Evaluating factors that affect copper tasting sensitivity in drinking water

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(Abstract)

Corrosion of household copper plumbing infrastructure can cause pipe failure and lead to elevated levels of copper in drinking water which can exceed the USEPA health based standard for copper in drinking water of 1.3 mg/L Cu. The purpose of this study was to determine taste thresholds of copper in different types of water, analyze how copper chemistry can affect tasting, determine if common disinfectants influence the taste of copper and evaluate genetic links to copper sensitivity. A one-out-of-five test was used to define thresholds, evaluate disinfectant influences, and examine copper chemistry differences. A difference from control test was used to analyze soluble copper tasting and a one solution test with visual classification was used to discriminate 6-n-propylthiouracil (PROP) taster status.

Solutions containing copper sulfate (0.05 – 8 mg/l Cu) were prepared in distilled water, mineral water of varying pH and mineral water with disinfectant added. Geometric mean copper taste thresholds were 0.48 mg Cu/l and 0.41mg Cu/l in distilled and mineral water pH 7.4 respectively. Logistic regression copper taste thresholds were 1.50 mg Cu/l and 1.96 mg Cu/l in distilled and mineral water pH 7.4 respectively. Soluble copper was readily tasted while particulate copper was poorly tasted. Chlorine and chloramines dosed at typical tap water levels had no significant effect on panelists' tasting abilities for water containing 1 mg/l total copper. Geometric mean copper thresholds values did not correlate with (PROP) status so PROP sensitivity would not be a good indicator for copper sensitivity.

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Author's Preface

This thesis is presented in the Virginia Tech manuscript format. The first chapter entitled "Introduction" and the second chapter "Literature Review" introduce the topic and review scientific literature. The third chapter "Evaluating factors that affect copper tasting sensitivity in drinking water" is a manuscript formatted for journal submission to Chemical Senses. The appendices contain raw data, scoresheets, panel observations, consent forms and questionnaires used.

1 Introduction

An estimated 70-80% of drinking water pipes currently in homes and being installed in new homes are made of copper (Townley, 2004). Corrosion of copper drinking water infrastructure is a major problem for homeowners with copper pipes. This corrosion can lead to two distinct problems. Increased corrosion can cause pipe failure and also produce drinking water that is not aesthetically pleasing and has the possibility of causing negative health effects. Pinhole leaks have been shown to be a major cause of copper drinking water pipe failure (Edwards et al. 2004). Copper in drinking water is monitored by the Lead and Copper rule. Historically copper has not been given priority in sampling protocols. This leaves the public with little means of protecting themselves from both copper pipe failure and increased by-product release that can cause negative health effects. This research set out to find what at what concentrations humans can taste copper in water and how changes in water quality can affect their tasting ability. If consumers are sensitive enough to low copper levels they could possibly identify copper corrosion simply by tasting their tap water.

Multiple sensory tests were conducted to investigate human copper tasting sensitivity and how changes in water chemistry can alter consumer sensitivity. A one-out-of-five test was used to conduct threshold testing while similarity and difference from control tests were used to address other characteristics of copper tasting. Many modifications to sensory protocols were used to deal with the complex problems encountered in tasting copper. Previous research evaluated human copper taste thresholds in water. These studies have reported various human thresholds and their differences can be attributed to changes in chemistry, various sensory methods and project objectives.

Chlorine and chloramines are two popular forms of residual disinfectant used at drinking water treatment plants. Their role in copper tasting was explored due to the possibility of interfering with copper tasting. An alternative method for testing sensitivity to copper was also explored.

2 Literature Review

2.1 Copper and Human Health

Copper is an essential dietary nutrient and casual intake of copper is necessary for survival. Copper is required for the proper functioning of important enzymes and metabolic systems (Olivares, et al. 1996). Copper containing enzymes include: ceruplasmin, superoxide dismutase, cytochrome oxidase, tyrosinase, monoamine oxidase, lasyl oxidase and phenylalanine (WHO, 1998). Dietary copper is primarily absorbed in the large intestine through active and passive transport of binding sites (WHO, 1998). The liver also is affected by copper intake. In the human body copper can be stored (bound mainly to metallothionein), secreted to the plasma (bound to ceruloplasmin) or excreted in the bile (Bremner, 1987). The total body content in a 70 kg adult is about 110 mg Cu and recommendations are that adults consume about 30 μg/kg bodyweight per day (Dietrich et al. 2004). Menkes syndrome and Wilson's disease are two known medical conditions that are related to human copper intake. Menkes syndrome is an inherited defect that creates a copper deficiency (Olivares et al. 1996). Wilson's disease sufferers are unable to rid their body of excess copper and suffer from copper toxicity (WHO, 1998).

Human copper intake can occur through skin contact, respiration and eating but food and water are the primary sources of copper exposure. Daily dietary copper intake of 1-3 mg/day is common. Variation in water, food and vitamin supplement consumption can increase or decrease these dietary intakes of copper (WHO, 1998). Overexposure to copper can lead to negative effects in humans. At lower doses (a few mg/l) symptoms of excess copper exposure can include vomiting, diarrhea, abdominal cramps, gastrointestinal upset and headache. Higher levels of copper intake can result in gastrointestinal bleeding, haematuria, haemolysis, methemoglobinemia and renal failure. The lethal dose of copper lies somewhere between 4-400 mg/kg of body weight (WHO, 1998) but oral ingestion of high copper doses is uncommon due to its unpleasant taste at higher concentration (Olivares et al. 1996).

2.2 Copper in the environment

Copper is used for electrical wiring, pipes and fittings, paint coatings, algaecides, fungicides and food additives (WHO, 1998). Copper ranks third in the world consumption of metals, after iron and aluminum. In 2002, the United States had an estimated copper consumption from all sources of about 3.4 million tons. Of the total copper consumption, 2.4 million tons were mined and 1 million tons were from recycled sources (Jolly et al. 2003).

Humans can be exposed to copper through the atmosphere, fresh water as well as food sources. Copper in the atmosphere typically is derived from erosion of natural deposits and smokestack emissions (WHO, 1998). Atmospheric copper tends to be at trace concentrations and is usually not a major source of exposure. Reported mean copper concentrations in United States ground and surface waters were 0.075 and 0.066 mg/l respectively (USEPA, 1991). A United Kingdom river had mean copper concentrations (range 0.003-0.019 mg/l) while an Asian river had mean copper concentrations range from 0.0008 to 0.010 mg/l (WHO, 1998). Copper content in food is variable and depends on the amount of copper available in the soil as well as other factors. Red meat, seafood, nuts and a variety of fruits and vegetables contain variable amounts of copper (Lonnerdal 1996).

Unlike the relatively low naturally occurring levels, copper in drinking water can vary greatly and can be present at mg/l concentrations (NRC, 2000). Copper drinking water pipes have been in use since the 1920's (Anonymous, 1987). Copper as a plumbing material can have a long life as illustrated by original copper drinking water plumbing still in use at a house built in 1937 (Copper Development Association, 2004).

Health and aesthetic based drinking water standards have been set to prevent overexposure to copper in drinking water. The World Health Organization (WHO) standard in drinking water is set at 2 mg/l to prevent adverse affects of copper exposure (WHO, 1998). These WHO guidelines also state that a long term intake of copper between 1.5 - 3 mg/l has no adverse health effects and water >5 mg/l can impart an undesirable bitter taste. The Unites States Environmental Protection Agency (USEPA) developed a health based standard of 1.3 mg/l Cu in drinking water (USEPA, 1991). The USEPA also has an aesthetic based standard of 1 mg/l Cu, because copper in drinking water above this level can stain plumbing fixtures and laundry as well as contribute to metallic or bitter tasting water (USEPA, 1997).

The amount of copper in drinking water can be influenced by water pH, source water, mineral content and type of piping system (Olivares et al. 1996). Elevated copper levels in drinking water are primarily due to corrosion of copper piping within the home drinking water system. Soluble copper is typically the form of copper measured at the tap and elevated copper levels tend to be found in water that has been sitting stagnant for an extended period of time and not in free flowing water (Dietrich et al. 2004). The copper corrosion process can be affected by a multitude of physical, biological and chemical properties and is very complex due the differences in source water chemical composition. However, changes in the distribution system that alter water chemistry can suddenly change a once stable equilibrated system and trigger increased pipe corrosion (Edwards et al. 2004).

High levels of copper in drinking water have been observed throughout the world. USEPA Safe Drinking Water Information System (SDWIS) database tracks copper action level violations (>1.3 mg/l) from drinking water taps throughout the Unites States. In 2003, 471 water systems serving over 622,000 people violated the 1.3 mg/l drinking water action level (USEPA, 2003). United States drinking water copper concentrations have been measured from 0.005 to 18 mg/l (USEPA, 1991). In Holland, copper levels from water stagnant for 16 hours ranged from 0.2 to 3.2 mg/l, although typical flowing levels for this water was between 0.04 and 0.69 mg/l. In Canada, two separate sampling periods found maximum copper levels in drinking water of 0.27 and 0.56 mg/l (WHO, 1998). Petersen et al. (1998) found copper levels of 25-41 mg/l after excess acid was inadvertently released into a Connecticut distribution system causing accelerated corrosion. In Sweden the mean copper level found in standing water was 0.72 mg/l, while the drinking water had a mean concentration of 0.32 mg/l (Pettersson et al. 1999). In the early 1980's a Vermont family became ill after drinking water that contained copper between 2.8 -7.8 mg/l (Spitalny et al. 1984). During 1992 and 1993 the Wisconsin division of health conducted five case studies of increased copper levels in drinking water. Maximum copper levels from these five studies include 8.1, 7.8, 7.7, 5.3 and 1.2 mg/l (Knoebloch et al. 1994). In 1992 investigations found 90th percentile copper samples of 0.71 and 0.94 mg/l at two U.S. utilities. These same utilities then had 90th percentile copper samples of 0.38 and 0.37 mg/l in 1999. These reductions were likely from corrosion control measures put in place to decrease copper corrosion (Burlingame et al. 2004). Blue water is a phenomenon associated with corrosion of copper pipes that turns drinking water a blue color. This phenomenon is not well understood and can be caused by many different factors. The blue color imparted to the water is from various copper precipitates that form in the pipe and are flushed out of the tap. Blue water can contain 500-1000 mg/l copper, however 3-10 mg/l is more common (Edwards et al. 2000). Obviously copper corrosion in drinking water systems occurs at high enough levels to affect human health and also have aesthetic impacts.

Source water is typically not a major source of copper. However, copper sulfate can be used as an algaecide in drinking water reservoirs to combat algal problems that can cause taste and odor events. Most of the copper sulfate algaecide forms a precipitate and sinks into the sediment but some copper may stay in suspension and be measured at the water outlet. One study of a Colorado reservoir found that 20% of the copper used as an algaecide was exported out of the reservoir over a 70 day period after initial treatment (Haughey et al. 2003). Copper from algaecide sources has the potential to increase copper content of drinking water sources. Continued use of copper containing algaecides may cause chronic sediment and water quality problems. Algal control alternatives that do not contain copper are currently being investigated (Grizzard, 2003 and Haughey et al. 2000).

2.3 Lead and Copper Rule

Drinking water sampling for copper is required under the EPA Lead and Copper Rule (LCR). The LCR was first implemented in June 1991. The LCR has a 90th percentile action level (if more than 10 % of utilities samples are above the action level then there is a violation) of 1.3 mg/l total copper. Consequently a utility may have samples that exceed 1.3 mg Cu/l and do not violate the LCR. This occurs when those samples above 1.3 mg Cu/l do not comprise at least 10% of the samples. When LCR action levels are exceeded additional treatment techniques and sampling are required. The treatment techniques may include water quality parameter monitoring, source water treatment, corrosion control treatment and other procedures (USEPA, 1991).

The LCR requires that sampling be done at home cold water taps throughout the distribution system. The samples must be drawn after water has been allowed to have contact time with home plumbing. Typically, these samples consist of first flush water taken in the morning after the water has been stagnant in the pipes all night. The sampling site requirements of the LCR follow a three tier process to prioritize sampling locations (USEPA, 2002). If a utility can not find enough Tier 1 sampling sites then Tier 2 sites are used. If a utility can not find enough Tier 2 sampling sites then Tier 3 sites are used.

Tier 1) Homes that contain copper pipe with lead solder installed after 1982 or contain lead pipes

Tier 2) Homes that are served by a lead service line

Tier 3) Homes that contain copper pipe with lead solder installed before 1983

The number of samples a utility must take depends on the number of people they serve. The maximum number of samples that must be taken is 100 (for utilities that service over 100,000 people) and decreases to 5 (for utilities that service <100). After utilities have been in compliance with the LCR for several monitoring periods the monitoring requirements are then reduced and sampling is only required every 2 or 3 years based on service population and compliance history (USEPA, 1991).

Lead solder was banned for use on copper drinking water pipes in 1986 (USEPA, 2002). Therefore, homes that qualify for tier 1 sample sites were likely built between 1982 and 1986 and are approximately 20 years old. These nearly 20 year old homes have had an opportunity to develop a scale on the pipe wall that can limit copper by product release from the pipe surface through solids aging and insoluble scale formation (Edwards et al. 2001). Newer homes are not a sampling priority based on the LCR sampling requirements (USEPA, 2002) even though these newer homes are typically good candidates for copper by-product release to the tap. Research demonstrates that soluble copper hydroxide and copper carbonate solids tend to form on the inner pipe surface of copper pipes as pipes age (Edwards et al. 2001). The soluble scale layers in equilibrium with the water surface have a higher likelihood, when being compared to insoluble copper scales, of releasing soluble copper into solution and eventually to drinking water (Powers et al. 2000).

Copper corrosion and increased copper by product release may be a greater problem than many realize due to the fact that newer homes are not typically sampled. Utilities may gain additional insight pertaining to their water corrosivity if newer homes were given more priority. Early discovery of copper pipe corrosion may prevent long term infrastructure failure and save homeowners and water utilities pipe replacement costs.

2.4 Copper Chemistry

Copper is a transitional metal and has an atomic weight of 63.55. Copper has two oxidation states, cuprous (Cu^{+}) and cupric (Cu^{++}). In natural water the cupric ion is the most common oxidation state (Beguin-Bruhin, 1983). Various ligands found in natural waters can bind to cupric (Cu^{++}) to form soluble complexes or particulates including SO_4^{2-},OH^{-}, PO_4^{3-}, HCO_3^{-}, NO_3^{-}, CO_3^{2-}, S^{2-}, O^{2-} as well as multiple combinations of these and other ligands (Dietrich et al. 2005). Cuprous (Cu^{+}) complexes such as CuCl, while not as common, are found in aqueous solutions (Edwards et. al 1994 and WHO, 1998).

Metals and ligands can have interactions in water and form a variety of soluble complexes, insoluble precipitates or remain uncomplexed under a specific set of conditions. Free uncomplexed cupric copper (Cu^{++}) concentrations, assuming no solid formation and considering only hydroxo complexes (OH^-) can be modeled. (Figure 2.1) represents the copper species formed at various pH's. Addition of other ligands and increasing the copper concentration can change the species present in an aqueous system (Jensen, 2003).

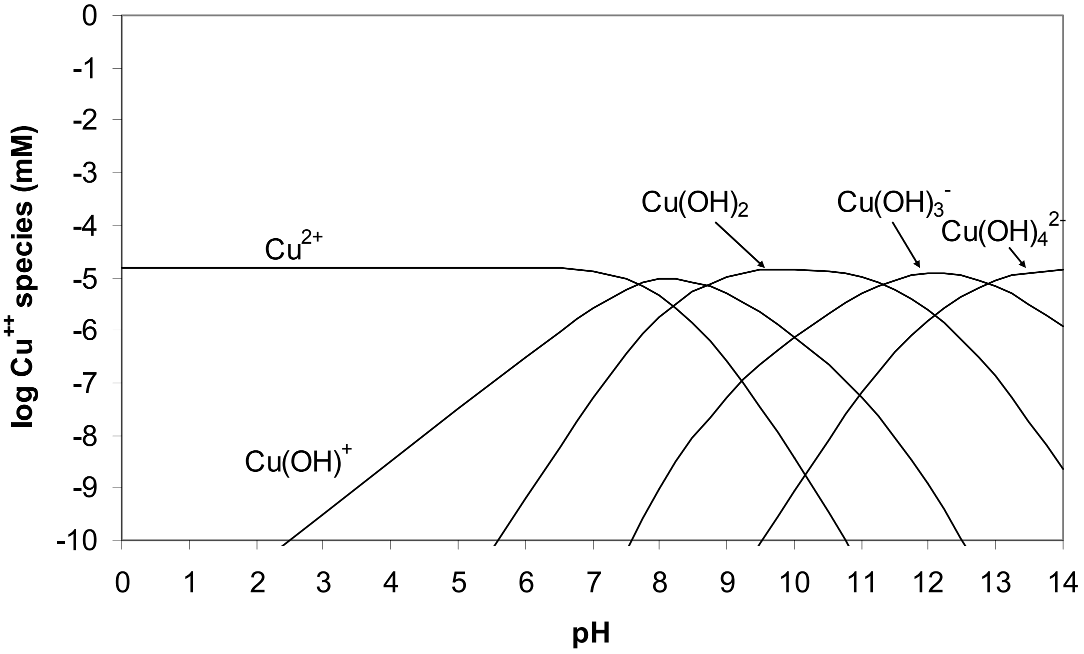

Figure 2.1 Theoretical copper speciation for C_t = 1 mg/l, USEPA secondary standard

Precipitation reactions may be fast or slow and cannot be assumed to be at equilibrium. Solubility constants, which predict at what concentration a metal will precipitate under standard thermodynamic conditions, have been evaluated for common metal complexes.

How copper solubility will change in pure water, assuming the water is free of CO_2 and therefore considering only hydroxo complexes, can be modeled. Previous research has shown that $CuOH_{2\,(s)}$ is the typical solid that controls solubility in newer copper pipes (Edwards et al. 1994). In (Figure 2.2) the $CuOH_{2\,(s)}$ line divides the solubility diagram into two regions. Conditions on or inside the line indicate a solid will form, while conditions outside the line indicate formation of soluble complexes. The copper solubility pattern is similar to many other metals. They are soluble at low pH's where species with *fewer* OH^- than in the solid dominate and soluble at high pH's where species with *more* OH^- than in the solid dominate. Precipitates tend to favor formation at an intermediate pH levels (Jensen, 2003).

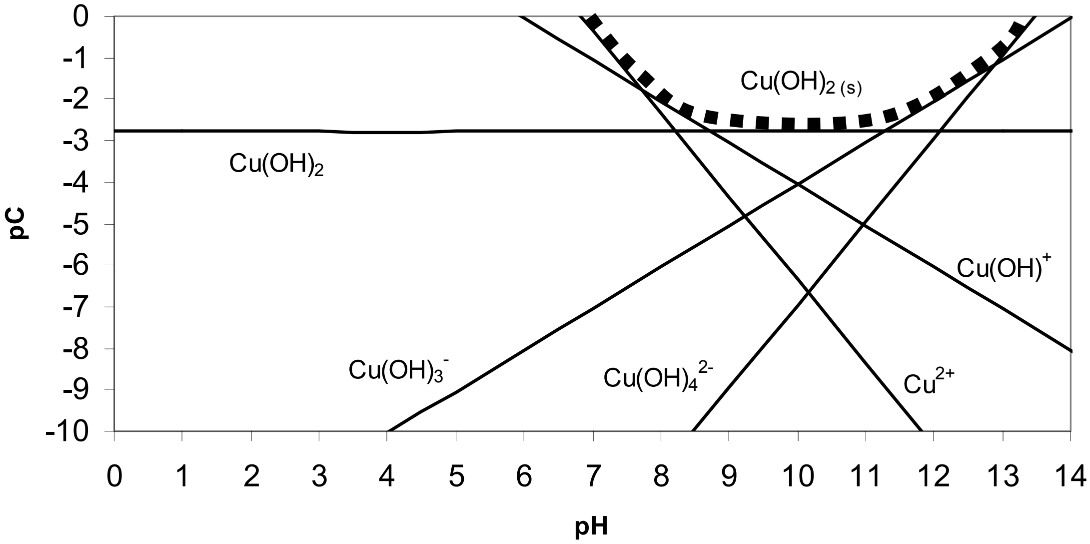

Figure 2.2 Cupric solubility diagram

Introduction of other possible ligands can have various effects on copper solubility including formation of multiple solids at various pH levels. In general adding additional ligands to a system, assuming a constant pH and the same solid phase controls, will increase metal solubility. It is possible to have multiple solid phases existing together in equilibrium. However, the least soluble solid phase for a given set of conditions controls solubility (Jensen, 2003). These rules of aquatic chemistry are well accepted and understood. They serve as general guideline to how solubility and precipitation should occur. However, due to the large variability and complexity of drinking water contents and distribution system variations copper solubility is not always so predictable.

2.5 Scale aging

Copper metal (Cu^0) is thermodynamically unstable in aerobic medium and will spontaneously react under aerobic conditions to form the cupric (Cu^{++}) ion according to the following redox reaction (Edwards et al. 2000).

$$\mathbf{\tfrac{1}{2}O_2 + Cu^0 + H_2O \rightarrow Cu^{2+} + 2OH^-}$$

Other oxidants, such as chlorine can also contribute to oxidation in copper pipe (Edwards et al.1994).

Copper pipe (Cu^0) must first be oxidized to the cuprous (Cu^+) or cupric (Cu^{++}) ion to allow the formation of free, soluble complexes or particulate copper. These forms of copper can either stick to the inside of the pipe wall and develop a scale or be released into the water and flow to the tap (Edwards et al. 2000).

The identity of the scale that is on the inner pipe wall and in contact with the water is a key factor controlling the maximum soluble copper concentration in the water. It has been established that cupric hydroxide, $Cu(OH)_2$, is the scale that controls solubility in new pipes. However, at least 20 other different copper compounds can form on the pipe as it ages under various circumstances, and each has its own color, rate of dissolution and equilibrium soluble copper concentration (Edwards and Schock et al. 1996). Over the pH and temperature range common in drinking water, equilibration with $Cu(OH)_2$ can lead to maximum soluble copper concentrations of 2-10 mg Cu/l (Dietrich et al. 2005). If more soluble solids on the pipe wall are replaced with less soluble solids, the concentration of soluble copper in drinking water will decrease (Powers et al. 2000).

High levels of equilibrated soluble copper can persist for years in home plumbing systems as opposed to a few days for $Cu(OH)_2$ solids in laboratory experiments. This is likely due to the complexity of components in natural waters that interfere with transitions to insoluble copper solids (Dietrich et al. 2005). Research has looked at how different copper solids age and transitions over time with addition on different anions. Powers et al. (2000) studied the effect that sulfate, bicarbonate and orthophosphate had on transitions from soluble copper to insoluble copper solids. Addition of various anions to cupric nitrate resulted in different solids formation (Table 2.1).

Table 2.1 Copper solids formed from various anion addition to $CuNO_3$ (adapted from Powers et. al 2001)

Anion added	Solid formed	Ksp @ 720 hours	Reported* Ksp
HCO_3^-	Malachite - $Cu_2CO_3(OH)_2$	6.24	5.48
SO_4^{2-}	Brochantite - $Cu_4(SO_4)(OH)_6$	-17.32	-15.38
PO_4^{3-}	$Cu_3(PO_4)_2$	30.23	36.86

*Reviewed by Schock et al. (1995)

The water containing sulfate solids was stabilized within a few minutes whereas the bicarbonate and phosphate addition took up to 4 days to equilibrate. Further testing with these solutions showed that the production of solids with lower solubility was the likely explanation for decreasing soluble copper with time (Dietrich et al. 2005). Powers et al. (2000) also looked at the effect of adding anions to already formed $Cu(OH)_2$ solid. In (Figure 2.3) sulfate addition rapidly (within 1 hour) formed insoluble copper solids, bicarbonate did not equilibrate into insoluble precipitates until 1 month of aging and phosphate initially formed moderately insoluble solids but then hindered formation of highly insoluble solids (Powers et al. 2000).

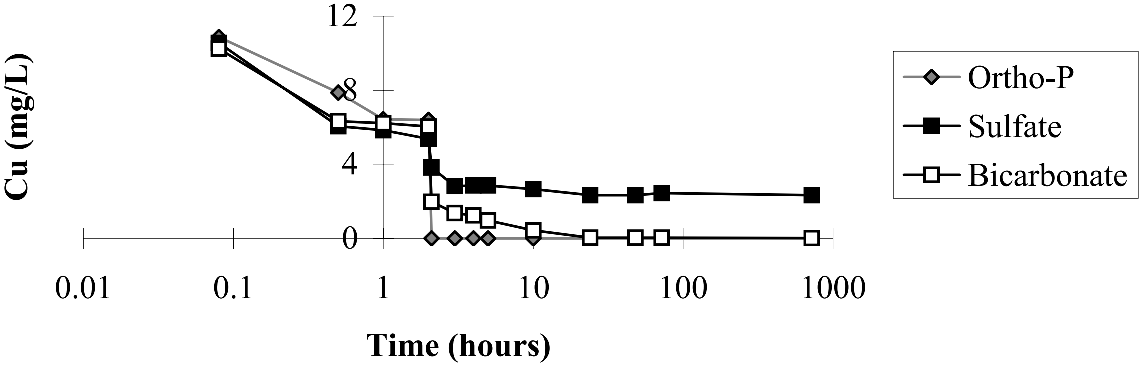

Figure 2.3 Soluble copper concentration for copper hydroxide solid in the presence of orthophosphate, sulfate or bicarbonate. For the time period 0-2, copper hydroxide solid formed in the presence of the corresponding anion. At time = 2 hours, 1.0 mM more of the tested anion was added to a solution containing preformed Cu(OH)2 (adapted from Powers et al. 2005)

Figure 2.4 presents a model of copper solid solubility. In drinking water systems, anions which accelerate the transition from $Cu(OH)_2$ to tenorite (CuO) would be beneficial whereas anions that delay such transitions would be unfavorable.

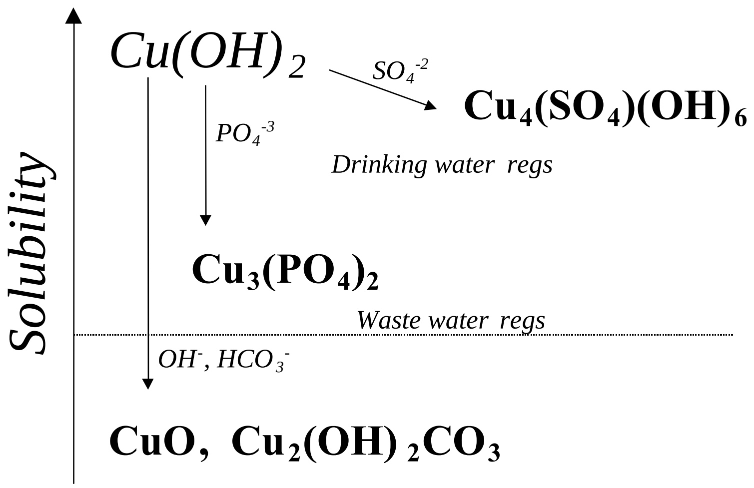

Figure 2.4 Model of equilibrated soluble copper in the presence of various anions (from Edwards et al. 2000)

Edwards et al. (2002) studied copper by-product release and aging from copper pipes of varying water quality. In copper pipes with 72 hour stagnation times, total copper by-product release decreased from 5.5 -0.3 mg/l in pH 7.2 water after 120 days of aging. This same study also found total copper decreases of more than 1 mg/l with 8 hour stagnation times. These decreases are classic examples of soluble complexes transitioning to more insoluble complexes over time.

As a general rule soluble copper concentrations depend on the type of scale controlling solubility. The results from these studies show that copper solubility can change drastically over time and soluble-particulate fractioning must be monitored when trying to control soluble and particulate copper concentrations.

The chemistry of copper pipe interactions with water is very complicated and can be different for each utility due to the large variation in source waters, distribution system composition and treatment techniques. Copper corrosion can be grossly broken into two categories, uniform corrosion and pitting corrosion (Edwards et al. 1994). Uniform corrosion attacks the entire pipe surface at an equal rate and can be visually characterized by loose powdery scales covering the entire inner pipe wall. These scales can be a variety of colors based on water composition. Pitting corrosion attacks the pipe in small localized areas and is a major contributor to pinhole leaks and pipe failure (Edwards et al. 1994). Pinhole leaks are relatively rare but are still the most common cause of residential copper pipe failure (Edwards and Rushing, 2004). These two distinct corrosion types, while sharing the same primary reactions, do not necessarily share the same corrosion mechanisms (Edwards et al. 1994). This research, while not focused on specific corrosion mechanisms, is concerned with both corrosion types due to their ability to release copper by-products into consumer's drinking water pipes. The rate of copper by-product release depends on the type of scale, corrosion type, pipe age and other variables.

Many studies have been conducted to assess the effect of different water parameters on corrosion trends. A wide range of physical, biological and chemical parameters can have an effect on copper byproduct release. Temperature, pH, ion contents, dissolved oxygen, microbial activity, disinfectant, flow rate, organic matter, pipe age, stagnation time and others have been shown to play a role in copper corrosion by-product release (Dietrich et al. 2004). The following is a brief summary of some studied interactions that can affect copper by-product releases.

Recently chlorine and aluminum solids together have been shown to have synergistic effects on increasing copper pitting corrosion (Edwards et al. 2004). Rushing et al. (2004) found flow rate effects copper corrosion in water with chlorine by increasing the corrosion potential of the water with the start of the flow. Free chlorine generally increases corrosion rates but has been demonstrated to decrease aspects of copper corrosion (Reber, 1989 and Edwards et al. 1993). Zhang et al. (2002) found that monochloramine can increase copper leaching to water. Natural organic matter (NOM) content of water can have variable effects on copper by-product release. NOM in water can both increase and decrease copper corrosion under varying conditions (Edwards et at. 2001 and Edwards et al. 1994). Edwards and Hidmi (2002) studied how various forms of phosphate (polyphosphate and orthophosphate) can affect copper corrosion. Both copper corrosion increases and decreases were shown with phosphate addition depending on time scale, concentration added and other water quality parameters. Water pH certainly can affect copper corrosion and lower pH has been shown to increase copper by-product release (Edwards and Boulay 2001). Temperature has also been shown to affect copper release, with higher water temperatures releasing a higher percentage of particulate copper than lower water temperatures (Edwards and Boulay 2001). Edwards and Rushing (2004) studied the effects of thermogalvanic currents on copper corrosion and found the currents may play a role in copper by-product release. Edwards and Rushing (2004) also looked at temperature gradients and showed they can effect copper corrosion from temperature related solubility changes. Copper by-product release has been shown to increase in water with lower pH’s with no disinfectant, higher pH’s with Cl_2, higher temperatures and the presence of organic matter (Edwards et al. 2000). Temperature, pH, alkalinity softness and chlorine have been shown to effect copper corrosion (Edwards and Boulay 2001). Biofilms have shown varied effects on copper pipes. *Acidovorax delafieldii, Cytophaga johnsonae* and *Micrococcus kristinae* can increase copper release in pipes while *Xanthomonas maltophilia* and *Rhodococcus sp.*can decrease copper concentrations (Critchley et al. 2002).

Due to the complexity of the chemistry and different conditions present for each of the different studies, only basic trends of increasing or decreasing copper by-product levels were cited and specific conditions were not delineated for the various studies. However the two applicable concerns of this research are that corrosion does occur which is obvious from the many citations on increased levels in drinking water and that certain water quality parameters can increase or decrease copper by-products in drinking water pipes.

2.6 Taste

The sense of taste is complicated and involves many factors. The tasting sequence starts with taste buds coming in contact with a stimulant, the taste buds then process the information and send neurotransmitter molecules to nerves that are in contact with the taste buds, this information is then directed to the corresponding processing centers of the brain where a tasting sensation is produced (Lawless and Heymann, 1999).

Taste buds are comprised of 30-50 skin like cells grouped together in a layered ball. These taste buds are contained in various bumps and grooves on the tongue surface. Three primary structures, fungiform papillae, foliate papillae and circumvallate papillae, comprise the majority of taste buds on the tongue (Lawless and Heymann, 1999). The fungiform papillae are reddish mushroom shaped bumps that have two to four taste buds each and are located toward the front of the tongue. Normal adults typically have over one hundred fungiform papillae on each side of the tongue, therefore allocating several hundred taste buds (Miller and Bartoshuk, 1991). Several parallel grooves toward the back and on the each side of the tongue house the foliate papillae. Several hundred taste buds can be located in each groove (Lawless and Heymann, 1999). The circumvallate papillae, large button shaped bumps, are located on the back of the tongue. Several hundred taste buds are also located here and around the bumps (Lawless and Heymann, 1999). Taste buds can also be located on the soft palate as well as the root of the tongue and upper region of the throat. Those individuals with more taste buds tend to be more sensitive to various tastes (Bartoshuk et al. 1994). Four different nerves take messages from the taste buds and send them to the brain. The four nerves include the chorda tympani, glossopharyngeal nerve, vagus nerve and the petrosal nerve (Lawless and Heymann, 1999).

The classic diagram that shows the four basic tastes (salty, sweet, bitter and sour) being perceived in different distinct areas of the tongue is not accurate. All of the four tastes can be perceived on any area of the tongue (Lawless and Heymann, 1999). Metallic taste, as copper is commonly perceived, is not one of the four commonly accepted tastes but has occasionally proposed as a legitimate taste category (Lawless et al. 2004). Metallic sensations are not part of everyday tastes although they can be derived from metal foils and lipid oxidation (Lawless et al. 2005). Metallic taste has been used as a descriptor in phantom taste disorders which seem to come from nowhere (Lawless et al. 1983) and burning mouth syndrome (Miller and Bartoshuk, 1991). Multiple metallic taste origins have been investigated including olfactory and gustatory sensations (Lawless et al. 2004, 2005). Copper salts can produce multiple effects in the mouth including metallic, bitter and astringency (Lawless et al. 2004 and Beguin-Bruhin et al. 1983).

Nose clamps have previously been used in studies to show that olfactory and or retronasal mechanisms have no significant effect on the tasting sensation of copper. Zacarias et al. (2001) showed that there was no significant difference between tasting copper with the nose open or closed. Lawless et al. (2004) showed that nasal occlusion did not significantly reduce panelist's metallic, bitter and astringent ratings of copper spiked water. Lawless et al. (2005) hypothesizes that copper sensations are gustatory in nature and mediated by oral chemoreceptors. Multiple theories for the how copper is tasted have been proposed, however the true mechanisms and conceptual boundaries of what constitutes a metallic sensation is currently unclear (Lawless et al. 2004).

2.7 Sensory Variation

When performing sensory analysis with a human panel variation is implicit. Panelist's sensory abilities can change from hour to hour or day to day for many reasons. Eating certain foods, improper sleep habits, emotional stress, age, genetics, general health, cultural background, gender and others can all play a role in variability (Meilgaard et al. 1999 and Lawless and Heymann 1999). A variety of physiological factors can also influence sensory verdicts. Adaptation, suppression and time to intensity are physiological factors that have been shown to influence results when testing copper (Zacarias et al. 2001). Adaptation is a decreased sensation from constant or continued stimulation. This is an unwanted source of variability in thresholds and intensity ratings. The taste of copper tends to linger in the mouth and decrease sensitivity when testing multiple samples. Compounds sensed as strongly bitter may bind to receptor proteins and can remain for hours or days (Meilgaard et al. 1999). Suppression occurs when the presence of one substance decreases the perceived intensity of a mixture. Chlorine has been shown to suppress earthy-musty and other odors in drinking water (Worley et al. 2003). Chlorine could also have an effect on drinking water taste due to its retronasal interactions in the mouth. Time to intensity of certain products can vary over time. Copper has shown that it can take several seconds to a minute for the full sensation to be realized by panelists.

Psychological factors can also influence sensory verdicts. The following is a list of some psychological errors common to sensory testing (adapted from Worley et al. 2003):

- Expectation error or pattern effect: occurs when a panelist is given too much information about a test, possibly triggering some preconceived notion concerning the sample. For instance, classic threshold tests using ascending concentrations may cause a panelist to expect or anticipate his or her next response before it is actually perceived.

- Logical or stimulus error: a false response invoked by an association with a factor other than the one being evaluated. For instance, a weakening of sample color might cause a panelist to rate its sweetness lower than a sample of more intense color.

- Mutual suggestion: occurs when a panelist is influenced by other members of the panel.

- Presentational errors: caused by the order or manner in which samples are presented to the panelist. Incrementing sample codes (e.g. 1, 2, 3) can cause a panelist to inadvertently rate samples in a biased order

- Error of central tendency: the subconscious avoiding of extreme ends of evaluation scales. This often occurs when one uses intensity scales because panelists do not tend to use the extreme low or extreme high values of a scale. The error produces a falsely biased central response.

2.8 Sensory Methods

A threshold can be grossly defined as the minimum value of a stimulus that can be detected. Any value below the threshold cannot be detected, any value above can. Thresholds are used in multiple applications to determine the point at which known contaminants start to reduce product acceptability (Meilgaard et al. 1999). A good threshold determination requires hundreds of samples and results do not reproduce well. Published thresholds have been shown to vary by a factor of 100 or more (Meilgaard et al. 1999). Various data analysis methods are used to deal with the high variability in threshold testing.

Ascending concentration forced choice (AFC) is a method used to determine human taste or odor thresholds (Lawless and Heymann 1999). This method is used when the test objective is to determine if a sensory difference exists. Typical examples of 3-AFC method protocols are ASTM E-679-91 (rapid method) and E-1432-91 (intermediate method). Both methods have similar testing protocols, but the rapid method is typically used when there are limits on the test medium- such as lingering. Both ASTM protocols use three sample presentations, two controls and one spiked sample. The panelists must sample all three cups and try to differentiate the different sample. The AFC forces panelists to choose a sample even if they are unsure of the different one. Panelists are presented with a series of ascending concentrations. The range of concentrations given to panelists is to be predetermined by bench top testing. The intermediate method suggests a multiplier of 1.5 or 2 between concentration steps. The threshold is typically confirmed when panelists correctly distinguish the different sample several times in a row.

Various data analysis methods can be used to estimate thresholds. A group of 25 panelists or more are desired when estimating population thresholds for taste (Lawless and Heymann 1999). Geometric mean and logistic regression are two common methods used to quantify taste thresholds. The geometric mean method tries to reduce the significance of outliers in the reported threshold value. Individual thresholds are calculated by taking the geometric of the lowest concentration panelists can detect and the highest concentration they cannot. The lowest concentration they can detect is classified as the first of at least 3 correct choices in a row. The overall (group) threshold concentration for each test water is calculated as the geometric mean of the individual panelists threshold values.

Logistic regression is another threshold determination analysis method. Logistic regression predicts where a certain percentage of panelists will correctly identify the copper spiked sample. This analysis method factors in the possibility that panelists can guess correctly even if they were unable to correctly distinguish the correct sample. Previous work has shown that logistic regression can be a valuable threshold determination method and compares well with geometric mean calculations (Van Aardt et al. 2001).

The Hubaux and Vos method is commonly used in environmental analysis and can be adapted to address sensory thresholds (Hubaux and Vos, 1970). A calibration curve is calculated from observed measurements and confidence intervals are then applied to the calibration curve. This method does not have the statistical limitations as do the geometric mean and logistic regression techniques.

Similarity testing is conducted when the test objective is to determine if two samples are sufficiently similar to be used interchangeably. Generally at least 50 responses are needed when testing similarity. The test setup for similarity testing is identical to the triangle tests, 2 control and 1 spike, except you are trying to show that the two samples are similar and not different (Meilgaard et al. 1999). Statistical parameters are pre-selected to determine sample size and the number of responses needed to show similarity.

The difference from control (DFC) test is used when the test objective is to determine if a difference exists and to estimate the size of that difference. A reference sample is given to the panelist, a second sample is then given to the panelist and the second sample is rated with respect to how different it is from the reference. One of the second samples will be identical to the reference and is used to measure the placebo effect. The placebo estimate represents the effect by simply asking how different the samples are when no actual difference exists. The panelists are aware that this situation will occur for one of the sample pairs. A category scale was used for the DFC test (Figure 2.5). At least 20 sample presentations are suggested when determining a difference. A paired t-test is a sufficient analysis method when only sample is compared with the control. (Meilgaard et al. 1999)

☐	☐	☐	☐	☐	☐	☐	☐	☐
Extremely Weaker	Much Weaker	Moderately Weaker	Slightly Weaker	**Same as Reference**	Slightly Stronger	Moderately Stronger	Much Stronger	Extremely Stronger

Figure 2.5 Difference from control category scale

2.9 Previous Studies

Previous research has evaluated taste threshold levels for copper in water of various water qualities. Copper taste threshold results for previous studies have varied (Table 2.2).

Table 2.2 Human taste thresholds for copper in various water types

	Threshold mg Cu/l		
	Distilled	**Tap**	**Mineral**
Cohen et al. (1960)	6.6	NA	13
Zacarias et al. (2001)	2.5	2.6	3.5
Beguin-Bruhin et al. (1983)	2.4-3.2	NA	0.8-1

Various techniques and sensory protocols can have a dramatic effect on quantitative results. Concentration interval, statistical analysis, sensory test modifications, copper chemistry, population tested, test objectives and other methods varied for each study. These variations in addition to human deviation may be the reason the studies have inconsistent results.

Cohen et al. (1960) used the 3-AFC triangle test and administered 3 sets per sitting. This technique may have not addressed the adaptation common when testing copper. The panel size was relatively small (15-20) and some of the panel were noted as being smokers. These methods would likely increase threshold results. Not surprisingly, the

Cohen study had the highest threshold results out of the three previous studies. Solubility problems were addressed by adjusting pH to 6.0. This permitted soluble copper over the entire testing range even with the mineral water. Threshold results represent the concentration at which 50% of the panelists can taste the copper and 95% confidence intervals were used in the statistical analysis.

Beguin-Bruhin et al. (1983) used 5-AFC instead of 3-AFC, to decrease the likelihood of guessing correctly. Only one concentration was given per session to address adaptation. Benchtop testing was conducted to set appropriate concentration ranges. The copper range was 0.1 mg/l -20 mg/l Cu, but interval steps were not provided. To deal with lingering one minute wait periods were mandated between sample testing. A weak sucrose solution was used for the control water, instead of distilled water, due to the unpleasant taste of distilled water. Solubility issues were addressed by making solutions fresh before each test. 95% confidence intervals and guessing correction were applied to threshold results for statistical analysis. This study concluded that only soluble copper is of sensorial importance. However only two waters were tested and the pH in both water waters was low (5.9 and 6.4). These low pH conditions would not be likely to cause particulate formation until high copper levels (several mg/l). Therefore the effect of particulate copper may not have been thoroughly tested.

Zacarias et al. (2001) also used 5-AFC instead of 3-AFC, to decrease the likelihood of guessing correctly. Only one concentration was given per session to address adaptation. Benchtop testing was conducted to set appropriate concentration ranges. The copper range was 1 mg/l -8 mg/l Cu, with 1mg/l concentration steps. One minute wait periods were mandated between sample testing to deal with lingering. Copper chloride and sulfate salts were used to test differences between different salts. No significant difference was found between the two salts. Nose clamping was also used to determine its effect on copper tasting. No significant effect was shown by clamping the nose. Threshold results represent the concentration at which 50% of the panelists can taste the copper. However, neither confidence intervals nor guessing correction techniques were used. The objective of this test was to see if humans can protect themselves from the negative health effects caused by increased copper intake. Therefore, lower concentrations of copper were not tested. If lower concentrations were available for testing the threshold values reported may have been lower. This study was conducted in Chile where cultural differences may have an effect and background levels of copper in drinking water may be higher, both of these factors would likely increase threshold levels. The effect of pH on copper solubility was not addressed in this study even though the tap water had a specified pH of 7.4. Therefore the results from this study may include soluble and particulate copper.

The importance of using a well defined aqueous system where copper is present in only the dissolved form has been addressed (Cohen et al. 1960). Solubility control of copper can be achieved through pH adjustment. The results from Cohen et al. (1960) and Beguin-Bruhin et al. (1983) seem to represent completely dissolved copper, while the results from Zacarias et al. (2001) appears to include some particulate copper based on sample water pH and ion contents. The soluble-particulate interaction and the primary role that soluble copper plays in the tasting sensation has been explored (Beguin-Bruhin et al. 1983 and Cohen et al. 1960). Beguin-Bruhin et al. (1983) concluded that dissolved copper is the primary taste component.

Concerns have risen about consumer's ability to protect themselves from sickness by ingestion of water with increased levels of copper. Zacarias et al. (2001) reports that copper in drinking water >4mg/l can cause gastrointestinal (GI) upset. Zacarias also concludes that ~ 40% of consumers would likely be unable to detect copper in their tap water at concentrations that would be likely to cause GI. Other work found that oral ingestion of high copper doses (>4mg/l) would be uncommon due to the unpleasant taste at higher concentration (Olivares et al. 1996).

2.10 PROP tasting

It has been documented for over 70 years that individuals vary dramatically in their sensitivity to bitter compounds containing thiourea, in particular phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) (Prescott et al. 2004). This taste difference was accidentally discovered in a laboratory by A.L. Fox in 1931 while he was synthesizing a PTC compound (Zhao et al. 2003). This discovery later showed that human sensitivity to PTC was variable and that panelist's perceptions of the compound represented a trimodal or bimodal distribution. Trimodal distribution can be broken in to three taster groups: supertasters, tasters and nontasters. Individuals in each taster group perceive the intensity of PTC/PROP differently.

- Supertaster: extremely bitter
- Taster: bitter
- Nontaster: no taste

Bimodal distribution is simply broken into tasters and nontasters.

- Taster: bitter
- Nontaster: no taste

In the 1960's a trend was initiated from using PTC to PROP. This came as a result of the sulfurous odor that was characteristic of PTC (Prescott et al. 2004).

A current area of interest is how does sensitivity to PROP correlate with sensitivity to other compounds and sensations-such as copper or metallic taste? Attempts have been made to correlate PROP taster status to a variety of compounds that represent the four basic tastes (Prescott et al. 2004). Can human sensitivity to copper be predicted simply by taster status? Finding answers to these types of questions could significantly reduce extensive sensory testing of products and sensitivity could be predicted solely by taster status (Prescott et al. 2004).

A large body of research has focused around PROP tasting and many studies have been completed. A central problem exists of trying to compare results from multiple studies that used different analysis and classification techniques for grouping panelists into taster groups. Currently attempts are being made to try and find a unified method so that in the future results from different studies can be compared (Prescott et al. 2004). Several methods of testing have evolved to try and discriminate taster groups including one, three and five solution tests. The one solution test, one PROP and one control solution, has been used before and has shown to be a representative method to classify PROP taster groups (Prescott et al. 2001). PROP classification involves panelists tasting PROP and ranking its' intensity on a labeled magnitude scale (LMS) (Green et al. 1996). The LMS (Figure 2.6) has been used as an effective scale to record PROP intensity ratings (Prescott et al. 2001, Tepper et al. 2001, Delwiche et al. 2001 and Horne et al. 2002). This PROP intensity ranking can then be compared to a control stimulant. NaCl has been used successfully as a control stimulant (Prescott et al. 2004 and Tepper et al. 2001, 1997 and Yackinous et al. 2001).

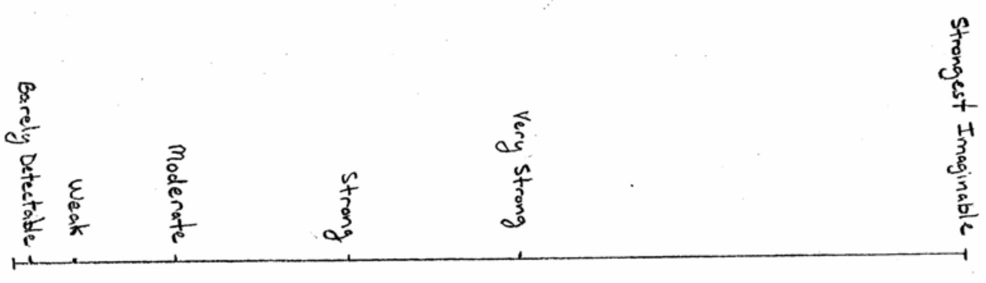

Figure 2.6 Labeled Magnitude Scale used for intensity rating

Typical results of PROP testing represent a bimodal or trimodal (Figure 2.7) distribution of the PROP covering the entire length of the LMS while the control stimulant tends to be normally distributed toward the bottom of the LMS (Figure 2.8).

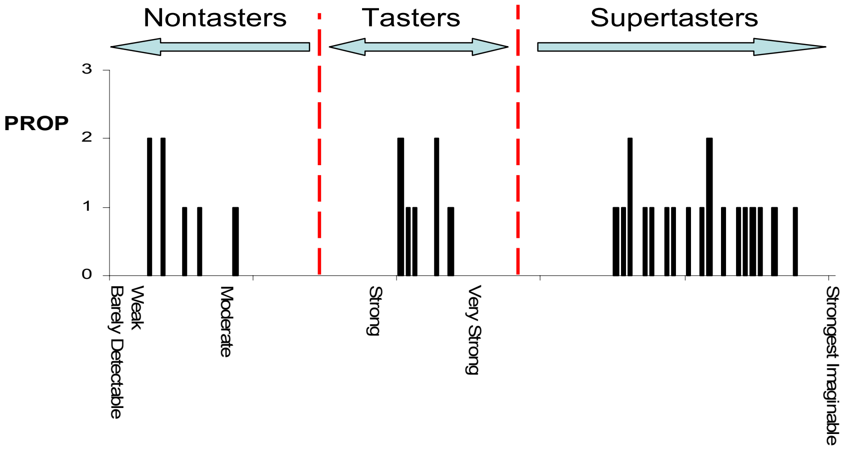

Figure 2.7 Trimodal distribution for PROP tasting on LMS scale

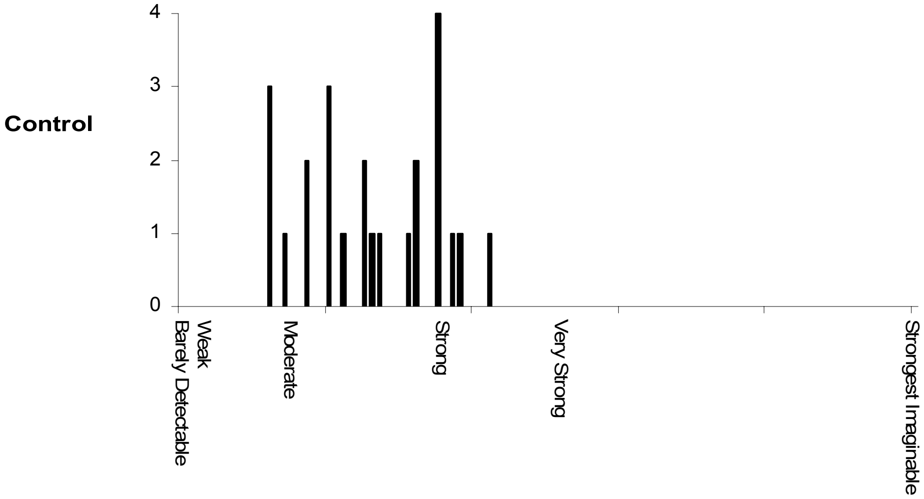

Figure 2.8 Normal distribution for control tasting on LMS scale

The panelist's rankings of the PROP and NaCl solutions are analyzed and broken into separate categories based on their perception of the PROP compound. The following taster grouping method is called visual classification and has been shown to have 82% test retest reliability with one solution tests (Prescott et al. 2004).

- *Nontaster*: PROP ratings much lower than NaCl ratings
- *Taster*: PROP ratings similar to NaCl ratings
- *Supertaster*: PROP ratings much higher than NaCl ratings

The visual classification technique has been used before to successfully segregate taster groups (Tepper et al. 2001, 1997 and Yackinous et al. 2001). Other studies have also broken the data into a bimodal distribution which usually results in higher test-retest results (Prescott et al. 2004).

PROP taste status has been shown to correlate with having a higher number of taste pores and fungiform papilla (Miller et al. 1990). Therefore nontasters have fewer fungiform papillae than tasters and tasters have fewer than supertasters. Collings et al. (1974) found thresholds for bitter, which is how PROP is typically perceived, are lower on the front of the tongue. A hereditary genetic link has also been established for PROP tasting. Some research suggests the classic Mendelian two allele trait concept, with recessive insensitive ***t*** and dominant sensitive ***T***, mode of inheritance (Bartoshuk et al. 2001 and Merton 1958). Other investigations have led researchers to advocate multiple alleles, multiple loci and incomplete dominance to help explain results (Ramana et al. 1992 and Olsen et al. 1989 and Reed et al. 1995).

The ability to taste PROP is a characteristic shared by approximately 70% of the US adult Caucasian population. Supertasters and nontasters comprise this 70% while the remaining 30% of the population are called nontasters (Tepper et al. 2001). PROP status has also been shown to vary with gender type. Females tend to be more sensitive to PROP and therefore are more likely to be tasters/supertasters than males (Bartoshuk et al. 1994). Ethnic differences have also been shown to have an effect on PROP group classification. Previous work on different ethnic groups found that PTC nontasters comprised 30% of Caucasians, 6% of Chinese and 10% of American Indians (Parr, 1934 and Kalmus et al. 1971). Research has also attempted to show associations between PROP taste status and traits not related to taste including: diabetes, eye disease, dental disease, obesity, thyroid disorders, personality disorders and immune system strength.

2.11 Chlorine and chloramines

Chlorine has been used as a water disinfectant since the late 1800's and is currently a popular method of disinfection for water utilities (Connell 1996). Chlorinous taste and odors in tap water are the number one source of customer dissatisfaction (Piriou et al. 2004). Water utilities can regulate disinfectant levels in the water to decrease customer taste and odor complaints but a residual level of disinfectant is required to deliver microbiologically safe tap water. Free chlorine residual concentrations can vary based on proximity to the water utility but levels in the U.S. ranging from 1.0-3.0 mg/l as Cl_2 would not be uncommon (Piriou et al. 2004). Chlorine has been shown to mask earthy-musty taste and odors in drinking waters (Worley et al. 2003). American thresholds for chlorine are below typical free residuals (1.0-3.0 mg/l). If humans can detect the presence of Cl_2 in their water by tasting, it is possible that chlorine could have the ability to interfere with the tasting process.

Alternatives to chlorine have been evaluated due to the unpleasant taste and odor that can be associated with higher levels. Taste and odor issues led to the use of chloramines as a disinfectant in the late 1920's (Connell, 1996). Chloramines, principally monochloramine, tends to impart less of a taste and odor that chlorine (Kirkmeyer et al. 1993). Chloramine chemistry can be complicated and multiple forms of chloramines can form based on chlorine-ammonia dosing ratios and pH. Monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and trichloramine ($NHCl_3$) are the three forms of chloramine that can form when chlorine and ammonia are combined in water. Dichloramine and trichloramine are generally less desired due to their ability to produce a more noticeable chlorinous taste and odor (Kirkmeyer et al. 1993). These two undesired forms have been shown to have lower flavor and odor thresholds than hypochlorous acid or the hypochlorite ion (Krasner and Barrett 1985). Piriou et al. (2004) looked at how thresholds differ between consumers of different nationalities (

Table 2.3). The French were 5 times more sensitive to chlorine and 10 times more sensitive to chloramines than Americans (Piriou et al. 2004). Decreased American sensitivity is likely due to adaptation from higher levels of disinfectant in their tap water. Free chlorine residuals of 0.1-0.2 mg/l are common in France while residuals of 1.0-3.0 mg/l can be found in the U.S (Piriou et al. 2004).

Table 2.3 Taste and Flavor thresholds for chlorine and chloramines

	Taste* , Flavor** Threshold (mg/l as Cl_2)				
	HOCl	OCl^-	Chlorine	NH_2Cl	$NHCl_2$
Krasner and Barrett*	0.24	0.3		0.48	0.13
Pirou et al. Americans**			1.1	1.8	
Pirou et al. French**			0.2	0.18	
Mackey et al.*			0.8	3.7	

Approximately 75% of water utilities using chloramines as a disinfectant have a residual between 1-3 mg/l entering the distribution system (Kirkmeyer et al. 1993). Krasner and Barrett (1985) found monochloramine taste thresholds <0.5 mg/l as Cl_2. Therefore, if humans can detect the presence of monochloramine in their water by tasting, it is highly possible that monochloramine could have the ability to interfere with the tasting process. Chloramine dosing can be regulated to try and inhibit free ammonia. Free ammonia is unwanted in drinking water because of it can sustain nitrifying bacteria in the distribution system. However, free ammonia can be present in the distribution system from, natural sources, improper chloramines dosing and chloramine degradation. A free ammonia concentration of 0.2 mg/l NH_3-N can be caused in drinking water from improper chlorine-ammonia dosing ratios alone (Kirkmeyer et al. 1993). A free ammonia concentration of 0.3-0.4 mg/l NH3-N at the tap would not be uncommon considering multiple sources.

3 Evaluating factors that affect copper tasting sensitivity in drinking water

3.1 Abstract

Corrosion of household copper plumbing infrastructure can cause pipe failure and lead to elevated levels of copper in drinking water which can exceed the USEPA health based standard for copper in drinking water of 1.3 mg/L Cu. The purpose of this study was to determine taste thresholds of copper in different types of water, analyze how copper chemistry can affect tasting, determine if common disinfectants influence the taste of copper and evaluate genetic links to copper sensitivity. A one-out-of-five test was used to define thresholds, evaluate disinfectant influences, and examine copper chemistry differences. A difference from control test was used to analyze soluble copper tasting and a one solution test with visual classification was used to discriminate 6-n-propylthiouracil (PROP) taster status. Solutions containing copper sulfate (0.05 – 8 mg/l Cu) were prepared in distilled water, mineral water of varying pH and mineral water with disinfectant added. Geometric mean copper taste thresholds were 0.48 mg Cu/l and 0.41mg Cu/l in distilled and mineral water pH 7.4 respectively. Logistic regression copper taste thresholds were 1.50 mg Cu/l and 1.96 mg Cu/l in distilled and mineral water pH 7.4 respectively. Soluble copper was readily tasted while particulate copper was poorly tasted. Chlorine and chloramines dosed at typical tap water levels had no significant effect on panelists' tasting abilities for water containing 1 mg/l total copper. Geometric mean copper thresholds values did not correlate with (PROP) status so PROP sensitivity would not be a good indicator for copper sensitivity.

3.2 Introduction

Corrosion of copper pipe does occur as evidenced from reported levels in tap water samples. USEPA databases showed that in 2003 there were 471 individual water systems that violated the copper action level of 1.3 mg/L Cu and potentially affected 622,000 people (USEPA, 2003) Clearly elevated levels of copper are present in drinking water.

Recent problems with pinhole leaks in Maryland and Washington, D.C. have raised awareness and concerns as to the specific causes of copper pipe corrosion, specifically pinhole leaks (Edwards et al. 2004). An estimated 70-80% of drinking water pipes currently in homes and being installed in new homes are made of copper. While many copper pipes do not readily corrode, certain water quality conditions cause corrosion (Edwards et al. 2004). Copper corrosion can be grossly broken into two categories, uniform corrosion and pitting corrosion (Edwards et al. 2004). These two distinct corrosion types, while sharing the same primary reactions, do not necessarily share the same corrosion mechanisms. However, both types of corrosion can release copper into solution. A variety of water quality parameters including pH, mineral content, pipe age, type of scale, oxygen content, natural organic matter, stagnation time as well as many other variables can affect the rate of copper corrosion (Dietrich et al. 2005). It is also not uncommon for water quality, particularly low pH and low alkalinity, to prevent a scale and therefore increase corrosion more than if a protective scale was present (Edwards et al. 2004).

Testing for copper in drinking water is accomplished through the lead and copper rule (USEPA, 1991). Due to the insignificant health risks associated with copper when being compared to lead, sampling sites where lead leaching is likely are given priority under the lead and copper rule. Therefore water utilities may not be aware of increased copper corrosion that can cause pipe failure as well as negative health effects from ingestion. So tasting copper in drinking water may be the only defense many customers have for protecting their pipes and themselves.

Previous research has evaluated taste threshold levels for copper in water of various water qualities. One study found copper taste thresholds of 6.6 mg/L Cu and 13 mg/L in distilled water and spring water respectively (Cohen et al. 1960). Another study found thresholds of 2.4-3.2 mg/l Cu and 0.8-1 mg/l Cu in distilled and mineral waters respectively (Beguin-Bruhin et al. 1983). Zacarias et al. (2001) found thresholds of 2.6 mg/L Cu for tap water, 2.5 mg/L Cu for distilled and 3.5 mg/L in mineral water. The differing results can be attributed to differences in test objectives, statistical analyses, analytical analyses, population tested and testing methods of the research. While these thresholds may seem high, due to the analysis typically done for reporting thresholds there may be a noteworthy population of people that can taste copper much lower than the reported levels.

Beguin-Bruhin et al. (1983) described copper speciation and the primary role that soluble copper likely played in the tasting sensation. Cohen et al. (1960) and Beguin-Bruhin et al. (1983) realized the importance of solubility in tasting and adjusted solution pH to control copper solubility. While the significance of soluble-free-particulate speciation in tasting is of interest, their exact roles are not clear.

Different copper salts have been used to assess the effect of different ions on copper tasting. Zacarias et al. (2001) found that use of either copper sulfate or copper chloride had no significant effect on copper tasting. The effect of nose clamping has also been applied to copper tasting. Zacarias et al. (2001) found panelists having their nose open or closed had no significant effect on copper tasting. Lawless et al. (2004) showed that nasal occlusion did not significantly reduce panelist's metallic, bitter and astringent ratings of copper spiked water.

The objectives of this research were:

- Determine at what levels humans can detect copper in drinking water
- Evaluate the role chemistry can have in tasting copper
- Analyze how disinfectants can affect tasting copper
- Examine the correlation between PROP tasting sensitivity and copper tasting sensitivity

3.3 Methods and Materials

Test samples were stored in 1 liter clear glass bottles purchased from Fisher Scientific. All glassware, pipettes, beakers and containers used were soaked in 10% trace metal grade nitric acid for 8 hours, rinsed with distilled water three times, rinsed with Nanopure® water three times and then air dried.

The copper salt for making the copper stock solutions was Copper (II) Sulfate Pentahydrate (Catalog# BP346, Fisher Scientific). A 100 mg/l Cu stock solution was used to spike the copper samples.

Distilled water solutions

The reagent grade water used for these experiments consisted of distilled water that was also deionized and carbon filtered by a Barnstead Nanopure® system. This system produces water with a chemical resistivity greater than 18 $M\Omega\cdot cm$. The pH of this water was 5.5.

Synthetic mineral water solutions

A mineral water was made using an ionic recipe designed to simulate a typical drinking water from the eastern U.S. Its chemical composition was 21 mg/l Na, 1.5 mg/l NO_3^--N, 41 mg/l SO_4^{2-}, 20 mg/l Na, 8 mg/l Mg^{2+}, 4 mg/l K, 12 mg/l Ca^{2+}, 34 mg/l HCO_3^- (as $CaCO_3$), 2.6 mg/l Si. The pH of the mineral water was 9.3. The pH was adjusted with 1 M HCl or 1M NaOH. Solids aging effects were prevalent with the copper spiked mineral samples so all samples were made fresh daily.

Copper concentration verification

Copper concentrations were verified using atomic absorption spectrophotometry (AAS) (Perkin Elmer 5100 PC). Total and soluble concentrations were measured according to direct air-acetylene flame standard methods for copper. Solutions were measured before and after the testing was complete to ensure no change occurred in the solutions over time. To determine total copper, samples were fixed with nitric acid to $pH < 2$ and then analyzed by atomic absorption. To determine soluble copper samples were filtered through a 0.45µm pore size filter, fixed to $pH < 2$ and then analyzed by AAS. Particulate copper was then calculated by subtracting soluble copper from total copper. To measure free copper (Cu^{++}) an ion specific electrode was used (Accumet Cupric Combination Cat.# 13-620-547). A series of standards were made using a certified cupric standard (0.1M Thermo Orion Cat.# 94206) dissolved in distilled water. Ionic strength activator (1ml of 42% $NaNO_3$) was added to the standards and the copper test solutions. These standards were measured with the electrode and the mV readings were constructed on a semilog plot to develop a calibration curve. The free copper concentrations were then determined from the standardized calibration curve. The potential of all solutions was calculated in mV.

Panelists

Thirty-six healthy adults, with no previous copper taste threshold experience, participated in the copper tasting studies. The panel was composed of 15 males and 21 females ranging from 22 – 54 years of age. A group of 25 panelists or more are desired when estimating population thresholds for taste (Lawless and Heymann 1998). Panelists were comprised of 10 different ethnic groups, and the majority of panelists were of American nationality. Two of the panelists were moderate smokers but no one reported using chewing tobacco. The majority of panelists never reported having any phantom taste experiences. Informed consent was obtained and daily incentives were given after each testing session. The sensory protocol was approved by the Institutional Review Board at Virginia Tech.

pH

The pH values of all samples were closely monitored and adjusted to ensure specific pH levels before the panelists tasted the water. Solutions of 1 M HCl or 1M NaOH were used to adjust water samples to desired pH levels. The pH was monitored closely to ensure each panelist had similar soluble-particulate copper fractioning within the copper spike solutions.

3.4 Similarity testing to analyze pH effects on sensory perception

Background

Water chemistry changes were achieved through altering pH by adding HCl or NaOH. A test was developed to demonstrate that pH alone was not interfering and causing changes in panelist's perceptions. This documented that test results for copper sensitivity were not related to pH differences but only from changes related to copper interactions.

Procedure

A 3-AFC triangle test was used to test for similarity of different pH water. Mineral water of pH 7 and pH 9 were used. This test used balanced presentation order (AAB, ABA, BAA, BBA, BAB, ABB) and 2 sets were given to each panelist. The samples were coded with 3 digit random numbers. Panelists were instructed to taste the sample from left to right and indicate which sample they thought was different. All test samples were presented at room temperature.

Results

Statistical parameters were pre-selected (Table 3.1) to determine sample size and the number of responses needed to show similarity (Meilgaard et al. 1999). Alpha values can be set high in similarity testing because you are not worried about finding a difference when one does not exist (Type I error). Beta values are set low for this test to avoid not finding a difference when one exists (Type II errors). Proportion of distinguishers (Pd) in similarity testing is variable based on what constitutes a meaningful difference. A value of 30% is considered moderate. Out of 53 respondents, 15 correctly picked the odd sample but 21 responses were needed to show significance (Meilgaard et al. 1999).

Table 3.1 Similarity testing statistical parameters for testing pH effect in pH 7 and 9 water

	Value		Response	Number
α	0.3		Correct	15*
β	0.01		Incorrect	38
Pd	30%		* 21 needed for difference	

We concluded with 99% confidence that the proportion of the population who can perceive a difference was less that 30% and probably much lower. Therefore, it can be concluded that any sensory difference between these two samples is sufficiently small and can be ignored and waters with pH of 7 and 9 were sufficiently similar to be used interchangeably.

The two waters in this test had a pH difference of 2 units which is equal to the largest difference between any two samples directly compared in this research. We concluded that a pH difference of ≤ 2 had no effect on tasting and that pH alone did not have an effect on any test comparisons of this research. Therefore any differences in sensory testing of copper perception were linked to copper based interactions.

3.5 Evaluation of taste thresholds of copper in various water types

Background

Threshold experiments were conducted to quantify the levels where the population can begin to taste copper in drinking water. These experiments allowed investigation into the possibility of consumers tasting copper in drinking water and therefore serving as an early warning signal for copper corrosion. As evidenced from EPA databases, concentrations >1.3 mg/L Cu can be present in drinking water so consumers with thresholds below this level are likely to taste the copper and possibly complain to the water utility.

Procedure

A screening test was performed with five panelists in order to define a concentration range and interval. The range of concentrations used for testing was (0.025, 0.05, 0.1, 0.5, 1, 1.3, 2, 2.5, 3, 4, 5, 6, 7, 8 mg/l Total Cu). ASTM threshold methods recommend five or more concentration scale steps, with each step varying from the previous by a factor of 2-4 (ASTM 1991). The multiplying factors used for this research were not uniform. Small interval steps were used to allow panelists to show more sensitivity at lower levels considering the 8 mg/l ceiling due to health risks. A ceiling of 8 mg/l was implemented due to health concerns because panelists were swallowing and not expectorating the samples. Panelists were instructed to swallow the samples as many panelists reported tasting low concentrations on the back of the tongue and throat. All panel members underwent an initial training session to familiarize them with the expected taste of copper and the one-of-five test methods. Copper concentrations used during the training sessions were above anticipated thresholds.

Threshold determinations were conducted on three different water types: distilled pH 5.5, mineral pH 7.4 and mineral pH 6.5. Individual taste thresholds were evaluated using the one-of-five test, a modification of the 3-AFC triangle test. Five samples (4 controls and 1 spiked) instead of three were used to decrease the chance of guessing correctly. Usually in threshold testing multiple concentrations, in an ascending or descending presentation order, of the compound of interest are tested at one sitting.

Typically only one session was given per day and the panelists did not know if the sample was stronger or weaker than the sample given the previous session. Preliminary testing indicated that carry-over was prevalent with copper. This study only tested one copper concentration per session. This modification was implemented to avoid the lingering effect of copper taste in the human mouth on subsequent sample evaluations. Zacarias et al. (2001) and Beguin-Bruhin et al. (1983) used similar modifications.

Five 3-oz white plastic sample cups were placed on a tray and filled with water. Rinse water was also provided and it did not contain any copper. Four of the sample cups contained a control water and one sample cup contained the copper solution. For each test, the control, rinse water and the copper spiked solution were the same pH before tasting. The only difference, in every test, was that one of the 5 sample cups contained copper at a pre-determined concentration. The cups were coded with a 3 digit random number and placed in random orders (ABBBB, BABBB, BBABB, BBBAB, BBBBA, with “A” containing the copper solutions and “B” the control water). All samples were presented at room temperature. The goal of the test was for the panelists to identify the copper containing sample by tasting it. Each panelist began the taste session by rinsing with the provided rinse water then tasting the samples, while rinsing in between samples to decrease lingering. The test protocol required the panelists to taste the samples from left to right and they were instructed to taste each sample only once. Panelists were familiar with the time delay of the copper taste and waited at least 20 seconds in between samples. All test waters were presented at room temperature and all sensory testing was done in a controlled atmosphere with minimal noise or odor influence. Large dividers were used when multiple panelists participated in the same room to reduce testing anxiety and prevent outside influence on the results.

Individual thresholds were confirmed when panelists correctly identified 3 ascending concentrations in a row. Those panelists that did not begin detecting copper until higher concentrations may not have had the opportunity to get 3 in a row correct. Replication of thresholds is recommended to ensure that results are within 20% of each other (ASTM 1997). Replication was not done in the same water but thresholds were conducted in two similar waters. Threshold variability in the two waters was 14.6%. This variability is less than 20% therefore replication in the same water was not performed.

An arbitrary value of 10 mg/l was used for panelists who did not give a positive response even at 8 mg/l. 10 mg/l was chosen because all panelists previously tasted copper within the 8 mg/l ceiling in the distilled water. Therefore their threshold was likely close to 8 mg/l.

Threshold Analysis

A threshold can be defined as the minimum value of a stimulus that can be detected. Any value below the threshold cannot be detected, any value above can. Thresholds are used in multiple applications to determine the point at which known contaminants start to reduce product acceptability (Meilgaard et al. 1999). A good threshold determination requires hundreds of samples and results do not reproduce well. Published thresholds have been shown to vary by a factor of 100 or more (Meilgaard et al. 1999). Various data analysis methods are used to deal with the high variability in threshold testing. Population thresholds in this study were calculated by geometric mean, logistic regression and the Hubaux and Vos method.

The geometric mean is a common threshold determination analysis method and is recommended in ASTM E-679. Geometric mean thresholds are based on where panelist's detection abilities break down (Van Aardt et al 2001). The geometric mean method tries to reduce the significance of outliers in the reported threshold value.

Logistic regression is another common threshold determination method and is recommended in ASTM E 1465-91. Van Aardt et al. (2001) showed that logistic regression can be a valuable threshold determination method and compares well with geometric mean calculations. Logistic regression predicts where a certain percentage of panelists will correctly identify the copper spiked sample. The logistic regression first calculates the probability of a correct response for each concentration. This probability is then transformed by the logit function and regressed against concentration.

The Hubaux and Vos method is commonly used in environmental analysis and can be adapted to address sensory thresholds (Hubaux and Vos, 1970). Population thresholds are calculated from a calibration curve of measured versus known concentrations with a corresponding prediction interval at some specified confidence level. The prediction interval is the range of the measurements where a new value of y will fall at a given single new x measurement. As applied to sensory data, the logistic regression serves as the calibration curve. The intercept of the upper confidence interval serves as the critical concentration, and the threshold limit is the concentration value corresponding to critical level on the lower confidence interval. This method suffers from none of the statistical limitations of the existing methods.

Results

Geometric mean individual thresholds (Table 3.2) were found by taking the geometric mean of the highest concentration sample they did not detect and the lowest concentration they did detect. The lowest concentration detected was classified as the first of at least 3 correct choices in a row. The population thresholds, 0.48 mg/l distilled and 0.41 mg/l mineral, were calculated as the geometric mean of the panelists individual threshold values.

Table 3.2 Individual geometric mean copper taste thresholds in distilled and mineral pH 7.4 water

	Geometric Mean Threshold mg/l Cu	
	Distilled	**Mineral pH 7.4**
Panelist 1	6.48	*10.00
Panelist 2	0.22	0.04
Panelist 3	0.07	0.04
Panelist 4	4.47	1.60
Panelist 5	0.71	0.22
Panelist 6	0.71	0.04
Panelist 7	2.73	*10.00
Panelist 8	1.14	1.60
Panelist 9	2.64	*10.00
Panelist 10	0.71	0.71
Panelist 11	2.64	0.22
Panelist 12	0.22	0.07
Panelist 13	0.22	0.22
Panelist 14	0.07	0.22
Panelist 15	0.07	0.04
Panelist 16	0.22	0.07
Panelist 17	6.48	*10.00
Panelist 18	0.22	0.22
Panelist 19	0.22	0.22
Panelist 20	2.24	*10.00
Panelist 21	0.22	0.04
Panelist 22	0.04	2.24
Panelist 23	0.71	0.07
Panelist 24	0.22	0.07
Panelist 25	0.07	0.71
Panelist 26	0.07	0.22
Panelist 27	0.22	0.07
Panelist 28	0.22	0.71
Panelist 29	0.71	0.71
Panelist 30	0.04	0.04
Panelist 31	0.07	0.04
Panelist 32	2.74	*10.00
Panelist 33	1.61	0.22
Panelist 34	1.14	1.14
Panelist 35	6.48	*10.00
Panelist 36	1.14	0.71
Population Threshold	**0.48**	**0.41**

***10 mg/l was used when panelists did not detect copper within range**

Logistic regression was used to report population thresholds. The population threshold concentration is represented as some arbitrary value (typically 50%) above the probability of guessing by chance as calculated by Abbbott's formula.

$$\text{adjusted proportion correct} = \frac{\text{observed proportion - chance}}{1 - \text{chance}}$$

For example, using 50% as the criterion and a one-out-of-five, where the probability of guessing by chance is 20%, the probability used to define the population threshold is

$$0.5 = \frac{x - 0.20}{1 - 0.20} \rightarrow x = 0.60$$

Logistic regression population thresholds (Figure 3.1) were 1.50 mg/l and 1.96 mg/l in distilled and mineral water respectively.

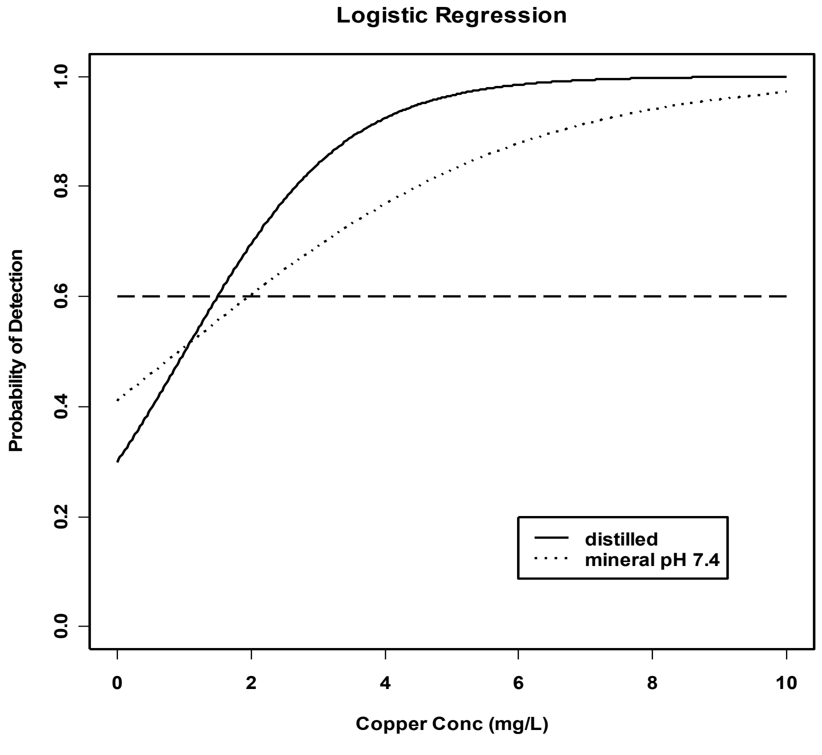

Figure 3.1 Logistic Regression population copper taste thresholds for distilled and mineral water (Probability =60%)

The Hubaux and Vos method was also used to analyze the threshold results (Figure 3.2). The logistic regression served as the calibration curve and 95% confidence intervals were used. Hubaux and Vos population thresholds were 0.67 mg/l and 1.23 mg/l in distilled and mineral water respectively.

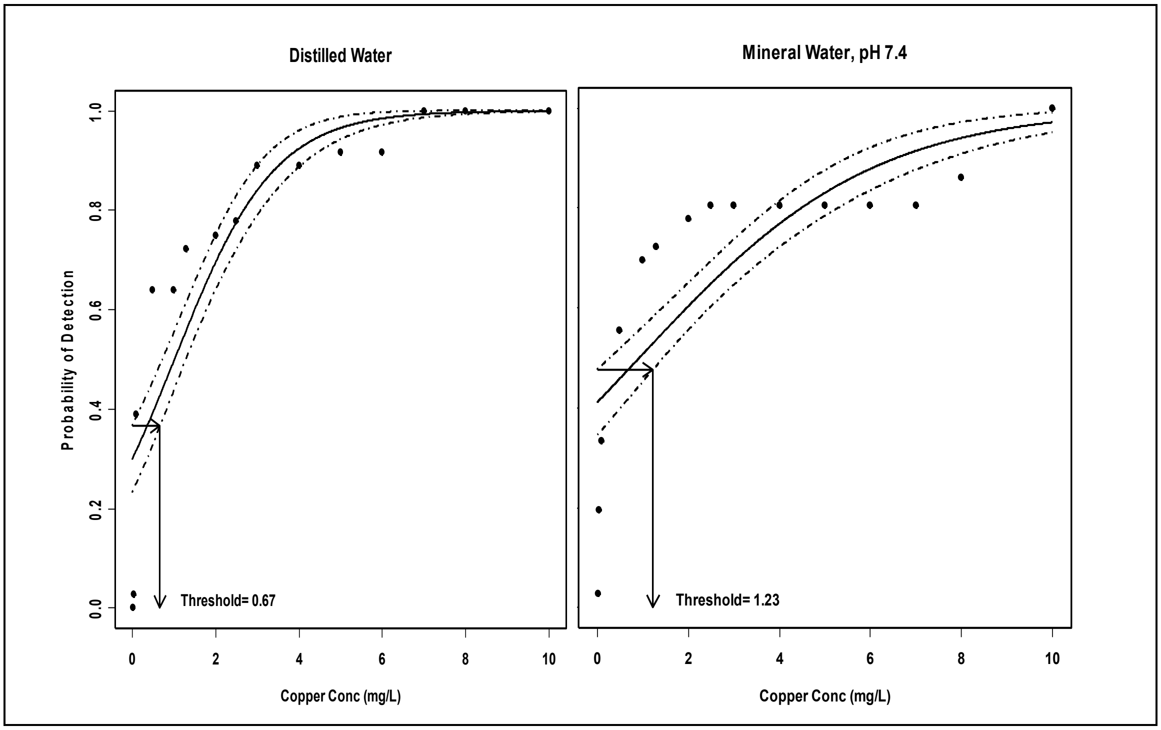

Figure 3.2 Hubaux and Vos population copper taste threshold with 95% confidence intervals for distilled and mineral water

Threshold results usually have high variability, especially with complex tastes such as copper. Reporting a range that represents the dynamics of the results may be more informative than a specific value (Lawless and Heymann 1999). Multiple data analysis methods were conducted to show the range of results that can be reported by these different approaches (Table 3.3). When analyzing reported threshold values it is important to know the way the threshold was calculated and the strengths and weaknesses of that method.

Table 3.3 Comparison of copper taste population thresholds

	Copper population thresholds (mg/l Cu)			
Source Water	**Geometric Mean**	**Logistic (p=60%)**	**Hubaux & Vos 95% CI**	**Range**
distilled	0.48	1.50	0.67	0.48 - 1.50
mineral pH 7.4	0.41	1.96	1.23	0.41 - 1.96

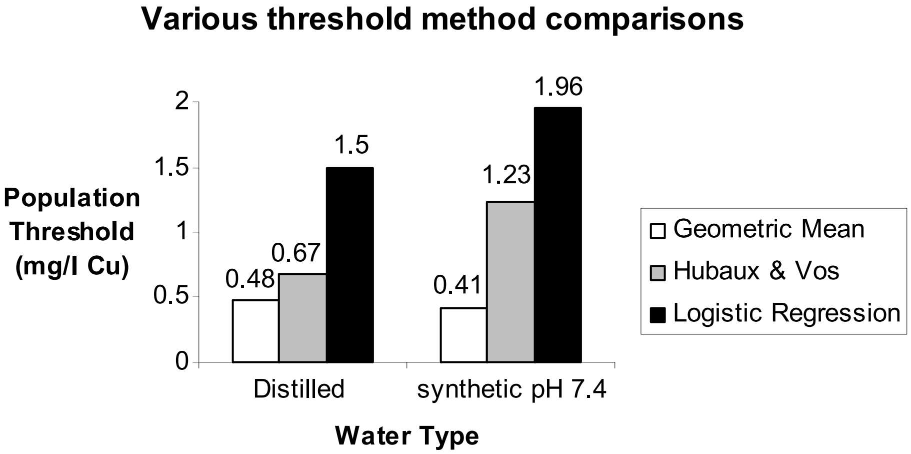

Figure 3.3 Comparison of copper taste population thresholds

Previous research has evaluated taste threshold levels for copper in water of various water qualities. In general threshold results from this research were lower than other studies. Copper taste threshold results for previous studies have ranged from 1-13 mg/l. Concentration interval, statistical analysis, sensory test modifications, copper chemistry, population tested, test objectives and other methods varied for each study. These variations in addition to human deviation may be the reason previous studies have results that differ form this study.

3.6 Evaluation of Copper Chemistry

Introduction

Changing water quality allowed thresholds results to be verified in water that had varying water chemistry. This allowed investigation into the effect that copper speciation can have on copper tasting sensitivity. Copper can have interactions in water and form soluble complexes, particulates or remain the free cation (uncomplexed) depending on the specific conditions. The existence of each of these forms is dependent on a variety of water quality factors (Figure 3.4 Copper chemistry speciation).

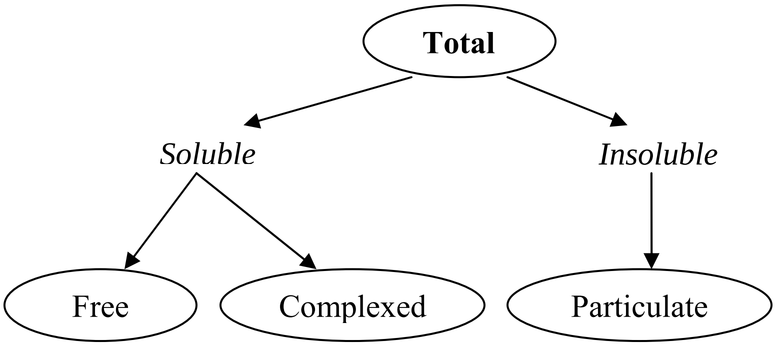

Figure 3.4 Copper chemistry speciation

The four water types that were used to evaluate chemistry affects on copper taste thresholds were:

- distilled pH 5.5
- mineral pH 6.5
- mineral pH 7.4
- mineral pH 8.5

Changing water composition and pH were the two approaches used to effectively manipulate the water chemistry and evaluate the effect it had on the taste thresholds of copper. The differences in copper speciation could then be compared to changes in taste sensitivity. Analyses of the previous threshold experiments in distilled pH 5.5 and mineral pH 7.4 provided some insight as to how these chemistry changes affected copper tasting. Another threshold experiment was conducted with pH 6.5 mineral water to further analyze the chemistry relationships.

Free copper analysis

Free copper, uncomplexed cupric ion (Cu^{++}), is considered soluble. Free copper can be present in water and is favorable at low pH levels and when there is a lack of anions to form complexes. The distilled water, as demonstrated by chemical analyses, had conditions that allowed all the copper within the tested range to remain free (Figure 3.5). Various ligands including: SO_4^{2-}, OH^-, PO_4^{3-}, HCO_3^-, NO_3^-, CO_3^{2-}, S^{2-}, O^{2-} as well as multiple combinations of these and other ligands can bind to cupric and form complexes. If an anion is attached to Cu^{++} then it is considered a complex and is no longer free. The mineral pH 7.4 water, as demonstrated by chemical analyses, had conditions that favored complex formation and did not allow a significant percentage of the copper to remain free (Figure 3.5).

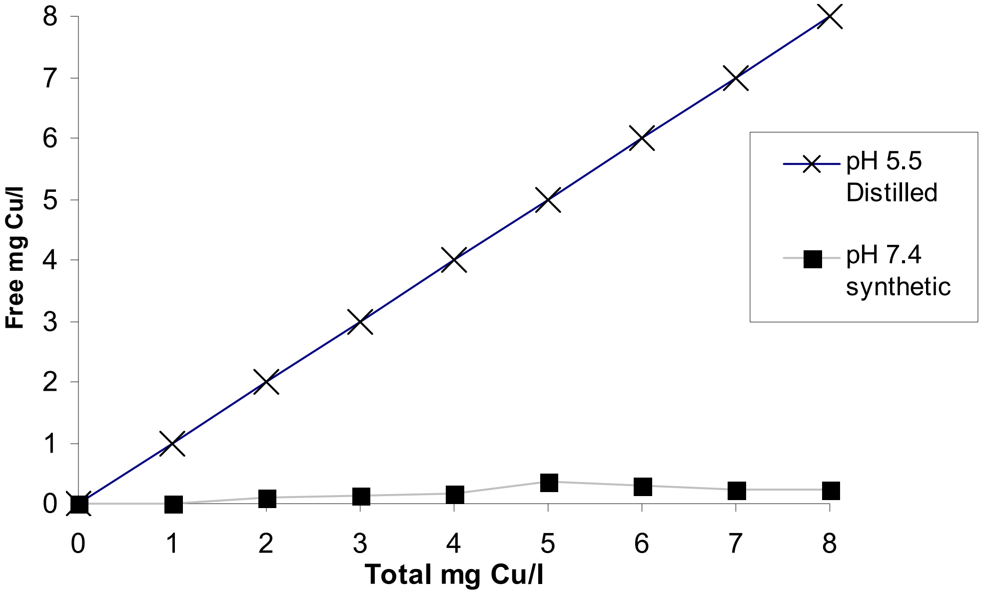

Figure 3.5 Measurement of free copper in distilled and mineral water by ion specific electrode

Inspection of the copper chemistry showed that the free copper was much different for the distilled and pH 7.4 mineral water (Figure 3.5 Measurement of free copper). Wilcoxon nonparametric paired t-tests were used to compare the geometric mean individual copper taste threshold values for the two water types (Table 3.2). The distilled water did not have a different mean threshold when compared to pH 7.4 mineral water (p-value = 0.3577). Thus, the thresholds of the two waters were the same even though the copper speciation was different indicating that free copper does not play the sole role in copper tasting.

Closer inspection of the results was of interest. The majority of panelists had thresholds <1.5 mg/l Cu and most of the copper in both waters <1.5 mg/l was soluble (Figure 3.6).

Considering soluble copper can be composed of both free and complexed, as long as the copper was soluble panelists were able to taste the copper.

Soluble vs. Particulate Analysis

When copper binds to an anion it can remain soluble or precipitate out of solution and become particulate. This is dependent on copper concentration, pH, kinetics of formation of individual complexes as well as other factors. Chemical analysis of the distilled and mineral pH 7.4 waters showed that all the copper in the distilled water was soluble but only about 1.5 mg/l Cu would remain soluble in the pH 7.4 water (Figure 3.6). Seven panelists, with thresholds above drinking water standards, were of particular interest (Table 3.4). All seven panelists tasted copper between 2-7 mg/l in distilled water (all soluble copper). Conversely none of the seven could taste copper in the pH 7.4 mineral water (soluble maximum of 1.5 mg/l). Evaluation of the panelist's thresholds and relating them to chemistry indicates that if a panelist did not detect copper while it was soluble then they were not likely to detect it at all. This analysis allows for hypothesizing that soluble copper controls tasting and particulate copper is not tasted.

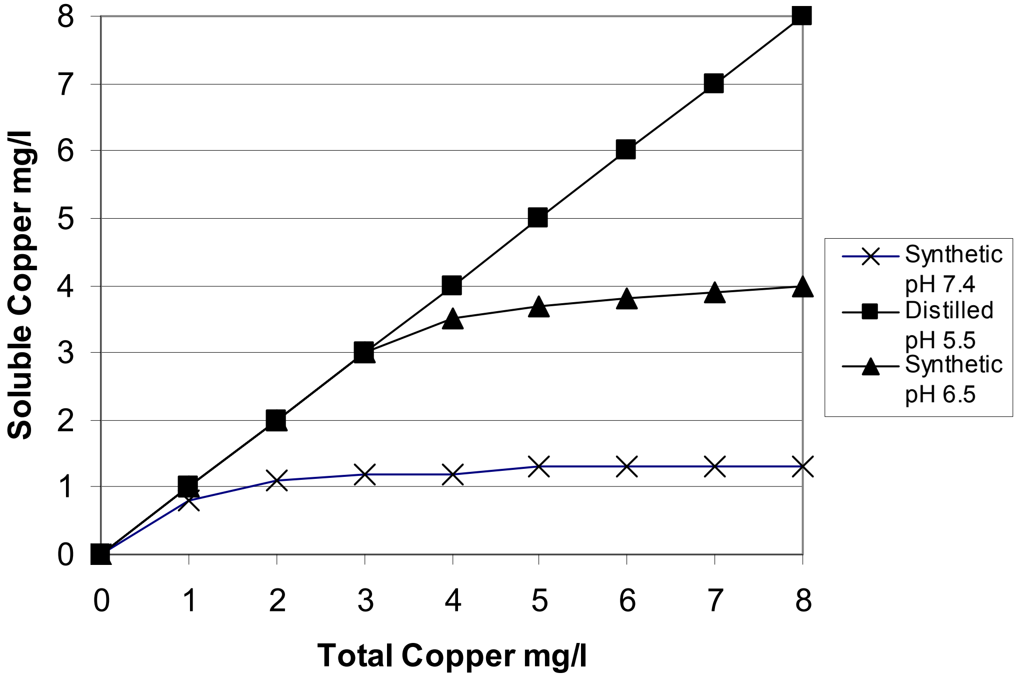

Figure 3.6 Measurement of soluble copper in distilled and mineral water by AAS

Table 3.4 Individual copper taste thresholds in distilled and mineral water

	Individual Geometric Mean Threshold mg/l Cu	
Panelist	**Distilled (8 mg/l max soluble)**	**Mineral Water pH 7.4 (1.5 mg/l max soluble)**
1	6.48	>8
2	2.24	>8
3	2.73	>8
4	2.64	>8
5	6.48	>8
6	2.74	>8
7	6.48	>8

Particulate Copper Analysis

The distilled and pH 7.4 mineral water threshold studies demonstrated that soluble copper is readily tasted by panelists. Soluble copper can be composed of both free and complexed copper. Results indicate that both free and complexed portions of soluble copper are tasted. Thus, waters containing 1 mg/l free copper or 1 mg/l soluble complexed copper would produce the same copper taste intensity.

Both soluble and particulate copper are known to occur in drinking water so an important question becomes – Can particulate copper be tasted? The pH 6.5 and 7.4 mineral water produced both soluble complexes and particulates while the distilled water only produced soluble (free) copper within the tested range. This difference allowed investigation into the role that soluble and particulate have in tasting copper.

To further test the proposal of solubility controlling tasting, a threshold study was conducted on pH 6.5 mineral water and the results were compared to the mineral pH 7.4 water. A select group of 18 panelists (11 sensitive and 7 insensitive) were chosen to evaluate these interactions. The goal of this test was to see if the 7 insensitive panelists, none of whom tasted copper up to 8 mg/l Cu in pH 7.4 water, would again taste copper in the pH 6.5 water if more soluble copper was available to them (Figure 3.6). The 11 sensitive panelists had thresholds below the level where copper solids began to form (~ 1.5 mg/l Cu) so they would be tasting all soluble copper for both tests and their threshold would not be expected to change. These 11 sensitive panelists served as the control.

Results

Geometric mean thresholds were determined for the 18 panelists.

Table 3.5 Individual geometric mean copper taste thresholds in mineral pH 7.4 and pH 6.5 water

	Individual geometric mean thresholds mg/l Cu	
Panelist	**Mineral pH 7.4 (1.5 mg/l max soluble)**	**Mineral pH 6.5 (4 mg/l max soluble)**
Panelist 1	10	10
Panelist 2	10	10
Panelist 3	10	5.48
Panelist 4	10	3.46
Panelist 5	10	2.24
Panelist 6	10	2.24
Panelist 7	10	1.6
Panelist 8	1.61	0.71
Panelist 9	1.61	0.71
Panelist 10	0.71	0.71
Panelist 11	1.14	0.22
Panelist 12	0.22	0.22
Panelist 13	0.22	0.22
Panelist 14	0.22	0.22
Panelist 15	0.22	0.07
Panelist 16	0.22	0.07
Panelist 17	0.04	0.04
Panelist 18	0.04	0.04
Population Geometric Mean	**1.16**	**0.63**

Wilcoxon nonparametric paired t-tests were used to compare the individual geometric mean threshold values of the 18 panelists in the two waters. Most of the threshold variation occurred at higher copper levels where the soluble copper is different between the two waters. These two means were found to be significantly different (p-value = 0.003805). Closer inspection of the results shows that thresholds decreased as more soluble was available This indicates that only soluble copper plays a role in tasting and supports previous conclusions of soluble controlling tasting. The number of test subjects within the required tasting range was small so conclusions regarding the extent of the roles that soluble or insoluble copper play are not concrete. Further investigation into the magnitude of this role is of interest.

*More appropriate ways of testing this hypothesis may come to mind but the limiting factor was copper chemistry and different panelist thresholds. Also the test medium represented typical drinking water contents and pH. These restrictions prevented a test that would have compared two samples, 1 with high total copper all particulate and 1 with high total copper and all soluble. In order for this to occur the two test waters would have to have much different composition and that would prevent doing threshold or difference testing because not only would the copper be different but the water contents would have to be different. So if a difference was detected you would be unable to differentiate what would be causing the difference.

3.7 Further evaluating the roles that soluble and insoluble copper play in copper tasting sensitivity

Background

Previous results have shown that particulate copper is not readily tasted by panelists. To further assess this, a test was developed to evaluate the role of particulate copper in tasting. A pH 8.5 mineral water was prepared that had 4.7 mg/l particulate copper and 0.3 mg/l soluble copper (Table 3.6).This water was compared to a control water that had 0.3 mg/l soluble copper and no particulate copper. 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.

Table 3.6 Water Quality parameters for control and sample

	Control	Mineral pH 8.5
pH	7.4	8.5
Total Cu mg/l	0.3	5
Insoluble Cu mg/l	0	4.7
Soluble Cu mg/l	0.3	0.3

Procedure

Testing protocols, 5-AFC, for the pH 8.5 water were identical to the protocol used in previous experiments with the exception of only testing 1 concentration of copper and not a range. Only testing one concentration provided a binary output of "yes" or "no" for the panelist's ability to taste copper in mineral pH 8.5 water at one copper concentration. A best of three method was used to produce a "yes" or "no" verdict. If the panelist identified the correct sample at least two times then they were designated "yes" for tasting the copper. If the panelist did not identify the correct sample at least two times then they were designated "no" for tasting the copper.

The results from the pH 8.5 mineral sample water were then compared to the mineral pH 7.4 control water. The control was not actually tested. The panelist's threshold results in pH 7.4 mineral water from previous threshold experiment served as the control verdicts. These pH 7.4 thresholds were analyzed and 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.

The pH 8.5 water used for this experiment produced enough blue copper precipitate that it could be seen by panelists in the white sample cups. A modification was used that presented the samples in semi-transparent blue 16-oz cups. These blue cups prevented panelists from discriminating the samples through their sense of sight. The size of the cup also caused the depth of water to be shallower which helped to conceal the blue precipitate.

Results

McNemar's test contingency table analysis for paired data was used to test the effect that increasing insoluble copper had on tasting (Table 3.7). The pH 7.4 control was significantly different (p-value = 0.02334) from the pH 8.5 mineral 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. Therefore particulate copper may play some role in copper tasting and the extent of the role would be of interest.

Table 3.7 Contingency table for control vs. mineral pH 8.5 (n=36)

		Tasted Copper in pH 8.5 mineral water	
		No	Yes
Tasted Copper in Control (pH 7.4)	No	**9**	**7**
	Yes	**0**	**20**

Closer inspection of the results is of interest. Five of these 7 panelists that changed their verdict with addition of particulate copper had thresholds relatively close to the soluble limit of the water. This shows that particulate copper may have some role in tasting but it may not be to a great degree since most of the panelists that were affected by particulate copper addition had thresholds close to the copper concentration that would be present without particulate addition. A possible explanation for this is that the particulate copper may have interactions with human saliva and become soluble. The panelists may actually only taste soluble copper. Further experiments exploring this issue and the specific mechanisms of what is occurring in the mouth would be of interest.

3.8 Evaluating the magnitude that soluble copper plays in tasting

Background

Previous experiments have shown that soluble copper is the predominantly tasted form of copper and particulate copper is only tasted somewhat if at all. These previous experiments have been discrimination tests so the magnitude of tasting soluble copper could not be concluded. A difference from control (DFC) test was developed to evaluate the magnitude of the role that soluble copper plays in tasting. To test this, a pH 7 mineral water and pH 9 mineral water were prepared (Table 3.8). This pH difference produced waters with different amounts of soluble copper. A similar test, comparing water with varying amounts of soluble cooper, was completed in a previous experiment. However, this previous test was only able to evaluate if a difference existed and not how big the difference was.

Table 3.8 Water quality of the two test waters used for the difference from control test

	Reference	Sample
1M HCl	0	150 µl
pH	9	7
Total Cu mg/l	1	1
Soluble Cu mg/l	0.25	1
Insoluble Cu mg/l	0.75	0

Procedure

Two 3-oz white plastic sample cups were placed on a tray and filled with the test samples. One cup contained the reference sample, the second cup contained the comparative sample. Panelists were asked to compare the second sample to the first sample with respect to how different it was from the reference for the copper taste attribute. Two of these tests were administered. For one test the second sample was identical to the reference and was used to measure the placebo effect. The placebo estimate represents the effect by simply asking how different the samples are when no actual difference exists (Meilgaard et al. 1999). The panelists were aware that this situation would occur for one of the sample pairs. For the other test the second sample was different to the reference and was used to compare the differences between the two identical samples. A category scale (Figure 3.7) was used for the DFC test.

☐	☐	☐	☐	☐	☐	☐	☐	☐
Extremely Weaker	Much Weaker	Moderately Weaker	Slightly Weaker	**Same as Reference**	Slightly Stronger	Moderately Stronger	Much Stronger	Extremely Stronger

Figure 3.7 Category scale used in difference from control test

.

Due to the lower copper concentration present in these water samples only a select group of 21 sensitive panelists were allowed to participate. Those panelists that had previously demonstrated to have thresholds above 1 mg/l Cu were not tested. If they would test it would be unlikely that they would be able to taste the copper intensity in the reference sample.

The DFC is not recommended if samples have strong lingering characteristics but a test was needed to explore the magnitude of soluble copper tasting interactions. Five minutes rests were required between samples. This 5 minute break allowed enough time for the panelists to rinse the excess copper out of their mouth but not forget the intensity of the first sample.

Results

When only two tests are conducted and each sample is tested by each panelist a paired t-test is appropriate to analyze the results. Because we were interested in finding if the sample with more soluble copper is perceived more strongly we can analyze the results using a one tailed hypothesis.

Ho: pH 9 = pH 7

Ha: pH 9 < pH 7

(pH 9 less soluble copper, pH 7 more soluble copper)

Using a mean difference of 1 the two means are statistically different but using a mean difference of 2 the two means are not statistically different. A mean difference of 1 corresponds to a verbal descriptor of slightly while a mean difference of 2 corresponds to a verbal descriptor of moderate.

Table 3.9 Statistical parameters of difference from control test

Mean Difference	α value	Tcalc	Tcrit (one tailed)	p value (one tailed)	Significance
1	0.05	2.6	1.7	0.009	Significant
2	0.05	-0.27	1.7	0.395	Not significant

This result shows that a soluble copper increase of 0.75 mg/l soluble copper was perceived as between slightly and moderately stronger and further validates that soluble copper in tasted.

Time to intensity observations

During the DFC test many panelists noted obvious differences that were not related to the test objective. A majority of the panelists stated that the sample with more soluble copper was perceived much quicker than the samples with less soluble copper. Also the samples with more soluble copper were described as lingering longer in the mouth. These observations can help to further explain the roles soluble and particulate copper play in tasting. Particulate copper seems to able to be tasted but not as strongly or as quickly as soluble copper. Again, this could be explained by hypothesizing that particulate copper interacts with saliva and is eventually solubilized. So are panelist actually tasting particulate copper or are chemical reactions occurring in the mouth that are solubilizing some of the copper and allowing panelists to taste soluble rather than particulate copper? This question remains unanswered and would be of interest.

3.9 Evaluation of the affect common water disinfectants have on tasting copper at the EPA aesthetic based standard (1 mg/l Total Cu).

Background

Disinfectants have previously been shown to mask earthy-musty taste and odors in drinking waters (Worley et al.2003). The ability of chlorine and chloramines to mask the taste of copper in drinking water is of interest because these are the most common residual disinfectants used by water utilities. Water used for testing that has disinfectant added would be representative of actual tap water that consumers may be drinking. If disinfectants effectively masked copper then humans may not be able to taste copper even at concentrations high enough to cause negative health effects. To test the masking affect of disinfectants on copper tasting, chlorine and chloramines dosed at concentrations that mimicked eastern U.S. tap water were added to water containing 1 mg/l total copper. These results were then compared to control water that did not have any disinfectant added. All disinfectant water samples tested for this experiment contained the same amount of copper as the control and had similar soluble-insoluble copper fractioning (Table 3.10). The only difference in the test waters were that the disinfected samples contained either chlorine or chloramines while the control did not.

Table 3.10 Water Quality parameters for control and disinfectant water

	Control	Chlorine	Chloramine
pH	7.4	7.4	7.4
Total Cu mg/l	1	1	1
Soluble Cu mg/l	0.8	0.8	0.8
Ion contents	same	same	same
Free Chlorine mg/l as Cl2	0	1	0
Monochloramine mg/l as Cl2	0	0	1.75
Free ammonia mg NH3-N /l	0	0	0.3

Chlorine Water

A 500 mg/l as Cl_2 stock solution of NaOCl was prepared by adding concentrated NaOCl to distilled water. This stock solution was then used to spike all chlorine test solutions. All test solutions were made from mineral water. Due to rapid chlorine decay a fresh stock solution was made daily. After chlorine addition the pH was adjusted to 7.4 using 1

M HCl or 1 M NaOCl. Free Chlorine concentrations were verified using a Hach pocket colorimeter II.

Chloramine Water

A 0.15 molar stock solution of NH_4OH was prepared in distilled water. Volumes of NH_4OH stock solution and concentrated NaOCl were then added to the test solutions to allow for 1.75 -2 mg/l monochloramine (NH_2Cl). All test solutions were made from mineral water and adjusted to pH 7.4. A 5:1 Cl_2: N mass ratio was used to try and limit free ammonia while preventing dichloramine formation. Total chorine, Free Chlorine, Monochloramine and Free ammonia concentrations were verified using a Hach DR-2400 portable spectrophotometer.

Procedure

Testing protocols, 5-AFC, were identical to the protocol used in previous experiments with the exception of only tasting one copper concentration and not a range. To prevent panelists from becoming fatigued, chlorine and chloramines were not in the rinse water for this experiment. The best 2 of 3 binary verdict of “yes” or “no” was applied to this test. Panelists able to discriminate the copper sample in water with chlorine or chloramines were designated as “yes” while those unable to discriminate the copper sample with disinfectant added were designated as “no”. These results were then compared to the control which was mineral pH 7.4 water without disinfectant.

The control was not actually tested. The panelist’s results in the pH 7.4 mineral water from previous experiments served as the control verdicts. Panelists with thresholds greater than 1mg/l Cu were designated as “no” unable to taste copper and panelists with thresholds less than 1mg/l Cu were designated as “yes” able to taste copper. The panelists with thresholds greater than 1mg/l Cu would test the enhancement effect of the disinfectants while those panelists with thresholds less than 1 mg/l Cu would the masking effect of the disinfectants.

Results

McNemar’s test contingency table analysis for paired data was used to test the effect that disinfectants had on tasting copper. Neither the chlorinated (p-value = 1) or the chloraminated (p-value = 0.4795) waters were different from the control. These results show that adding disinfectants at levels typical in tap water would not affect consumers discrimination abilities enough to prevent them from tasting 1 mg/l copper in drinking water.

Table 3.11 Contingency table for tasting copper in chlorinated water vs. control (n=36)

		Tasted copper in Chlorinated water	
		No	Yes
Tasted copper in Control (no chlorine)	No	**9**	**2**
	Yes	**3**	**22**

Table 3.12 Contingency table for tasting copper in chloraminated water vs. control (n=31)

		Tasted copper in Chloraminated water	
		No	Yes
Tasted copper in Control (no chloramine)	No	**6**	**2**
	Yes	**0**	**23**

Closer inspection of individual panelist's results are of interest. 9 of 36 or 25% of the panelists (Table 3.11) were unable to taste copper in either the control or chlorine water. 6 of 31 or 19% of the panelists (Table 3.12) were unable to taste copper in either the control or chloramine water. Most of these insensitive panelists had thresholds greater than 2 mg/l Cu so they would most likely be unable to protect themselves from ingesting copper at levels greater than health based standards simply by taste. This would lead them to rely on visually detecting copper particulate at high levels to signify a water quality problem. However, it would not be unusual for particulate copper present at levels greater than health standards (1.3 mg/l) to be undetectable by consumers just by sight. This creates a scenario of consumers unknowingly ingesting copper at concentrations high enough to cause negative health affects.

24 of 36 or 67% of the panelists were able to taste copper in both the control and chlorine water. 25 of 31 or 80% of the panelists were able to taste copper in both the control and chloramine water. These sensitive consumers would be able to avoid negative health effects from copper in drinking water by rejecting the unpleasant taste.

3.10 Evaluating the genetic link to copper tasting abilities

Introduction

It has been documented for over 70 years that individuals vary dramatically in their sensitivity to bitter compounds containing thiourea, in particular phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) (Prescott et al.2004). This taste difference was accidentally discovered in a laboratory by A.L. Fox in 1931 while he was synthesizing a PTC compound (Zhao et al.2003). This discovery later showed that human sensitivity to PTC/PROP was variable and that panelist's perceptions of the compound represented a trimodal or bimodal distribution. Trimodal distribution can be broken in to three taster groups: supertasters, tasters and nontasters. Individuals in each taster group perceive the intensity of PTC/PROP differently.

- Supertaster: extremely bitter
- Taster: bitter
- Nontaster: no taste

Bimodal distribution is simply broken into tasters and nontasters.

- Taster: bitter
- Nontaster: no taste

The ability to taste 6-n-propylthiouracil (PROP) is an inherited characteristic (Prescott et al. 2004). Research has shown that approximately 70% of the US adult Caucasian population can taste PROP while the remaining 30% of the population perceive PROP as weak or tasteless (Prescott et al. 2004).

Materials

The compound used for the PROP testing was 6-Propyl-2-thiouracil (Catalog# P3755, Sigma Aldrich). A 0.32 mM solution of PROP was prepared by dissolving the compound in distilled water. This solution had to be heated in order to allow the PROP to totally dissolve in the water. The pH of this solution was 6.2. NaCl was used for the salt solution (Catalog# 7647-14-5, Fisher Scientific). A 0.1M salt solution was prepared by dissolving NaCl in distilled water. The pH of this solution was 6.3.

Background

Copper taste testing can be problematic and getting reliable results can be time consuming. A quicker alternative method for determining copper sensitivity would be of interest. PROP sensitivity can be established in several days as opposed to several months with copper. Evaluation of the possibility that PROP sensitivity can serve as a predictor for other compounds and tastes has been investigated but specific links to copper tasting have not been explored (Prescott et al.2004). If PROP sensitivity was found to correlate with copper tasting sensitivity then a significant amount of sensory testing could be reduced.

Procedure

Panelists used a one solution test to rate the solutions, which has previously been shown to be a valid protocol to determine PROP status (Prescott et al.2004). Two 3-oz white plastic sample cups were placed on a tray and filled with the test samples. One cup contained the NaCl solution and the other cup contained the PROP solution. Rinse water, Dannon® bottled water, was also provided to the panelists. The goal of the test was for the panelists to taste the samples, NaCl first and PROP second, and then individually rate them on the labeled magnitude scale (LMS). The NaCl solution was used as the control to aid in classifying panelists into taster groups. The LMS used was a vertical scale marked with verbal labels to indicate taste intensities (Figure 3.8) and panelists were instructed to make their ratings relative to sensations they experience in everyday life. Two identical tests were conducted in order to examine the test-retest efficiency factor of the taster group breakdown.

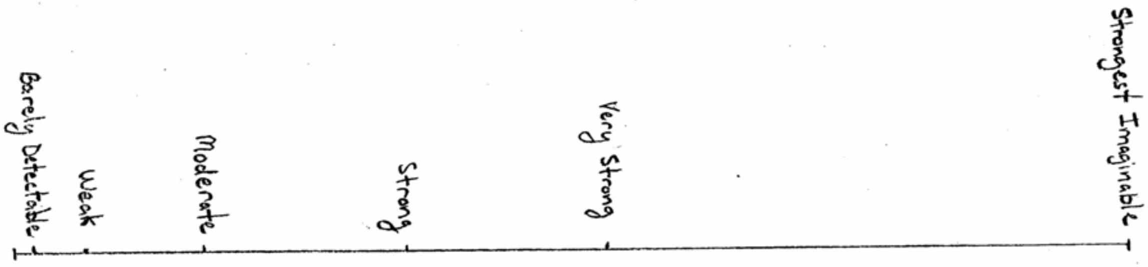

Figure 3.8. Horizontal version of the Labeled Magnitude Scale used for intensity rating. *Actual scale given to panelists was oriented vertically

Sensory Controls

Panelists were instructed to expectorate the samples to reduce lingering effects and prevent possible negative health affects. Each panelist began the taste session by rinsing with the provided rinse water and then rinsing in between samples to decrease lingering. The test protocol also made panelists taste the NaCl sample before the PROP sample. This control was implemented to deal with the lingering effects of PROP. All test waters were presented at room temperature and all sensory testing was done in a controlled atmosphere with minimal noise or odor influence. Large dividers were used when multiple panelists participated in the same room, to reduce outside distractions.

Results

Panelist's markings for NaCl and PROP intensity on the LMS were given a quantitative score based on a 0-100 numerical scale. Taster groups were decided based on visual classification of scores. Visual classification examines each panelists response individually. Panelists ratings of PROP are compared with their NaCl rating. Breakdown of taster groups is as follows:

Nontaster: PROP ratings much lower than NaCl ratings
Taster: PROP ratings similar to NaCl ratings
Supertaster: PROP ratings much higher than NaCl ratings

Test retest reliability of the visual classification technique was calculated for the 3 taster group and 2 taster group distribution. The test-retest percentage is calculated by taking the number of subjects who were classified in the same taster group for both replications and dividing by the total number of subjects.

Table 3.13 Test-retest reliability of visual classification

	test-retest	Supertasters	Tasters	Nontasters
3 group	81%	14	3	8
2 group	100%	23		8

Once PROP status was determined, the copper taste thresholds of the various taster groups were analyzed to determine if PROP status would be a good indicator for copper sensitivity. Kruskal-Wallis nonparametric ANOVA was used to compare geometric mean copper taste thresholds of distilled and pH 7.4 mineral water by taster type. Panelists were nested by PROP status. Analysis was conducted (Figure 3.9) Figure 3.9 PROP status copper sensitivity analysis for trimodal and bimodal classificationfor three group taster status as well as two group taster status.

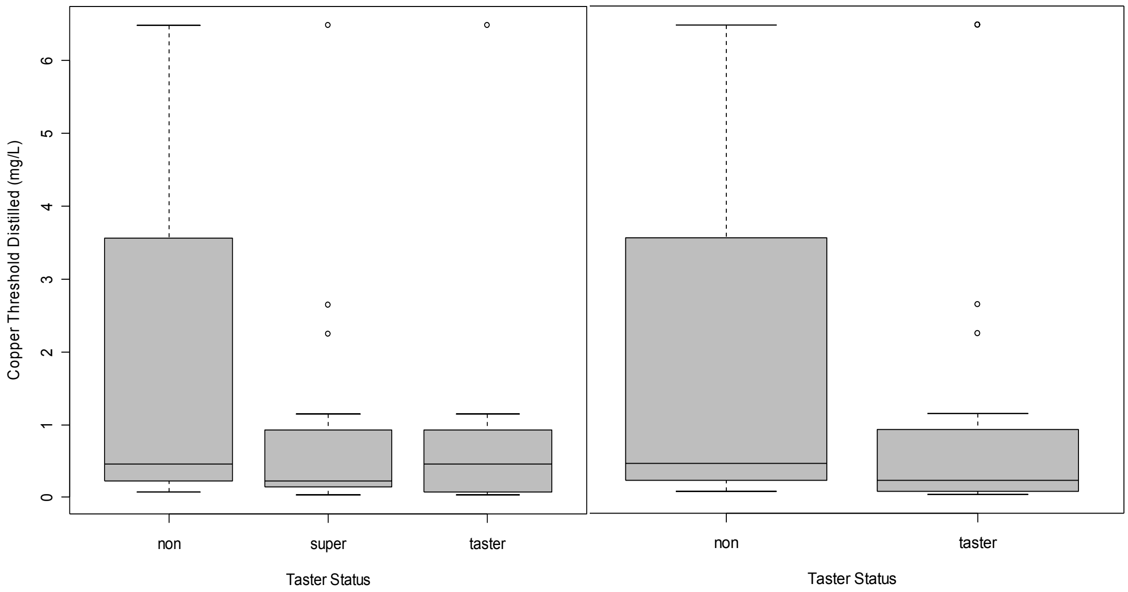

Figure 3.9 PROP status copper sensitivity analysis for trimodal and bimodal classification

No significant differences were found for either 3 group or 2 group PROP status for either water. Therefore PROP status does not correlate well with copper tasting and would not be a good indicator for sensitivity to copper.

Females tend to be more sensitive to PROP and therefore are more likely to be tasters/supertasters than males (Bartoshuk et al.1994). This study found a higher percentage of female supertasters.

Table 3.14 Gender differences in taster status

	Super	Taster	Non
Female count	11	3	4
Female %	61%	17%	22%
Male count	4	5	4
Male %	31%	38%	31%

Age and cultural background were not analyzed due to lack of statistically representative populations.

3.11 Conclusions

- Sensitive consumers (~75% of the population) should be able to taste copper in drinking water at low levels (<1mg/l)
- Sensitive consumers could indicate copper corrosion and prevent negative health effects by rejecting the water
- Various statistical interpretations exist for threshold testing. When analyzing reported threshold values it is important to understand the way the threshold was calculated and the strengths and weaknesses of that method. A reported number or range for a threshold may not be representative of how sensitive certain people are to tasting.
- Taste thresholds for copper in drinking water are variable based on copper chemistry. Having the copper soluble is an essential criteria for determining tasting and it does not matter if the copper is free or complexed.
- Particulate copper is poorly tasted and its taste may be influenced by dissolution in the oral cavity.
- If water quality favors particulate copper, significant copper corrosion may be occurring and consumers would not be able to detect it by taste.

- A significant percentage of the population tested (~25%) have thresholds above the health based standard of 1.3 mg/l Cu even when all copper is soluble. These panelists would likely unknowingly consume copper in drinking water art levels high enough to cause negative health effects.
- If a consumer can taste copper at levels below where negative health effects begin to develop, then water disinfected with typical levels of chlorine or chloramines is unlikely to mask the taste of copper enough to prevent them from protecting their health.
- Genetic PROP taste classification is not a good indicator for sensitivity to the taste of copper.

3.12 Future Considerations

Further evaluating the role that particulate copper has in tasting would be of interest. However there will likely always be some amount of soluble copper in drinking water considering the water conditions typically found in the distribution system. Therefore testing done to analyze this may have to be conducted in a different medium and those test results may or may not correspond well to what can happen in drinking water.

Further evaluating the role that disinfectants have in tasting copper would be of interest. To examine this relationship, different levels of disinfectant could be added to a copper concentration just above a panelist’s threshold. This would show what concentration of disinfectant can begin to effect copper tasting. Testing different levels of copper with common disinfectant levels would also be of interest. This would show how copper thresholds change with a typical disinfectant dose.

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Appendix A. Panelist Comments

Most panelists involved in this research tested copper 20-30 separate times and became very trained on what types of sensation that copper typically gave them. The following information is a compilation of quantitative feedback from the panelists.

Distilled samples

Many panelists complained of the metallic-like taste that the nanopure samples and rinse water gave them. For this reason it would be suggested that an ion free water (distilled, deionized, reverse osmosis etc.) not be used as a control or rinse when using test samples that have a metallic like taste. The distilled water tasting metallic may be from the reactions it has with the saliva and taste buds. The lack of ions in the water may cause the mouth to attempt to equalize the osmotic pressure. These reactions may be what causes the metallic sensation panelists described when tasting the distilled water.

Copper Descriptors

The descriptors used to classify the copper solutions were: copper, penny, bitter, metallic, astringent, rusty and bloody.

Disinfectant Delay

Time to intensity for copper samples was variable. Several panelists mentioned that the delay was noticeably longer when the disinfectants were used.

Difference form Control Test

During the DFC test many panelists noted obvious differences that were not related to the test objective. A majority of the panelists stated that the sample with more soluble copper was perceived much quicker than the samples with less soluble copper. Also the samples with more soluble copper were described as lingering longer in the mouth. These observations can help to further explain the roles soluble and particulate copper play in tasting. Particulate copper seems to able to be tasted but not as strongly or as quickly as soluble copper. Again, this could be explained by hypothesizing that particulate copper interacts with saliva and is eventually solubilized. So are panelist actually tasting particulate copper or are chemical reactions occurring in the mouth that are solubilizing some of the copper and allowing panelists to taste soluble rather than particulate copper? This question remains unanswered and would be of interest.

Daily Variation

It is well documented that your daily routine can have a drastic effect of your perception of taste. This effect was shown when single concentration testing was performed. During single concentration testing the panelist would receive identical copper spiked samples, but just on different days. The copper content was measured daily to verify that the samples had the same copper content. Some panelists would mention that one sample seemed much stronger than the last one while other panelists would mention that one sample was much weaker that the previous sample. These observations were given even though the samples contained the same amount of copper.

Appendix B. Informed Consent Forms

Copper Tasting Form

VIRGINIA POLYTECHNIC INTITUTE AND STATE UNIVERSITY

Informed Consent for Participants of Investigative Projects

Thresholds for Metals in Drinking Water by Taste-and-Odor Testing

Investigators: Dr. Andrea Dietrich and Jonathan Cuppett

PURPOSE: Approximately 65 people will perform taste and odor analysis of distilled and tap water solutions containing copper. This will provide data on threshold levels for this metal. The data may be used by the researchers and the drinking water industry to help understand sensory characteristics of metals that are present in water from corrosion of plumbing materials.

PROCEDURES: Panel members will use their senses to evaluate water samples at different metal concentrations and water qualities. Panelists will attend up to 50 meetings over the period of several months lasting 5-10 minutes each meeting. During a typical meeting, panelists will evaluate sets of five samples (triangle tests), which include two odor/taste free water samples, and one metal containing solution. About 30 ml will be consumed for each test solution. Copper concentrations will not go above 8 mg/l. Panelists will evaluate all of the samples independently; the group may discuss their evaluations. The maximum foreseeable copper intake for the highest testing session is 0.24 mg of copper. The EPA health based standard for daily copper intake is 2.6 mg. Also keep in mind that you will only be tasting the maximum concentration once every 10 trials. The average mass of copper through all trials will be approximately 0.05 mg (2% of EPA's health based standards).

RISKS: The risk level is anticipated to be minimal for the panelist. Sample concentrations will be selected based on concentrations found in drinking water. U.S. Environmental Protection Agency (USEPA) water quality standards will be used as guidance to determine concentrations of copper and chlorine. Because EPA sets regulatory guidelines for components of health concern, most of the parameters to be investigated do not have primary water quality standards; for this project, only copper and chlorine has USEPA Primary Standards. The USEPA Primary drinking water standards are set based on health consideration and assume that an individual drinks 2000 mL of water per day with that concentration for 70 years (lifetime exposure).

USEPA Current Primary Drinking Water Standards (EPA 816-F-02-013July 2002)

Maximum Contaminant Level (not to be exceeded)
Chlorine: 4.0 mg/L as Cl_2

(NOTE: if 2 liters per day were imbibed and the water contained at 4.0 mg/Las Cl_2, then a person would consume 8 mg as Cl_2.)

Action Level – (will be exceeded)
Copper: 1.3 mg/L Cu
(NOTE: If 2 liters per day were imbibed and the water contained at 1.3 mg/L Cu, then a person would consume 2.6 mg Cu.)

For tasting chlorine, less than 8 mg as Cl_2 per day will be consumed by panelist during a daily test session. For tasting copper, less than 2.6 mg Cu per day will be consumed by panelists during a daily test session.

BENEFITS: Personal benefits for the human subjects include getting trained in standard sensory procedures used in the food and beverage industry. In general, the research will benefit the drinking water industry by providing a better understanding of when individuals can taste/smell corrosion by-products in drinking water.

EXTENT OF ANONYMITY AND CONFIDENTIALITY: Panelists may discuss their individual evaluations, and the subject matter is not of sensitive nature. For these reasons, we do not feel that taking steps to conceal individual identification during testing will be necessary. For any reports, the individuals will not be identified by name, but rather "panelist 1,2,3" etc. We may include the gender/age of the subject in report, but again, this will be done anonymously.

COMPENSATION: No compensation will be earned.

FREEDOM TO WITHDRAW: Subjects are free to withdraw from this study at any time without penalty.

APPROVAL OF RESEARCH: This research project has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University, by the Department of Civil Engineering.

SUBJECT'S RESPONSIBILITIES: I voluntarily agree to participate in this study. I have the following responsibilities:

1. To attend taste/smell the metal solutions as instructed and to provide and honest answer on how they taste/smell to me.

To attend as many sessions as possible (dates to be announced).
Confirm that I am not pregnant (pregnant women are excluded from the protocol).

I have read and understand the Informed Consent and conditions of this project. I have had all of my questions answered. I hereby acknowledge the above and give my voluntary consent and participation in this project. If I participate, I may withdraw at any time without penalty. I agree to abide by the rules of this project.

______________________________	______________________
Name	Email
______________________________	______________________
Signature Date	

Should I have any questions about this research or its conduct, I may contact:

Jonathan Cuppett	552-4515, jdc217@vt.edu
Investigator(s)	Telephone/e-mail
Andrea Dietrich	231-5773, andread@vt.edu
Faculty Advisor	Telephone/e-mail
William Knocke	231-7532, knocke@vt.edu
Departmental Reviewer/Department Head	Telephone/e-mail
David M. Moore	540-231-4991/moored@vt.edu
Chair, IRB	Telephone/e-mail

Office of Research Compliance
Research & Graduate Studies

This Informed Consent is valid until February 20, 2005.

Panelist Copy

I have read and understand the Informed Consent and conditions of this project. I have had all of my questions answered. I hereby acknowledge the above and give my voluntary consent and participation in this project. If I participate, I may withdraw at any time without penalty. I agree to abide by the rules of this project.

Name	Email
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Office of Research Compliance
Research & Graduate Studies

This Informed Consent is valid until February 20, 2005

PROP FORM

VIRGINIA POLYTECHNIC INTITUTE AND STATE UNIVERSITY

Informed Consent for Participants of Investigative Projects

Determining taster status for correlation with Copper thresholds

Investigators: Dr. Andrea Dietrich and Jonathan Cuppett

PURPOSE: Previous research shows that the human population can be broken into 3 basic categories; nontaster, taster and supertaster. The compound 6-*n*-propylthiouracil (PROP) has been shown to differentiate humans into these 3 categories. Individuals responses to PROP can be categorized as follows: intensely bitter - supertasters, bitter – tasters and barely anything – nontasters. This research will attempt to classify individuals by taster status. The data may be used by the researchers, food industry and the drinking water industry to help understand metal thresholds and tasting status correlations.

PROCEDURES: Approximately 35 people will perform taste analysis of (PROP), which is a standard chemical used in human sensory research. Panel members will take a 30 ml volume of 0.32mM PROP solution into their mouth and then expectorate it. They will then assign an intensity to the solution based on a predetermined scaling system. Panel members will take 2 of these tests and their scores will be averaged for statistical analysis. Anticipated PROP dosage per test if swallowed is 0.75 mg. Panel members will also take a 30 ml volume of 0.1M NaCl (table salt) solution into their mouth and then expectorate it. They will then assign an intensity to the solution based on a predetermined scaling system. Panel members will take 2 of these tests and their scores will be averaged for statistical analysis

RISKS: From time to time, concerns have arisen about the toxicity of thiourea compounds and their appropriateness for taste research. Thiourea compounds are widely distributed in plants including cabbage, broccoli, brussels sprouts, and kale. These compounds have well-known antithyroid properties by inhibiting thyroid hormone biosynthesis. High dietary levels induce goiter in experimental animals and are associated with reduced thyroid function in humans. But the latter effects are limited to certain isolated human populations consuming marginal diets, as has been noted in native West African populations subsisting on cassava. The pure derivative, propylthiouracil (PTU), also has therapeutic uses. PTU is routinely used to treat children, young adults, and pregnant women with hyperthyroidism due to Grave's disease. The therapeutic safety and efficacy of PTU are well established. PTU is not considered as a human carcinogen or teratogen. The concentration of PROP encountered in a typical taste study is several orders of magnitude lower than a single therapeutic dose of PTU (300 mg/day). The mass of PROP in the solution volume is expected to be 0.75 mg—approximately three orders of magnitude lower than the therapeutic dose. Data on the physiological effects of exposure to low concentrations of PTU can be extrapolated from studies in human infants. Breastfed infants of mothers treated with PTU are estimated to consume 41–460 mg/day PTU. Breastfeeding has been shown to have no effect on neonatal thyroid function even in mothers taking up to 750 mg/day PTU. Thus, it seems unlikely that casual exposure to PROP in a taste study poses a foreseeable risk to human subjects. (Zhao, Tepper et al. Physiology and Behavior. 2003)

BENEFITS: Personal benefits for human subjects include being informed on their tasting status and getting trained in standard sensory procedures used in the food and beverage industry. The research will also benefit the drinking water and food industry by providing a better understanding of metal tasting and tasting status.

EXTENT OF ANONYMITY AND CONFIDENTIALITY: For any reports, the individuals will not be identified by name, but rather "panelist 1,2,3" etc. We may include the gender/age of the subject in a report, but again, this will be done anonymously.

COMPENSATION: No compensation will be earned.

FREEDOM TO WITHDRAW: Subjects are free to withdraw from this study at any time without penalty.

APPROVAL OF RESEARCH: This research project has been approved by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University and by the Department of Civil and Environmental Engineering.

SUBJECT'S RESPONSIBILITIES: I voluntarily agree to participate in this study. I have the following responsibilities:

1. To taste the solutions and rate their taste intensities.

I have read and understand the Informed Consent and conditions of this project. I have had all of my questions answered. I hereby acknowledge the above and give my voluntary consent and participation in this project. If I participate, I may withdraw at any time without penalty. I agree to abide by the rules of this project.

____	________________________
Name	Email
____	________________________
Signature Date	

Should I have any questions about this research or its conduct, I may contact:

______Jonathan Cuppett_____________	____552-4515, jdc217@vt.edu
Investigator(s)	Telephone/e-mail
______Andrea Dietrich______________	____231-5773, andread@vt.edu
Faculty Advisor	Telephone/e-mail
______William Knocke________________	____231-7532, knocke@vt.edu
Departmental Reviewer/Department Head	Telephone/e-mail
David M. Moore	540-231-4991/moored@vt.edu
Chair, IRB	Telephone/e-mail
Office of Research Compliance	
Research & Graduate Studies	

This Informed Consent is valid until December 1, 2005

I have read and understand the Informed Consent and conditions of this project. I have had all of my questions answered. I hereby acknowledge the above and give my voluntary consent and participation in this project. If I participate, I may withdraw at any time without penalty. I agree to abide by the rules of this project.

Name	Email
Signature	Date

Should I have any questions about this research or its conduct, I may contact:

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Research & Graduate Studies	

This Informed Consent is valid until December 1, 2005

Appendix C. Scoresheets

5-AFC Scoresheet

Name:______________________ Date:___________

Circle the sample that is different

Set 1	138 216 647 469 881

3-AFC Scoresheet

Name:______________________ Date:___________

Circle the sample that is different

Set 1	206 847 659

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

PROP Scoresheet

Name: ____________________

Date: ________________

In making your judgements, you should rate the stimuli relative to other tastes of all kinds you have experienced. Thus, *'Strongest Imaginable'* refers to the most intense sensation you can ever imagine experiencing. This includes such varied sensations as those produced by a fresh lemon, a piece of celery or spicy mustard. Note that *'taste'* does not mean the pain produced by a physical trauma like biting or burning your tongue. Simply rate the samples relative to tastes that you experience in daily life.

Directions:
1) Rinse
2) Taste Salt sample
3) Expectorate Salt sample
4) Rate Salt sample
5) Rinse
6) Taste Prop sample
7) Expectorate Prop sample
8) Rate Prop sample

Salt

Strongest Imaginable
Very Strong
Strong
Moderate
Weak
Barely Detectable

Prop

Strongest Imaginable
Very Strong
Strong
Moderate
Weak
Barely Detectable

Appendix D. Panelist Questionnaire

Background Information

Name:____________________ Age: ___ Sex: ☐Male ☐Female

Nationality _________ Years living in Blacksburg Area ____

What was the date of your last significant dental work (other than normal cleaning)? ___________

Are you currently suffering chronic health problems (including oral or nasal conditions) that would affect your performance of this test? Y N

Have you had previous experience with sensory testing? Y N

If you use tobacco, check all that apply ☐Cigarettes ☐Pipe
☐Cigars ☐Chewing Tobacco

If cigarettes, average per day ____
If cigarettes, years you have been smoking regularly ____

Have you ever had a tonsillectomy? ☐Y ☐N

Do you ever have persistent salty, sweet or bitter tastes in your mouth?
☐Never ☐Occasionally ☐Sometimes ☐Always

Have you ever sustained a serious head injury?
☐No
☐Yes, but did not result in concussion or loss of consciousness
☐Yes, resulted in concussion and or loss of consciousness

What types of water do you drink? Check all that apply.
☐Tap ☐Filtered ☐Bottled ☐Well

Do you have a water preference? ☐ Y ☐ N
If so which?
☐ Tap ☐Filtered ☐Bottled ☐Well

Glasses (8 oz.) of water consumed per day (not including beverages made with water i.e. tea, juice etc.)? ☐0-1 ☐2-4 ☐More than 4

VITA

Jonathan Cuppett was born on May 19, 1980. He grew up in Johnstown Pennsylvania and graduated from Conemaugh Township High School. He received a Bachelors Degree from The Pennsylvania State University in Environmental Resource Management in 2002. Jonathan then worked at Concurrent Technologies Corporation for 7 months before returning to school pursue a graduate degree in the Fall of 2003. Jonathan graduated from Virginia Polytechnic Institute and State University in the spring of 2005 with a Master's of Science in Environmental Science and Engineering