group thresholds were calculated as the geometric mean of the individual threshold values for each panelist ($n = 36$). Geometric mean group thresholds were 0.48 and 0.39 mg/l in distilled–deionized and pH 7.4 mineralized water, respectively. Logistic regression group thresholds were 0.77 and 0.75 mg/l in distilled and mineralized waters, respectively. The factor of 2 difference between the geometric mean thresholds and logistic regression-based group thresholds is not a substantial difference given the different criteria for calculating the group thresholds. The logistic regression group threshold is based on when 50% of the group could taste copper; the geometric mean group threshold is based on individual thresholds that required a panelist to correctly taste copper at three consecutive concentrations.

In summary, threshold results from this research were lower than previous studies. Taste thresholds of copper for our study, depending on the threshold method, ranged from 0.4 to 0.8 mg/l Cu, and similar values were obtained for both distilled and mineralized water by each threshold method. Previous studies had threshold estimates ranging from 1 to 13 mg/l Cu and varying results concerning the role of mineral water. Variations in concentration interval, statistical analysis, sensory test conditions, conditions affecting copper chemistry, and test objectives all likely to play a role in producing variation in test results among studies. Our results show that soluble copper can be readily tasted whether it is free or complexed with anions. Thus, waters containing 1 mg/l free copper or 1 mg/l soluble complexed copper would produce the same copper taste intensity.

Experiment 2: threshold testing in pH 6.5 mineralized water to evaluate the role of particulate copper

Results from Experiment 1 indicated that the majority of panelists could taste copper at concentrations where the chemistry allowed the copper to be soluble. Thus, this experiment did not directly allow assessment of the role of particulate copper. In Experiment 1, there were seven panelists who had individual thresholds greater than 8 mg/l Cu in the pH 7.4 water, which had a maximum of 1.3 mg/l soluble copper and therefore up to 6.7 mg/l Cu in particulate form. These panelists had thresholds in distilled–deionized water that contained all free and soluble copper from 2.2 to 6.5 mg/l Cu. This group of seven was labeled "insensitive" panelists, and their thresholds are presented in Table 2. In order to evaluate the role that particulate copper plays in the taste of copper, another threshold study was conducted with pH 6.5 mineralized water to increase the amount of soluble copper to a maximum of 4 mg/l Cu in the presence of minerals (where soluble copper includes both free and complexed copper). It was hypothesized that the seven insensitive panelists would taste copper at <8 mg/l in the pH 6.5 water because there would be more soluble copper available for detection.

The seven insensitive panelists were compared to a group of 11 "sensitive" panelists who all had individual thresholds below the level where particulate copper began to form (~1 mg/l Cu). The 11 sensitive panelists would detect the taste of copper in a soluble form at pH 5.5, 6.5, or 7.4, and thus this group served as the control.

Table 2 Individual geometric mean thresholds for pH 5.5, pH 6.5, and pH 7.4 waters

Panelist number	Individual geometric mean thresholds, mg/l Cu		
	Distilled–deionized pH 5.5 (8 mg/l max soluble)	Mineralized pH 6.5 (4 mg/l max soluble)	Mineralized pH 7.4 (1.3 mg/l max soluble)
Insensitive panelists			
1	6.48	>8	>8
2	6.48	>8	>8
3	6.48	5.48	>8
4	2.74	3.46	>8
5	2.24	2.24	>8
6	2.74	2.24	>8
7	2.24	1.6	>8
Sensitive panelists			
8	1.14	0.71	1.61
9	4.47	0.71	1.61
10	0.71	0.71	0.71
11	1.14	0.22	1.14
12	0.07	0.22	0.22
13	0.22	0.22	0.22
14	2.64	0.22	0.22
15	0.22	0.07	0.22
16	0.035	0.07	0.22
17	0.04	0.04	0.04
18	0.22	0.04	0.04

Test water sample preparation

The pH 5.5 distilled–deionized water and pH 7.4 mineralized water were the same as for Experiment 1. The mineralized water was adjusted to pH 6.5 with HCl, which increased the maximum soluble copper concentration to 4 mg/l and a maximum free copper concentration to 3 mg/l (Figures 3 and 4).

Sensory procedure

The threshold procedure for the pH 6.5 mineralized water was identical to the one-of-five test described in Experiment 1. A select panelist group (18) that participated in Experiment 1 [11 with individual thresholds $\lesseqgtr$ 1 mg/l copper (sensitive) and 7 with individual thresholds >2 mg/l in pH 7.4 water (insensitive)] was chosen to participate in Experiment 2. Individual geometric mean thresholds in the pH 6.5 mineralized water were determined for all 18 panelists in this experiment. These results were then compared to the individual geometric mean thresholds from the mineralized water pH 7.4 from Experiment 1.

Results and discussion

Five of the seven insensitive panelists were able to taste copper in the pH 6.5 mineralized water that provided them with 2.7 mg/l more soluble copper than was available to them in the pH 7.4 water (Table 2). This result indicates that soluble copper plays an important role in the taste sensation and particulate copper is poorly tasted if at all.

The 11 sensitive panelists were able to detect the taste of copper in all three water samples (pH 5.5, 6.5, and 7.4) that had maximum soluble copper concentrations between 1.3 and 8 mg/l. Table 2 might suggest that panelists 8 and 9 did not detect copper at pH 7.4 when it was in the soluble form because their geometric mean threshold is 1.6 mg/l which is greater than the 1.3 mg/l Cu solubility limit, but this is an artifact of the geometric mean calculation. The 1.61-mg/l value is the geometric mean of the tested concentrations 1.3 and 2.0 mg/l Cu, and the 2.0-mg/l concentration would have had more soluble copper than the 1.3-mg/l sample.

A Wilcoxon nonparametric paired test was used to compare the individual geometric mean threshold values of the 18 panelists who evaluated pH 6.5 and pH 7.4 mineralized waters, in which the pH 6.5 water could have up to 2.7 mg/l more of soluble copper. The two means were found to be significantly different ($P = 0.004$). Most of the threshold variation occurred at higher copper concentrations where the amount of soluble copper was most different between the two water samples. Closer inspection of the results showed that thresholds decreased as more soluble copper was available. These data further support the conclusion that soluble copper controls tasting and particulate copper was poorly tasted.

Results of Experiments 1 and 2 demonstrated that soluble copper was readily tasted, while particulate copper was poorly detected. This is important for drinking water applications, where copper levels above the 1.3-mg/l USEPA's regulatory action limit are likely to be present as particulate copper and may not be tasted.

Experiment 3: evaluating the difference in perceived taste intensity of soluble versus particulate copper for a 1 mg/l Cu solution

The previous two experiments were discrimination tests, and consequently the relative intensity of tasting soluble or particulate copper could not be assessed. A difference from control test was performed to evaluate the effect of particulate copper on taste. Mineralized waters were prepared with 1 mg/l of total copper at pH 7 and pH 9 with 0 and 0.75 mg/l particulate copper, respectively. The objective of this experiment was to determine if panelists would perceive the sample with more soluble copper as having more copper taste.

Test water sample preparation

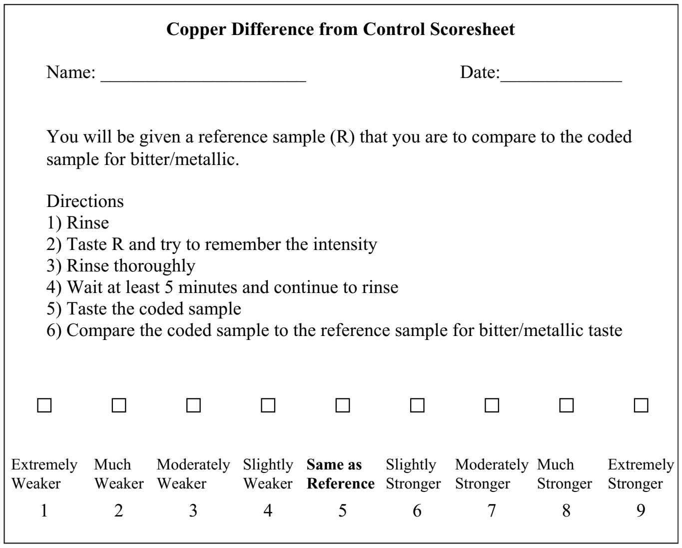
The constant "reference" sample consisted of pH 9 mineralized water containing 1 mg/l total copper, of which 0.25 mg/l was soluble and 0.75 mg/l was particulate. The comparative coded sample was either the same as the reference or consisted of pH 7 mineralized water containing 1 mg/l total copper that was all in soluble form; no particulate copper was present.

Sensory procedure

Two 3-oz white plastic sample cups were placed on a tray and filled with 20 ml of water. One cup contained the reference water and was labeled "R." The second cup contained the comparative sample labeled with a three-digit random code. Panelists first tasted the reference sample and then tasted the coded sample and compared the tastes to describe the difference in the copper taste attribute on the category scale. A category scale with verbal descriptors was used for this test (Figure 5). Two of these tests were administered. For one test, the comparative sample was identical to the reference and was used to measure the placebo effect. For the other test, the comparative sample was different from the reference and contained 0.75 mg/l more soluble copper than the reference. Panelists were familiar with the time delay of the copper taste. Panelists were given two copper-containing samples in one taste session and instructed to wait 5 min between tasting samples. Due to the 1-mg/l copper concentration present in these water samples, only those 21 panelists that had previously demonstrated thresholds at or below 1 mg/l Cu were tested.

Results and discussion

Figure 6 shows how each panelist rated the comparative sample to the reference sample on the category scale. Weighted averages were calculated by multiplying the number of responses by the numerical scale translation of the category scale and then dividing by number of panelists. A value of 5.2 was the weighted response for the reference compared to the reference. Because a value of 5.0 would equal "same as reference," the 5.2 value indicates that panelists perceived



Copper Difference from Control Scoresheet

Name: ____________________ Date:____________

You will be given a reference sample (R) that you are to compare to the coded sample for bitter/metallic.

Directions
1) Rinse
2) Taste R and try to remember the intensity
3) Rinse thoroughly
4) Wait at least 5 minutes and continue to rinse
5) Taste the coded sample
6) Compare the coded sample to the reference sample for bitter/metallic taste

☐	☐	☐	☐	☐	☐	☐	☐	☐
Extremely Weaker	Much Weaker	Moderately Weaker	Slightly Weaker	**Same as Reference**	Slightly Stronger	Moderately Stronger	Much Stronger	Extremely Stronger
1	2	3	4	5	6	7	8	9

Figure 5 Sample scorecard and category scale used in difference from control test to evaluate the role of soluble and particulate copper on the intensity of the bitter/metallic taste. Only the verbal scale was provided to panelists; the 1–9 numerical scale was used to translate the verbal scale for statistical analyses (e.g., extremely weak = 1, same are reference = 5, and extremely strong = 9).

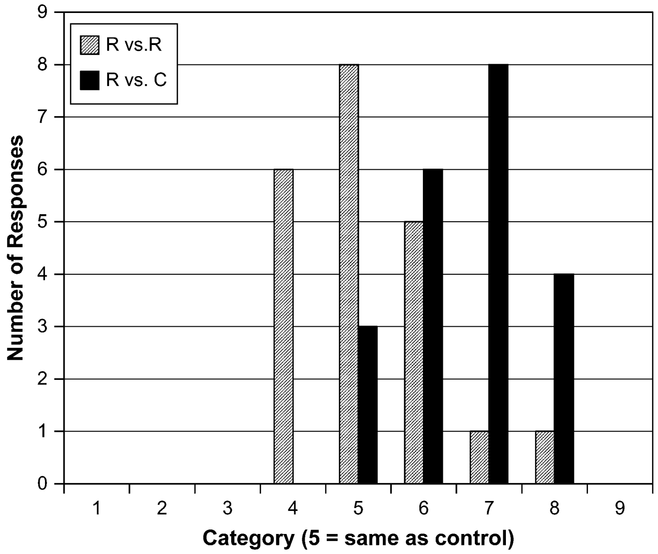


Figure 6 Individual panelist's ratings for the difference from control test for mineralized waters with the same amount of total copper but different amounts of soluble copper. Category scale corresponds to 1 = extremely weak, 5 = same are reference (no difference), and 9 = extremely strong (see Figure 5). R = "reference sample" that contained 0.25 mg/l soluble and 0.75 mg/l particulate copper. C = "comparative sample" that contained 1 mg/l soluble total copper with no particulate copper.

little difference when the samples were the same. The test where the comparative sample contained 0.75 mg/l more soluble copper but the same total copper as the reference resulted in "stronger" descriptors (between "slightly" and "moderately") and a higher weighted average of 6.6, indicating that the sample with more soluble copper had a more intense metallic taste than the sample with the same total amount of copper but only 0.25 mg/l soluble copper.

A one-sided Wilcoxon nonparametric paired test indicated that the comparative sample with more soluble copper had a more intense copper taste than the reference sample ($n = 21$, $P < 0.001$). This experiment showed that when the soluble copper concentration was increased but the total copper remained the same, the sample with more soluble copper was perceived as having a more intense copper taste, while particulate copper was poorly tasted.

Experiment 4: evaluating the role of particulate copper in taste sensitivity at a concentration of 5 mg/l Cu

Results to this point demonstrate that panelists do not readily taste particulate copper. However, the previous experiments were designed to evaluate the role of soluble copper and were not ideal for assessing the role of particulate copper. To specifically investigate the role of particulate copper in affecting taste perception, a pH 8.5 mineralized water with 4.7 mg/l particulate copper and 0.3 mg/l soluble copper was tested. The goal of this experiment was to determine if panelists with thresholds above the level in the control could taste copper if mostly particulate copper was added. If particulate copper was not tasted at all, then any panelist that could not taste copper in the control would not be expected to taste copper in the pH 8.5 water. If particulate copper has some role in tasting, then panelists would be able to taste copper in the pH 8.5 water and not in the control.

Test water sample preparation

The mineralized water pH 8.5 contained a total copper concentration of 5 mg/l, 4.7 mg/l particulate copper, and 0.3 mg/l soluble copper. The pH 8.5 water used for this experiment produced enough blue copper precipitate in the white sample cups that panelists could see the blue color. A modification was used that presented the samples in semitransparent blue 16-oz cups. The blue cup color and shallow depth prevented panelists from discriminating the copper-containing samples by sight.

Sensory procedure

The same 36 panelists who participated in Experiment 1 also participated in this study using a one-of-five protocol similar to that used in Experiment 1 with the exception that only one concentration of copper was tested. A best of three methods was used to produce a "yes" or "no" verdict. A correct verdict for two of the three tests was coded as a "yes," indicating that the panelist was sensitive to the taste difference between the samples. If the panelist did not identify the correct sample at least two times, then they were designated "no" for tasting copper. There is a 1 in 25 chance of guessing both samples correctly using this method.

The "yes" or "no" results from the pH 8.5 mineralized sample water were then compared to the mineralized pH 7.4 control water using panelist threshold results for pH 7.4 mineralized water from Experiment 1. These pH 7.4 thresholds were analyzed, and individual panelists with thresholds above 0.3 mg/l Cu were designated as "no" (unable to taste), and those panelists with thresholds under 0.3 mg/l Cu were designated as "yes" (able to taste).

Results and discussion

McNemar's test contingency table analysis for paired data was used to test the effect of increased amounts of insoluble copper on taste of copper (Table 3). Detection of a copper taste in the pH 7.4 control sample was significantly different ($P = 0.023$) from the pH 8.5 mineralized water. Seven panelists who did not taste copper in the control tasted it in the pH 8.5 water. This would not be expected if particulate copper played no role in tasting.

Closer inspection of the results is of interest. Five of the seven panelists who changed their verdict from "no" in the control to "yes" with addition of particulate copper had copper thresholds relatively close to the soluble limit of the pH 8.5 water (0.3 mg/l Cu). This suggests that particulate copper may have some role in taste perception, but it is not to a great degree. A possible explanation is that particulate copper may become soluble due to dilution and pH changes in the mouth in the presence of human saliva; this is an area for future research.

Summary discussion

The study produced group thresholds for copper in the range of 0.4–0.8 mg/l in both distilled and mineralized water. Like Zacarias *et al.* (2001), the thresholds were similar between distilled and mineralized water, which is a different result than observed by Cohen *et al.* (1960) and Beguin-Bruhin *et al.* (1983). The thresholds in our study were generally lower than those previously reported. This may be due to the concentration interval used that focused on testing

Table 3 Contingency table for control versus mineralized pH 8.5 ($n = 36$)

		Panelist tasted copper in pH 8.5 mineralized water (0.3 mg/l soluble Cu and 4.7 mg/l particulate Cu)	
		No	Yes
Panelist tasted copper in control (pH 7.4 and 0.3 mg/l soluble Cu)	No	9	7
	Yes	0	20

concentrations at or below 1 mg/l, the value for the USEPA aesthetic-based standard, and near the solubility limit of copper in a typical pH 7–8 drinking water.

The experiments in this study clearly demonstrate that both free copper ion and soluble copper complexes can be readily tasted. The results with particulate copper indicate that particulate copper is poorly tasted, especially compared to soluble copper species. Because the previous studies typically tested concentrations above the solubility limit, the higher threshold values for these studies may reflect the inability of panelists to taste the particulate form. The sensitivity tests indicated that adding a large amount of particulate copper only increased the copper taste by a slight to moderate amount.

Concerning human health, this study indicates that 70% of the panelists could taste copper at or below a concentration of 1 mg/l, while 75% of the panelists could taste copper at or below the health-based standards of USEPA or WHO. Those who could not detect copper at these regulatory levels would probably not be able to detect copper in a typical drinking water due to the solubility limits of copper. As the copper concentration increases above 1 mg/l, more and more of the copper will be in a particulate form that is poorly tasted. Therefore, a minority of the population could potentially ingest higher levels of copper than are recommended without having an adverse taste effect.

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