

REPLY TO GEORGE BREWER LETTER TO *JTEH* EDITOR (“TOXICITY OF COPPER IN DRINKING WATER”)

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Five articles on copper toxicity were recently published in a special issue of the *Journal of Toxicology and Environmental Health A* (Deveau, 2010; Krewski et al., 2010a, 2010b; Meek et al., 2010; Stern, 2010). Prof. George Brewer suggests that the risk of copper exposure from inorganic copper has been underestimated by the authors of these articles. By “inorganic” copper, he refers to copper not bound to protein. In serum, the most relevant form of unbound copper is that not bound to serum ceruloplasmin (Cp), an iron-oxidase and acute-phase reactant. The term “free copper” has also been used for copper not bound to Cp or other proteins. This terminology ignores the fact that that copper can be bound, often with very high affinity, to small proteins, peptides and amino acids and small molecules present in body fluids, serum, food, and beverages.

We here use the terms “non-Cp copper” or low-molecular-weight copper complex (“LMW copper”) when referring to copper not bound to protein.

Brewer suggests that typical intakes of copper present a risk to people over 50 years of age. More specifically, he proposes that low-level intake of LMW copper from drinking water or from vitamin supplements is a risk factor in Alzheimer’s disease (AD). This view is inconsistent with recent human trials on copper and AD (Kessler et al., 2008a, 2008b), as well as a large toxicity database recently summarized in the European Union (EU) Voluntary Risk Assessment of Copper (Cross et al., 2006), all three of which evaluated compounds being inorganic by Brewer’s definition. The current authors respectfully propose that Brewer’s “warnings” and recommendations could

420 inadvertently result in a disservice and a potential danger to the public, for reasons outlined in the following.

Brewer begins his argument by citing a study in hypercholesterolemic rabbits (Sparks & Schreurs, 2003) that evaluated the risks associated with high-cholesterol diets. The high-cholesterol feed was tested with and without copper (copper was never tested on its own). Signs consistent with AD pathology were seen in all animals given the high-cholesterol feed. Two major endpoints examined in this paper, the formation of senile plaques and neuronal a-beta levels, did increase with copper addition to the drinking water.

435 However, it is not clear that these endpoints are related to the induction or progression of dementia: The now famous “nun study” (Snowdon, 1997) has demonstrated that, in humans, plaque formation does not correlate with dementia (some nuns maintained high cognitive test scores, despite having abundant neurofibrillary tangles and senile plaques). It is now known that a-beta plays a role in copper homeostasis in the brain (see Hung et al., 2010, for a review of this issue), and the increase in neuronal a-beta levels in response to copper has been interpreted as a potentially normal, homeostatic response (Bush et al., 2003).

440 The Sparks studies referenced by Brewer examined high cholesterol status together with copper intake (Sparks & Schreurs, 2003; Sparks et al., 2006). This type of study in compromised animals is applicable to neither the derivation of the maximum contaminant level (MCL) for copper in drinking water, nor its extrapolation to the no-observed-adverse-effect level (NOAEL) for copper in humans. This work is cited by Brewer out of context, ignoring the high-cholesterol preconditioning and incorrectly deducing that copper exposure (from drinking water) in itself can be a risk factor in AD etiology.

465 Moving on to human trials, Brewer correlates intake of copper from multivitamins with increased cognitive decline, citing a study by Morris et al. (2006). The effects of concern occurred only in those study subjects with concomitant high trans-fat and saturated-fat

intakes. It is not possible to separate the factors studied by these authors and implicate copper directly in the presence of high-fat intake, and the additional 30 ingredients of a typical multivitamin–multimineral supplement.

Overall, Brewer’s main concern appears to be what he calls “sharply increased toxicity” of inorganic copper. He supports his argument with his own early work, where radioactive copper was administered to Wilson’s disease patients and healthy controls (Hill et al., 1986). It was observed that 5–6% of the radioactivity appeared in the systemic circulation of healthy individuals within an hour, suggesting that it had bypassed the liver. This interpretation appears correct, but the conclusions drawn from it are not. First, Brewer calls this “a large part” of the copper, but, in actual fact, the physical amount of copper remains unknown, as the specific activity of the radioactive copper was not determined. Second, the copper was administered orally together with milk. In the milk, the radioactive copper most likely became bound to proteins and in some cases it likely exchanged with naturally protein-bound copper, so the actual physical state of the administered copper is unknown. In a similar study where radioactive copper was added to different types of milk and administered to suckling rats, it was shown that copper extrinsic label equilibrates with the intrinsic copper in the milk (Lonnerdal et al., 1985). We would suggest that, while interesting, the interpretation of the results from the cited study are complicated due to the issues just outlined.

We fully agree that non-Cp copper is toxic in Wilson’s disease patients, where it can cause liver damage (resulting in cirrhosis) and central nervous system (CNS) symptoms (Yarze et al., 1992). However, it has to be remembered that these patients suffer from a genetic disorder that prevents the secretion of excess copper by the normal route via the bile (Tanzi et al., 1993). Clearly, exposure to any form of copper presents a risk to Wilson’s disease patients and remains of great concern.

When making decisions about public health and setting standards for copper exposure, however, it is overly simplistic to postulate

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that high non-Cp copper in serum correlates with high copper in the brain and leads to neurodegenerative disease. Brain tissue of AD patients is in fact copper *deficient*—only the extracellular amyloid plaques are enriched in copper, along with iron and zinc (Deibel et al., 1996; Adlard & Bush, 2006; Religa et al., 2006; Magaki et al., 2007). Indeed, increasing intracellular copper bioavailability has been reported to be beneficial (Crouch et al., 2009), underscoring the notion of a localized copper imbalance. The paradox of extracellular copper excess and intracellular copper deficit in the AD brain remains unresolved.

Recently, an essential role for extracellular unbound copper has been discovered, which highlights the need for “free” copper and which may in part explain the detriment of copper being trapped in the extracellular plaques. It has been shown that the glutamatergic synapses in the cortex and hippocampus release copper into the synaptic cleft as an integral part of neurotransmission. This is, in our opinion, one of the more significant discoveries in the copper field in the last few years and highlights a critical need for copper for proper brain function (copper’s role in neurotransmission has recently been reviewed by Hung et al. [2010]).

The review just described also summarizes emerging data on copper uptake into the brain by the copper pump, ATP7A, and neuronal copper uptake by Ctr1 (copper transporter 1). Both systems transport copper in a regulated fashion, irrespective of its origin. Brewer’s notion that “inorganic” copper undergoes a separate pathway and penetrates the brain in an unregulated fashion lacks empirical support.

His statement that there has been no formal chronic toxicity testing on this form of copper (“inorganic,” LMW copper) is not correct. It is beyond the scope of this response to review the wealth of toxicity data on non-Cp copper and LMW copper in drinking water. The task of critically reviewing and generating toxicity data on copper (copper(II) sulfate pentahydrate, copper(I) oxide, copper(II) oxide, and dicopper chloride trihydroxide, all of them truly inorganic) has been accomplished by the European Union (EU) risk assessment of copper (Cross et

al., 2006). In a nutshell, the main conclusions, accepted by the European Commission and EU Member State experts, are:

- (i) The use of copper products is in general safe for Europe’s environment and the health of its citizens; 570
- (ii) the threshold value for acute effects in drinking water is 4.0 mg/l of copper, with the general public typically exposed to 0.7 mg/l. This is consistent with the 2.0 mg/l guideline for copper advised by the World Health Organization. 575
- (iii) For adults, the minimum daily dietary intake is 1 mg, with a maximum threshold of 11 mg. Actual intakes range between 0.6 and 2 mg, suggesting that deficiency may be of concern. 580

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While the geographic scope of this risk assessment was Europe (relevant, for example, for the risk assessments of soils and rivers there), the mammalian and human toxicity data are relevant for public health risk assessment worldwide. The mammalian toxicity database included several U.S. National Toxicology Program (NTP) studies (Hebert, 1993) and drew from all regions of the world, mainly the United States and Europe. 590

In addition to the EU Risk Assessment, we would like to highlight a human trial Brewer did not cite (Kessler et al. 2008a, 2008b). Kessler et al. conducted a prospective, randomized, double-blind, placebo-controlled phase 2 clinical trial involving copper. Patients with mild AD received either copper(II) orotate dihydrate (8 mg copper daily), an LMW copper complex, or placebo for 12 mo. The trial demonstrated that long-term oral intake of 8 mg copper can be excluded as a risk factor for AD, based on its lack of effect on cognition, as well as on the progression of Tau and phospho-Tau levels in the cerebrospinal fluid (CSF). On the contrary, CSF Aβ₄₂ levels (a diagnostic marker for AD) indicated that copper treatment had a positive effect, at least on one relevant AD biomarker (Kessler et al. 2008b). 600

Brewer cites human studies by Squitti et al., also carried out in AD patients (Squitti et al., 2005 2006, 2007). In these studies, patients were not exposed to copper directly. Instead, the researchers investigated serum copper levels and presence of non-Cp copper. They measured total serum copper, and deduced 615

“free copper” (non-Cp copper) values arithmetically by measuring Cp-bound copper and subtracting the value from total copper. There is a scientific consensus (voiced by seven experts in the field in a peer-reviewed article: Danzeisen et al. [2007]) that non-Cp copper determined by arithmetic method is not sensitive to copper status, and that its true value is very difficult to measure. Recent efforts have been made to measure non-Cp bound copper (McMillin et al., 2009), as it may indeed be a useful marker *when measured correctly*. These novel methods involve serum ultrafiltration, an approach not used in the studies by Squitti et al. Cp is an acute-phase protein (Frieden, 1980; Gruys et al., 2005), and any measurement incorporating Cp values will depend on the concurrent inflammatory and immunological status of the host. This makes such data extremely difficult to interpret, especially when obtained from patients who may likely be in an acute-phase state.

Brewer comments on some of the five papers in more detail (Deveau, 2010; Krewski et al., 2010a; Stern, 2010). The paper by Krewski et al. (2010a) explores the utility of an empirical modeling approach to analyze a pre-existing database on copper exposure-response that only included studies published before 2002. The paper recognizes this limitation and does not make any statements regarding an acceptable range of oral intake as defined by this preliminary analysis. The exercise was simply a means to explore the data requirements that would be needed to support such a complex empirical model. Future analyses of the copper database, which has since been updated, acknowledge that the current copper database includes only experiments wherein copper has been assessed alone without other dietary modifications.

Commenting on Deveau (2010), Brewer says that he does not think that “copper in drinking water should ever be thought of as positively contributing to nutritional requirements for copper.” Brewer may have misinterpreted the scope of Deveau’s article. Since drinking water is not typically considered as a source of essential metals in nutritional

assessments, the focus of the study was simply to determine whether certain nutritional groups should indeed consider drinking water as a source of essential metals in dietary exposure assessments. The study was intended to evaluate exposure only, and not to make conclusions about either essentiality or toxicity of these metals.

Regarding the Stern (2010) paper, Brewer disagrees with its conclusion that copper deficiency is a growing public health concern. However, health officials conclude that “available data indicate that humans worldwide are at greater risk of adverse health effects from deficiency of copper intake than from excess intake” (IPCS, 1996; Cross et al., 2006). This is a statement that should not lightly be ignored.

Recent support for these expert conclusions come from a U.S. population-based study assessing total copper intake from diet, drinking water, and air. The findings demonstrate that over 90% of individuals have intakes lower than the copper RDI of approximately 1 mg/d (Georgopoulos et al., 2006). Further, adult-onset copper deficiency-associated cases of irreversible progressive myeloneuropathies and of a range of reversible blood disorders are being reported in the literature with increasing frequency, now that copper status is being assessed more often in the differential diagnosis of neuropathies and unexplained blood abnormalities (Fong et al., 2007; Huff et al., 2007; Kumar et al., 2003; Kumar, 2006; Weihl & Lopate, 2006).

The “special cases” that Brewer cites as impairing copper absorption are bowel surgery and hyperzincemia induced by ingestion of denture creams. Bowel surgery is infrequently associated with acquired copper deficiency. Brewer is perhaps thinking of bariatric surgery or other gastric surgery, procedures that impair copper absorption and contribute to the risk of acquired copper deficiency (Juhasz-Pocsine et al., 2007; Prodan et al., 2009; Thaisetthawatkul, 2008). In the United States alone, the incidence of bariatric surgery is approaching 200,000 cases per year and increasing (Sinha 2008). The number of

715 individuals at risk for acquired copper deficiency and resulting neurological complications is appreciable (Juhász-Pocsine et al., 2007).

Brewer similarly dismisses recent public health concerns about a possibility of hyperzincemia, and secondary copper deficiency, that might result from the overuse of denture cream containing zinc (Nations et al., 2008; Hedera et al., 2009). While this concern must still be viewed as a hypothesis, given that over 35 million Americans currently wear removable dentures (NHANES 2009), even if only a small percentage of these individuals overapply a zinc-based denture cream, and only a fraction of this population develops copper deficiency-associated disorders, the problem could still potentially affect a the large number of individuals. Considering the growing population of post-bariatric surgery patients and denture cream users, adult-onset copper deficiency is al public health concern that merits serious discussion.

At the end of his letter, Brewer suggests a causal relationship between the use of copper plumbing and the prevalence of AD. Although the etiology of AD is not well understood (Heininger, 2000), current evidence suggests that genetic factors play a particularly important role in early-onset AD (Mayeux 2006), including APP, PS-1, and PS-2 genes. The ApoE 4 has been identified as an important predictor of the sporadic form of AD (Meyer et al., 1998). The greatest nongenetic risk factor for AD is advanced age (Alzheimer's Association, 2010). At this point in time we are not aware of any peer-reviewed scientific evidence that supports Brewer's conjecture that the use of copper piping is a risk factor for AD.

We acknowledge Brewer's very valuable contributions to the study of copper nutrition and Wilson's disease. However, the direct link between Wilson's disease and copper exposure cannot be extended to common diseases such as AD. Doing this can cause undue concern and potentially adverse "adjustment" behavior in the public, such as minimizing copper uptake. The public needs to remain aware of copper's essentiality for good health, and of the value of a balanced approach to diet and health.

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