



November 27, 2007

DEC - 5 2007

California Department of General Services, Real Estate Services Division
Professional Services Branch, Environmental Services Division
707 Third Street, Suite 3-400
West Sacramento, CA 95605
Attn: Valerie Namba, Senior Environmental Planner

REAL ESTATE
SERVICES DIVISION

RE: Environmental Impact Report on PEX Tubing

Dear Valerie,

The California Professional Association of Specialty Contractors, Inc. ("CALPASC") is a not-for-profit trade association composed of specialty or trade contractors, material suppliers to those contractors and affiliate members who are all located in California. CALPASC has 550 member companies, representing approximately 75,000 employees.

CALPASC has a large number of plumbing contractors as members. They are very much in support of having the option of utilizing PEX tubing on jobs. There are other trade contractors, such as concrete, framing, drywall, and finish carpenters, who also benefit from and are strongly in support of utilizing PEX tubing as an alternative material.

CALPASC sincerely appreciates the time and effort that you, Dave Walls, and Heather Halsey have taken to allow the public to interact with you on this issue, on a local basis. CALPASC strongly encourages this type of open communication for future issues as well. CALPASC members would like for you and your colleagues to be as educated as possible regarding the benefits of PEX tubing versus copper. Therefore, we have included an Appendix with this letter for your review containing important information regarding copper versus PEX tubing. If you have any additional questions regarding this Appendix, or would like additional information about PEX tubing, please contact Tom Price of Trilogy Plumbing. His phone number is (714) 745-3215. His email is zztomas@aol.com.

Additionally, CALPASC supports the items proposed for the Environmental Impact Report in the initial Notice of Preparation and Initial Study dated October 31, 2007. However, CALPASC respectfully requests the addition of the following items for consideration in that EIR:

1. Reviewing whether PEX tubing should be allowed to be installed under concrete slabs. The only exception might be for PEX tubing being inserted into an ABS sleeve for island sinks. It appears that the consensus of the industry is that PEX tubing should not be installed under slabs.
2. Reviewing the positive aspects of PEX tubing in regards to air quality issues. By substantially reducing the exposure to fires during installation, PEX tubing is good for the environment.

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3. Reviewing the positive aspects of PEX tubing in regards to water quality. If PEX tubing is damaged during installation, it will leak right away, causing minimal water damage to the housing unit and the environment. Other substances such as copper, may not begin to leak for two or three years, causing substantial damage to a finished housing unit, and potential mold problems.

CALPASC thanks you again for your time and thorough review of this matter.

Sincerely,



Dave Louden
CALPASC Director of Government Affairs



Bruce Wick
CALPASC Director of Risk Management

/gwh
Enclosure

Appendix - Copper Tubing Versus PEX Tubing

The reason plumbing contractors and builders are concerned about PEX tubing water piping being adopted are many. Copper tubing, which has been the product of choice for decades, is now developing pinhole leaks and failing at alarming rates. When copper fails, it is not defective; it is reacting to its environment. The water chemistry has changed over the last decade. Much of the change is directly related to EPA mandates to change the disinfectant product from chlorine to chloramine. The Metropolitan Water District (MWD), which supplies over 60% of the water to Southern California, uses chloramines as the exclusive disinfectant product. Chloramines are a mixture of chlorine and ammonia. The unintended consequence of this chemical change is that the ammonia reacts with the copper and causes the copper to develop pinhole leaks. The second source of water in addition to the MWD surface-collected, or reservoir water, is well water. Well water will get more aggressive during droughts because the water is pumped from lower in the well. The well water pH is many times lower than the pH 6.5 which is the level at which the water will no longer be compatible with copper. Often, the well water has an over abundance of dissolved gases such as oxygen or carbon dioxide. According to the Copper Development Association ("CDA"), water with CO₂ content of above 10 mg/liter or oxygen content above 4 mg/liter is not compatible with copper. Many water districts will blend MWD water with well water, giving us the worst of both worlds for copper.

The issue is liability. When copper leaks for whatever reason, the plumbers and builders get involved in a lawsuit with a homeowner or the homeowner's insurer. The CDA has a 50-year warranty on copper for it not to fail, but the exclusions from that warranty are aggressive soil, aggressive water or trade damage. Tom Price, a CALPASC member, states that in his 35 years of plumbing, he has only seen 2 pieces of defective copper. He has also never seen the CDA honor a warranty claim for failed copper. We need alternate materials. Effective January 1, 2008, we will be able to install CPVC plastic water piping, but we would also prefer to use PEX tubing, as it is easier to install and comes in 100 foot rolls which are flexible and will have fewer joints in the walls.

CALPASC suggests that Section 604.11.1 of the EIR standard be amended to include plastic insert fittings as well as metal; or, just remove the word metal. As worded, some building inspectors may read that section as meaning that only metal insert fittings are approved, which is not the case. CALPASC also suggests that the installation of PEX tubing be limited to above the slab only, to negate the permeability issues of the soil. Where PEX tubing is being installed for an island sink, the PEX tubing should be encased in a sleeve of ABS plastic pipe or corrugated flexible area drain piping to protect the PEX tubing from contact with pesticides or petroleum byproducts. This sleeve would also allow the piping to be placed through the sleeve after the slab is poured, eliminating the chance of damage or contamination during the slab pouring process.

PEX tubing is the most cost-effective product for water piping on the market. The cost per house ranges from \$500 to \$1,500 less expensive than copper, depending on the size of the house. PEX tubing is inert and will not react with aggressive water chemistry. PEX tubing is self insulating and does not transfer heat as copper does. PEX tubing is quiet and does not transfer water noise as copper does. PEX tubing is not subject to theft, as it has no value on the scrap market as copper does. There is no torch used on PEX tubing installations which reduces the threat of fire during the installation process.

There is also the health issue of using copper. In 2005 HCD published a 9 page document titled, "SUMMARY OF LITERATURE SEARCH ON COPPER LEACHING INTO DRINKING WATER FROM COPPER PIPE." There were 3 parts to the study:

- A. Known and suspected toxicity of copper to humans and other living organisms.
- B. Pipes leaching copper and other contaminants into the drinking water.
- C. Scientific community recommendations on copper in drinking water.

This study is excellent and should be provided to all homeowners. The study states that,

"It takes 2 to 5 years for copper to cure and form the Patina or Oxidation which is the protective barrier that makes copper last. If the Patina never forms, the copper will fail from the reaction to aggressive water. During the curing process, raw copper is leaching into the water systems. When copper is in drinking water, it can reach the blood stream within 15 minutes as water permeates through the stomach lining quickly. The Public Health Goal ("PHG") for copper content in drinking water is .17 mg/liter. Having said that, a very small percentage of drinking water ever meets the PHG."

The action level set by the EPA for copper content is 1.3 mg/liter. At the action level or above, a person drinking such water will get sick. The plumbing contractor members of CALPASC conducted a test of approximately 400 new homes that were 1 year old or newer. Homes from San Diego to Los Angeles were tested. Twenty percent of those new homes had copper levels in the drinking water that exceeded the action level. Over 95% of the homes exceeded the PHG. When water districts test their water to produce the Annual Water Quality Reports each year, they do not test the water at the homes to see water's reaction to copper or copper's reaction to water. They test at the well pump heads. There is no copper in the water system at that point. The purpose of explaining this is that Americans and Californians are needlessly being exposed to dangerous levels of copper contamination from copper water piping in their homes. Nobody is testing for it. As you will see from the HCD's 9-page document referenced above, the health risks are great and many researchers state that copper content in water may well be the cause of Alzheimer's Disease, Autism, ADD and many illnesses.

SUMMARY OF LITERATURE SEARCH ON COPPER LEACHING INTO DRINKING WATER FROM COPPER PIPE

The following summary has three components:

- A. Known and Suspected Toxicity of Copper to Humans and Other Living Organism.
- B. Pipes Leaching Copper and Other Contaminants Into The Drinking Water.
- C. Scientific Community Recommendations on Copper in Drinking Water.

A Known and Suspected Toxicity of Copper to Humans and Other Living Organism

1. From CalEPA's Office of Environmental Health Hazard Assessment:

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to adopt Public Health Goal (PHGs) for contaminants in drinking water based exclusively on public health considerations. The PHG technical support document provides information on health effects from contaminants in drinking water. The PHG describes concentrations of contaminants at which adverse health effects would not be expected to occur, even over a lifetime of exposure. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime:

A PHG of 170 ppb has been developed for copper in drinking water.

Copper does not appear to be carcinogenic in animals or humans, therefore the PHG is based on noncarcinogenic effects. The PHG is based on gastrointestinal effects in children, the sensitive group for this chemical. In one case report of a Vermont family that consumed drinking water with a copper concentration of 7.8 mg/L, a seven-year-old girl experienced abdominal pain and a five-year-old girl experienced episodes of vomiting and abdominal pain after drinking the water. To calculate the lowest-observed-adverse-effect-level (LOAEL) the water consumption of the two girls was estimated at one liter per day. An uncertainty factor of 10 was employed to extrapolate from an LOAEL to a no-observed-adverse-effect-level (NOAEL), and a relative source contribution of 80% was assumed. Based on these assumptions, OEHHA calculates a PHG of 0.17 mg/L (170 ppb) for copper in drinking water.

2. The following health and environmental problems have been identified by various health organizations and groups as potential toxic problems associated with copper in drinking water. The below comes from the Environmental Defense and have hyperlinks attached.

| <u>Human Health Hazards</u> | <u>Reference(s)</u> |
|---|-----------------------------------|
| <u>Cardiovascular or Blood Toxicant</u> | <u>KLAA</u> |
| <u>Developmental Toxicant</u> | <u>EPA-SARA</u> |
| <u>Gastrointestinal or Liver Toxicant</u> | <u>ATSDR DOSS KLAA RTECS ZIMM</u> |
| <u>Kidney Toxicant</u> | <u>MERCK</u> |
| <u>Reproductive Toxicant</u> | <u>EPA-SARA FRAZIER</u> |
| <u>Respiratory Toxicant</u> | |

3. The National Research Council (NRC)¹ stated that the current health level established by U.S. EPA for Cu is for acute exposure and is not suitable for establishing a health base level for MCLG (maximum contaminant level goal). The need to develop a chronic exposure level is necessary and will be much lower concentration.
4. The average absorption of Cu by the body is controlled by the liver function and is 30% to 40% and is influenced by age and genetic background. (see NRC 2000 Report)
5. Infants fed formula with tap water are much more sensitive to elevated copper in water because they have a higher absorption rate and reduced capacity to excrete copper as those of an older age. (see NRC 2000 Report)
6. There is the potential role of genetics that underlie infant and childhood copper toxicosis. (see NRC 2000 Report)
7. Evidence suggests that when the bodies ability to regulate Cu is surpassed by excess Cu, a large amount of Cu is released into the bloodstream damaging red blood cells and causing acute hemolytic anemia. (see NRC 2000 Report)
8. Because reproductive and development effects are affected by small amounts of Cu intrauterine devices by preventing embryogenesis by blocking implantation and blastocyst development that Cu exposure during the early postnatal period requires additional study to determine teratogenicity during pregnancy. (see NRC 2000 Report)
9. The ingestion of Cu should be cautioned against because of the possibility of the hepatic (liver) susceptibility. (see NRC 2000 Report)

¹ At the direction of Congress, U.S. EPA asked the National Research Council (NRC) to review independently the scientific and technical basis for U.S. EPA's health level for copper in drinking water. The Committee members were from the fields of toxicology, epidemiology, pathology, pharmacology, genetics, physiology, medicine, public health, exposure assessment, nutrition, chemistry, biostatistics, and risk assessment. The Committee reviewed available toxicological, epidemiological, and exposure data and made specific recommendations in their 2000 published report titled "Copper in Drinking Water," Committee on Copper in Drinking Water, Board of Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council.

10. There is an association between liver toxicity and copper in sensitive population (Wilson Disease-2% of population). (see NRC 2000 Report)
11. A Wisconsin Department of Health and Social Service's Division of Health study stated Health studies have found that copper in drinking water can add 4 to 45 percent more copper to a person's diet than what is in food sources.
12. In sensitive human populations, the majority target of chronic copper toxicity is the liver and neurological toxicity with those with Wilson disease. (see NRC 2000 Report)
13. The liver and brain are targets of copper toxicity in patients with Wilson disease. (see NRC 2000 Report)
14. Excess digestion of copper in drinking water can cause nausea, diarrhea, vomiting, and intestinal cramps. Severe cases of copper poisoning have led to anemia and to the disruption of liver and kidney functions. They also stated that individuals with Wilson's and Menke's disease (genetic disorders resulting in abnormal copper absorption and metabolism) are at a higher risk from copper exposure than the general public and can have serious health problems. (see NRC 2000 Report)
15. Chronic exposure to excess copper causes liver toxicity and a number of chronic cases of liver toxicity have been reported. (see NRC 2000 Report)
16. Dr Lewis Mehl-Madrona, M.D., Ph.D, Program Director, Center for Health and Healing, Beth Israel Hospital/Albert Einstein School of Medicine reported that studies suggest that environmental factors associated with learning disabilities such as Attention-Deficit/Hyperactivity Disorder (ADHA) have found correlations between certain toxic agents such as copper accumulating in brain tissue.
17. Dr. William Walsh, Ph.D., Co-Founder and Senior Scientist for "The Health Institute and Pfeiffer Treatment Center" suggested that studies point to a potential correlation for Autism Disorder and copper injustice that could impair neuronal development, especially in the first 30 months of life, which could result in incomplete maturation of the G.I. track and brain.
18. Research is being carried out by Ashley Bush, Harvard Medical School and the University of Melbourne and the PRANA Biotech School Melbourne that studies the a link of copper accumulation in the brain that causes a buildup of hydrogen peroxide which induces amyloid plaques in the brain i.e., Alzheimer's disease.
19. An old antibiotic, Clioquinoline, is now being tested on 50 Alzheimer's patients according to Dr. Ashley Bush of Massachusetts General Hospital and Harvard Medical School. The drug was effective in mice experiments because it removed copper and zinc in amyloid plaques in the brain that are a major feature of Alzheimer's. There was a 51 percent reduction in the plaques in the mice and the hope is that for humans it will aid the brain to "heal" itself to "clear out the mess" causing Alzheimer's.
20. In 1992 a 6 week old girl was diagnosed with methemoglobinemia induced by simultaneous exposures of copper at levels close to the federal drinking water standards. The study stated that drinking water that stands overnight in copper pipes may often contain copper levels that exceed federal drinking water standards and that the water should be flushed prior to drinking water that stands overnight in copper pipes according to the investigation by the Wisconsin Department of Health and Social Service's Division of Health.
21. Two Material Safety Data Sheet states that copper may cause anemia and other blood cell abnormalities and copper accumulates in various tissues and may result

- in liver, kidney, and brain damage. It has also been reported that copper poisoning has lead to hemolytic anemia and accelerates arteriosclerosis.
22. The Medical Toxicology Unit from Guy's and St. Thomas' Hospital stated that "Chronic poisoning with copper leads to gross hepatic copper overload with severe liver disease in young children. Indian childhood cirrhosis have reports of poisoning in young children as a result of high copper content in well water
 23. The NRDC cited a study (Sidhu, K 1995²) in its report that recommended decreasing the federal maximum allowed amount of Cu in drinking water in order to adequately protect children³. This study found that infants and children up to ten years of age have greater sensitivity due to the presence of normally high concentrations of copper in the liver during early life and the lack of a fully developed physiological mechanism for regulating levels of copper in the body. One study, recognizing this difference, recommended decreasing the federal maximum allowed amount of Cu in drinking water in order to adequately protect children.¹⁰²
 24. In a report by the International Programme of Chemical Safety titled "Environmental Health Criteria 200 Copper the following findings were made:
 - (1) Ingestion of excess copper is infrequent in humans and is usually a consequence of the contamination of beverages (including drinking-water) or from accidental or deliberate ingestion of high quantities of copper salts. Effects which occur at lowest levels are those on the gastrointestinal tract; for example, nausea, vomiting and diarrhoea. Doses which induce such effects have not been well characterized and confounders such as microbiological quality of water supplies or other potential causes of the symptoms have not been adequately considered. On the basis of available data, gastrointestinal illness appears to be associated with consumption of drinking-water containing several mg/litre of copper, but it is not possible to provide a precise number. Symptoms disappear following a change of water supply.
 - (2) Human health risks are risks associated with low intakes as well as high intakes of essential elements. The relationship between intake/exposure level and risk therefore has a U-shaped curve, wit risks from deficiency at low intakes and risk of toxicity at high intakes. There is a need to define an intake range that prevents both deficiency and toxicity for the general population. The range of acceptable intakes to meet the biological requirement, as well as prevent risk of toxicity, may be extremely narrow. A balanced and comparable scientific approach to assess risk from deficit as well as excess is needed when evaluating essential elements such as copper.
 - (3) When copper homeostatic control is defective and/or copper intake is excessive, copper toxicity may occur.
 - (4) People with Menkes and Wilson disease are at risk with copper contained drinking water.
 - (5) Menkes disease is an X-linked recessive disorder of copper metabolism that occurs in approximately 1 in 200 000 live births. Clinically the condition resembles a copper deficiency state and is characterized by skeletal abnormalities, severe mental

² Sidhu, K. et al., "Need to Revise the National Drinking Water Regulation for Copper." Regulatory Toxicology and Pharmacology 22, August 1995, pp. 95-100.

³ From NRDC's "Toxic Chemicals & Health: Kids' Health. In Depth: Report: Our Children At Risk: The 5 Worst Environmental Threats To Their Health. Chapter 7: Drinking Water Contamination.

retardation, neurological degeneration and death in early childhood. The symptoms of Menkes disease result from a deficiency of copper and its effects on the function of copper-dependent enzymes.

- (6) Wilson disease is the most extensively described inherited disorder of copper metabolism. The gene is distributed worldwide, having been demonstrated in virtually all races. Current global estimates indicate that the incidence rate of the disease is approximately 1 in 30 000 live births, with prevalency ranging from 15 to 30 per million. The gene frequency varies between 0.3 and 0.7%, corresponding to a h Copper (EHC 200, 1998)
- (7) Idiopathic copper toxicosis, or non-Indian childhood cirrhosis Scattered reports of early childhood cirrhosis similar to ICC, referred to as copper-associated idiopathic copper toxicosis (ICT) have appeared from some Western countries (Walker-Smith & Blomfield, 1973; Müller-Höcker et al., 1987; Adamson et al., 1992; Gormally et al., 1994). Copper (EHC 200, 1998)
- (8) In England a correlation study, with measurements made after diagnosis of coronary heart disease, has shown higher serum copper levels in cardiovascular disease patients (Punsar et al., 1975).
- (9) Available data in humans and animals are inadequate to assess the reproductive/developmental effects of copper compounds on humans..
- (10) Bioaccumulation of copper by microorganisms, plants or animals from their surrounding environment can be adverse and must be studied further.
- (11) Copper exhibits significant toxicity to some aquatic organisms, although the degree of toxicity is highly variable and the bioavailability of copper dictates its toxicity to a large extent.

B. Copper Water Pipes Leaching Copper and Other Contaminants Into The Drinking Water

1. The California Department of Health Services (DHS), Drinking Water Program, has a Water Quality CD available which is all of the historical drinking water data beginning in the late 1980's through the present. The data is the mandated sampling, testing and reporting of drinking water by water systems, primarily large water systems (more than 200 connections), drawn at designated delivery/sample locations before delivery to households. DHS was unaware of any governmental agency that had data on water samples taken from the tap in households.
2. According to the U.S. EPA⁴ and the National Research Council (NRC), metallic copper is unstable and subject to corrosion when in contact with water and it is a "mistake to" assume that copper metal (Cu) and its alloys "do not leach into the drinking water." This includes water termed non-corrosive or water treated to make it less corrosive. Copper occurs in drinking water primarily due to its use in plumbing materials. Copper leaching continues from installation until about 10 year of service.
3. A review of the Associated Laboratories data developed from July 17 through July 25, 2002 at the Murrieta Ranchos Development in the City of Murrieta, California showed copper concentrations from 22 homes ranging from a low of

⁴ USEPA Ground Water and Drinking Water Technical Fact Sheet on Copper.

- 146 ppb to a high of 2,400 ppb. The Public Health Goal for copper in drinking water as established by CalEPA's Office of Environmental Health Hazard Assessments is 170 ppb. 21 of these homes have exceeded this PHG level. The average pH of the water tested was 7.4 which is slightly basic not acidic.
4. A 1975 AWWA Journal reported that corrosion of household Cu plumbing
 5. Several states have measured CU concentrations in drinking water from Cu pipes that exceeds U.S. EPA MCLG levels. (See NRC 2000 Report)
 6. Water softeners using ion exchange are likely to have increase Cu contamination levels in the drinking water. (See NRC 2000 Report)
was a major source of Cu metal contamination in U.S. drinking water.
 7. The Agency for Toxic Substances and Disease Registry (ATSDR)⁵ created the "Public Health Statement for Copper" which states that you may be exposed to high levels of soluble copper in your drinking water, especially if your water is corrosive and you have copper plumbing and brass water fixtures. The average concentration of copper in tap water ranges from 20 to 75 parts per billion (ppb). However, many households have copper concentrations of over 1,000 ppb (near the upper limit of U.S. EPA's Maximum Contaminant Level. This is because copper is picked up from copper pipes and brass faucets when the water sits in the pipes overnight. After the water is allowed to run for 15-30 seconds, the concentration of copper in the water decreases.
 8. Copper pollution in the Town of Discovery Bay, California has lead to a NPDES permit issued by the California Regional Water Quality Control Board requiring the removal of Cu from the water supply through a "Pollution Prevention Plan." The major cause of copper pollution is from the use of water softeners that are installed on copper piping systems in homes of Discovery Bay. (see letter from Virgil Koehne, General Manager of the Town of discovery Bay, August 22, 2003.)
 9. In February 1997, the Office of Drinking Water for the U.S. EPA Environmental Criteria and Assessment Office reported that a majority of copper present in drinking water appeared to come from copper pipes and they were unable to estimate the number of individuals who regularly consume water that exceed safe MCL (Maximum Contaminant Level) levels for copper.
 10. An article published a recent issue of the Wisconsin Medical Journal detailed two separate cases in Wisconsin reported that ingestion of copper-contaminated drinking water resulted in numerous reports of nausea, vomiting and abdominal discomfort because of new copper piping systems. Samples analyzed showed copper level exceeding Federal MCL levels. In the following weeks, 251 families submitted first drawn water samples (after sitting overnight) and 48 had copper levels that exceeded federal limits. The homes were built in the past 10 years had the highest copper levels in the water.
 11. Washington State Department of Health stated that most Cu in drinking water comes from household plumbing and that copper contamination can accumulate overnight (called "first flush") and recommends that households flush their water before use for the first 30 to 45 seconds.

⁵ ATSDR is an agency of the U.S. Department of Health and Human Services whose purpose is to serve the public by using the best science, taking responsive public health actions, and providing trusted health information to prevent harmful exposures and disease related to toxic substances.

12. A Wisconsin Department of Health and Social Service's Division of Health study stated that "first flush" drinking water in Cu pipes often contain Cu levels that exceed federal drinking water standards and should be flushed prior to drinking.
13. Each year thousands of pounds of copper enter the San Francisco Bay, and the accumulation is harming aquatic life. Research shows that corrosion from newly installed copper pipes is 5 times higher than that from older systems. A Copper Action Plan for the NPDES permit for Palo Alto whose receiving waters are the San Francisco Bay estimated in 2002 that corrosion accounts for 60% of the estimated Cu sources. The Regional Water Quality Control Plant's discharge permit (Order No. 00-109) requires outreach to plumbers and designers to reduce corrosion of copper pipe via better design and installation.
14. Test carried out in Victoria in 1976 by the State Water Supply commission indicated that fluoride is involved in the corrosion of the copper pipes, which causes more leaching into the water. Leaving fluorinated water standing in the copper pipes for longer periods of times allows for more corrosion. Countries such as Switzerland, Belgium, Holland, Germany, and Sweden have terminated the use of fluoride due to its potential health hazard.
15. The presence of acidic water in household plumbing systems resulted in levels of copper or zinc up to 9,000 ppb being recorded in water from cold taps, and up to 22,500 ppb from hot taps. (IRC 2000, Section R324)
16. In the City of Highland, the Richmond Creek subdivision experienced 175-200 pin hole leaks in the 67 homes (Letter to Department from Richmond American Homes of California, March 1, 1993)
17. The initiation of pits in copper water tubing has been correlated to carbon films left on the surface during manufacturing, flux residues from soldering, debris left in tubes during installation and water chemistry parameters (Chester Neff, P.E. Chemist, July 31, 1991 Letter to DEC Consultants in San Diego)

C. Scientific Community Recommendations on Copper in Drinking Water

1. Office of Environmental Health Hazard Assessment (OEHHA) established in 1997 the current Public Health Goal of 0.17 mg/L (ppb) for copper which was based on acute gastrointestinal effects in children. OEHHA is currently reviewing the 1997 standard and staff has suggested that a target level of copper in drinking water, measured at the tap, should be in the range of 0.1 to 0.3 mg/L (ppb), i.e., at about the level of the current PHG.
2. Given the potential risk for toxicity in humans, quantification of copper toxicity should be undertaken and the MCLG for copper be re-evaluated. NRC stated the current health level established by U.S. EPA for copper is based on acute exposure to copper and is not suitable for establishing a MCLG (maximum contaminant level goal). A chronic exposure level is necessary for the MCLG.
3. The National Research Council (NRC)⁶ stated that because reproductive and development effects are affected by small amounts of Cu intrauterine devices by preventing embryogenesis by blocking implantation and blastocyst development that Cu exposure during the early postnatal period requires additional study to determine teratogenicity during pregnancy. The reproductive and development effects of excess copper is not well known other than small amounts of copper

⁶ See footnote #1

from intrauterine devices can prevent embryogenesis by blocking implantation and blastocyst development. The committee commended that copper exposure during the early postnatal period requires additional study to determine teratogenicity during pregnancy. (see NRC 2000 report)

4. The NRC recommended that increase in the ingestion of Cu should be cautioned against until the hepatic (liver) susceptibility is clearly identified.
5. The NRC recommended that studies be conducted to characterize the potential role of genetics that underlie infant and childhood copper toxicosis.
6. The NRDC cited a study (Sidhu, K 1995⁷) in its report⁸ that recommended decreasing the federal maximum allowed amount of Cu in drinking water in order to adequately protect children. This study found that infants and children up to ten years of age have greater sensitivity due to the presence of normally high concentrations of copper in the liver during early life and the lack of a fully developed physiological mechanism for regulating levels of copper in the body. One study, recognizing this difference, recommended decreasing the federal maximum allowed amount of Cu in drinking water in order to adequately protect children.¹²
7. The Agency for Toxic Substances and Disease Registry⁹ which created the "Public Health Statement for Copper" stated that because many households have copper concentrations of "first drawn" water over 1,000 ppb (near the upper limit of U.S. EPA's Maximum Contaminant Level that after the water is allowed to run for 15-30 seconds, the concentration of copper in the water decreases.
8. A Wisconsin Department of Health and Social Service's Division of Health study stated that drinking water that stands overnight in copper pipes may often contain copper levels that exceed federal drinking water standards and that the water should be flushed prior to drinking water that stands overnight in copper pipes according to the investigation.
9. A Washington State Department of Health and Social Division of Health Study stated that most copper in drinking water comes from household plumbing and that copper contamination can accumulate overnight (called "first flush") and recommends that households flush their water before use for the first 30 to 45 seconds.
10. The Nebraska Health and Human Services recommends flushing household water supply prior to use if it has stood in pipes for six hours or more and this water should not be used for drinking or cooking. If you live in an apartment complex, flushing may not be as effective for reducing copper levels. Water from the hot water tap shouldn't be used for drinking or cooking. If the results of water testing show elevated copper levels, flushing may not be adequate for children or infants and an alternate source of water may be needed.
11. The Regional Water Quality Control Plant's discharge permit (order No. 00-109) requires outreach to plumbers and designers to reduce corrosion of copper pipe.
12. Known Copper pollution in the Town of Discovery Bay, in Danville, CA that has a California Regional Water Quality Control Board NPDES permit with a

⁷ Sidhu, K. et al., "Need to Revise the National Drinking Water Regulation for Copper," *Regulatory Toxicology and Pharmacology* 22, August 1995, pp. 95-100.

⁸ From NRDC's "Toxic Chemicals & Health: Kids' Health: In Depth. Report: Our Children At Risk: The 5 Worst Environmental Threats To Their Health; Chapter 7: Drinking Water Contamination.

⁹ ATSDR is an agency of the U.S. Department of Health and Human Services whose purpose is to serve the public by using the best science, taking responsive public health actions, and providing trusted health information to prevent harmful exposures and disease related to toxic substances.

requirement for a Pollution Prevention Plan to remove copper from the water supply. The recommendation will be to replace the copper pipe for new homes with alternative plastic pipe.

13. The NRC recommended genetic animal models be used to determine the associations between liver toxicity and Cu in sensitive population (Wilson Disease-2% of population).
14. The NRC recommended that epidemiological studies of population who have been chronically exposed to elevated copper should be carried out to determine the nature and frequency of chronic effects, especially in sensitive populations.
15. Determine the bioavailability of dietary copper, particularly in vegetarian diets. Copper (EHC 200, 1998)
16. In human populations develop the methodology for identifying adverse effects of marginal copper deficiency and of intakes in excess of recommended levels. This should include an evaluation of stable isotope technology to define bioavailability and body stores of copper. Copper (EHC 200, 1998)
17. Determine the concentrations of copper and the other quality parameters of drinking-water that produce toxicity from single and chronic exposures (e.g. gastrointestinal effects). Copper (EHC 200, 1998)
18. Characterize the mechanisms that influence copper homeostasis including placental transfer of copper. Copper (EHC 200, 1998)

JUL 0 2004

~~STATE OF CALIFORNIA - BUSINESS, TRANSPORTATION AND HOUSING AGENCY~~

ARNOLD SCHWARZENEGGER, Governor

**DEPARTMENT OF HOUSING AND COMMUNITY DEVELOPMENT
OFFICE OF THE DIRECTOR**

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June 28, 2004

Mr. James Kanell, President
Casa Plumbing, Inc.
856 North Elm Street, Suite L
Orange, CA 92867

Dear Mr. Kanell:

Public Petition – HCD-P-1/04

Petition to the California Plumbing Code, Section 604.1.2,
Statewide Use of CPVC Water Pipe within All Residential Structures.

On April 27, 2004 the California Building Standards Commission (Commission) made a finding that your petition, identification number HCD-P-1/04, as submitted is in compliance with California Code of Regulation, Title 24, Part 1, Article 1-8 and is complete (henceforth all sections are referenced to this cite unless otherwise noted). Pursuant to Section 1-805(a) the Department of Housing and Community Development (Department) concurs with the Commission's April 27 finding.

Pursuant to the duties specified in Section 1-805, the Department is required to inform the petitioner in writing that it rejects, accepts, or approves the petition in part and may grant such other relief or take such other action as it may determine to be warranted by the petition. Because the petition HCD-P-1/04 includes an emergency clause, the Department is also required under Section 1-805(b) (4) to rule on the reasons posed for the emergency clause, and if it concurs that an emergency exists, the Department must schedule code development and adoption procedures on an emergency basis.

Petition HCD-P-1/04 consists of two parts. The first part petitions the Department to amend Section 604.1.2 of the California Plumbing Code (CPC), as an emergency rulemaking, to allow the use of chlorinated polyvinyl chloride (CPVC) in residential structures on a statewide basis with the continued requirement for worker safety and flushing requirements. The second part requests the Department to investigate the health effects associated with the leaching of copper in drinking water from copper water pipe.

The Department is authorized under Health and Safety Code Section 17921 to propose for adoption into the California Plumbing Code CPVC amendments that the Department finds necessary for the protection of the public health, safety, and general welfare of the occupants of

Mr. James Kanell

Page 2

residential structures. The Department has reviewed the information provided in the petition in conjunction with its own independent literature study and accepts the petition in part. The Department will propose regulations to discontinue Section 604.1.2(a) *Finding Required*, of the California Plumbing Code, consistent with the petition's request.

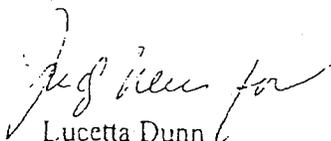
However, you are aware there exists a mitigated negative declaration concerning the limited use of CPVC in California. In addition, there is a current court order directing the Department and the Commission to comply with CEQA prior to the approval of CPVC. Therefore, prior to HCD's submission of any proposed expansion of the use of CPVC, the Department must initiate and complete the CEQA process. It is our intention to begin work on the initial study in July 2004. No final decisions can be made prior to the consideration of all relevant information during both the CEQA and rulemaking processes.

The Department's standard for proposing an emergency building standard to the Commission for their adoption is provided in Health and Safety Code Section 18937 which requires specific findings to be made pursuant to Government Code Section 11346.1. The specific findings required to be made by the Department are specific facts that "the adoption of a regulation..." is "necessary for the immediate preservation of the public peace, health and safety or general welfare."

The Department denies the emergency clause in the petition for cause. The Department relies on the national testing processes for the selection of codes and does not have the staffing, expertise, or the finances to verify the facts presented in the petition regarding the necessity for emergency. The Department relies on approved national testing laboratories such as NSF, ANSI, and ASTM, to provide approvals on various products. Currently copper is an approved material in all national codes as well as the California Plumbing Code and has been approved to meet the minimum standards for health and safety. Therefore, the Department denies the clause in the petition for an emergency rulemaking based on the California Building Standards Administrative Code, Section I-806(c) because the issue of testing materials or products for health safety is not within the Department's jurisdiction. Additionally, for all the reasons listed here the Department will not move forward with a Departmental investigation on the health effects of copper leaching in drinking water from copper pipes as requested in the second part of the petition.

If you have questions, you may call Bill Staack of my staff at 323-7288.

Sincerely,


Lucetta Dunn
Director

cc. Tom Morrisson, California Building Standards Commission
Norm Sorensen, HCD
Jim McGowan, HCD
Chris Anderson, HCD
Dave Walls, HCD
Rich Friedman, HCD
Bill Staack, HCD

Did you know?

- The method of water disinfection has changed significantly in the last few years.
- Aggressive water has caused the failure of copper plumbing systems across the country in alarming rates.
- Trace amounts of copper in your water supply have been potentially linked with health-related issues.

WHAT
Drinking Water Symposium

DATE
January 12, 2005

TIME
9am – noon

LOCATION
Atrium Hotel
18700 MacArthur Blvd
Irvine, CA (next to the airport)

CALPASC

ORANGE COUNTY / INLAND EMPIRE

Drinking Water Symposium

An educational discussion on the negative health effects that copper plumbing pipes can have on your health.

Welcome to the Drinking Water Symposium sponsored by CalPASC.

The residents of California do not have a choice when it comes to plumbing pipe. It is estimated that residents spend over \$100 million annually due to the restrictions placed on plastic plumbing pipe. This estimate is based on the number of new home starts per year, the material and labor savings of using a non-metallic piping material, and the elimination of repiping due to failed copper plumbing systems. This estimate does not include the cost of associated property damage, lower property value due to disclosure process, increases to insurance premiums and personal inconvenience.

More importantly, the method of water disinfection has changed significantly in the last few years. Aggressive water has caused the failure of copper plumbing systems across the country in alarming rates. And, the trace amounts of copper in the water supply can potentially be linked with health-related issues.

California is the only state in the U.S. that promotes the use of copper piping when installing a plumbing system by restricting the use of plastic piping alternatives. This symposium will begin with a discussion on how and why copper is getting into our water supply. This discussion will be followed by information linking even trace amounts of copper in the water supply to various health issues.

Featured speaker Marc Edwards, Ph.D. from Virginia Polytechnic Institute and State University will discuss water chemistry and its link to corrosion and pinhole leaks in copper pipes. Larry Sparks, Ph.D. from Sun Health Research Institute will discuss the negative effects of trace amounts of copper in drinking water and its link to Alzheimer's disease.

In April 2004, Casa Plumbing submitted a petition to the State of California documenting these issues and requesting the State to remove the restriction that local code bodies must approve the use of Chlorinated Polyvinyl Chloride (CPVC) pipe based on findings. As of today in the State of California, PEX plumbing material remains in a lengthy court battle and, while included in the California Plumbing Code, CPVC plumbing pipe remains constrained with costly and cumbersome restrictions. You can read more about the status of alternative piping material in this handout in the section titled State of California Update.

If at the conclusion of this symposium, you are motivated by the information presented and would like to elevate this issue to the Buildings Standards Commission, please consider signing the enclosed letter addressed to Fred Aguiar, Agency Secretary, State and Consumer Service Agency of the California Building Standards Commission.

Thank you for your attendance.

Sincerely,

Tom Price
Former Plumbing Contractor

Meet our Speakers:

Marc Edwards, Ph.D.
Professor of Civil and Environmental Engineering
Virginia Polytechnic Institute and State University (VA Tech)

Marc Edwards, Ph.D., is the Professor of Civil and Environmental Engineering at the Virginia Polytechnic Institute and State University (VA Tech) where he and his research group are currently emphasizing research on internal corrosion processes in home plumbing. This work is supported by the National Science Foundation (NSF), individual water utilities and homeowners' groups, the AWWA (American Water Works Association) Research Foundation, and the Copper Development Association (CDA).

Dr. Edwards has published more than 70 peer-reviewed journal articles, made more than 100 national and international conference presentations, and delivered four key note addresses. In both 1994 and 1995, Edwards received the Outstanding Paper Award in the Journal of American Water Works Association. Since 1995, undergraduate and graduate students advised by Dr. Edwards have won 14 nationally recognized awards for their research work on corrosion and water treatment.

The NSF and the White House honored him in 1996 with an NSF Presidential Faculty Fellowship, an award that is given to only 20 professors annually. In 2003, Edwards was awarded the Walter Huber Research prize from the American Society of Civil Engineers. He is currently President of the Association of Environmental Engineering and Science and Science Professors.

Drinking Water Symposium

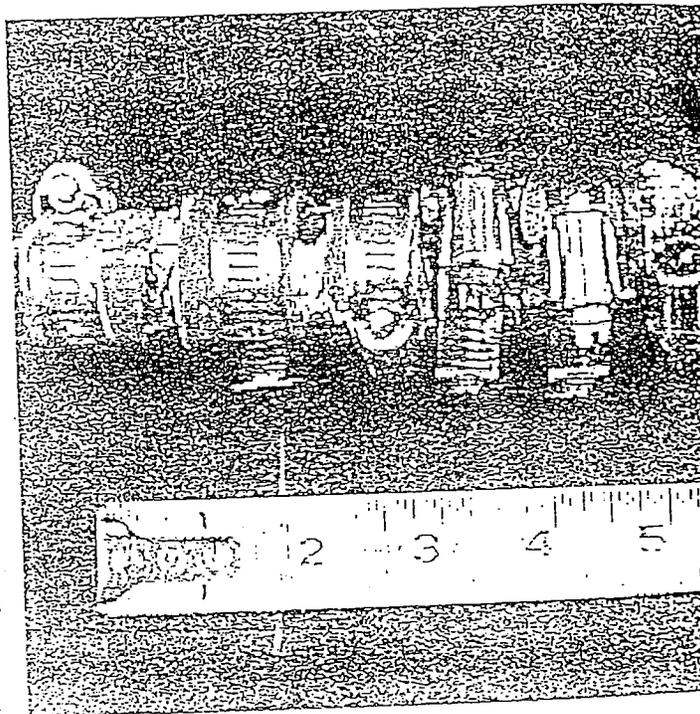
An educational discussion on
the negative health effects that
copper plumbing pipes can have
on your health.

New Research Pinholes in

A noted researcher reports that he has made a breakthrough in addressing an ongoing corrosion problem.

MATTHEW V. VEAZEY, STAFF WRITER

Could the infamous pinhole puzzle be solved? Regular *MP* readers may recall a December 2002 feature article discussing the problem of pinhole leaks forming in copper tubing in residential water distribution systems.¹ The article states that the leaks would form spontaneously in homes and apartment buildings, surprising unsuspecting residents and in many cases leading to destructive and expensive results. Some experts have hypothesized that the leaks, which became prevalent in the suburban Washington, D.C., area and elsewhere in the late 1990s and earlier this decade, were caused by tubing defects. Others have blamed stray current, lightning, or microbiologically influenced corrosion (MIC). The previous *MP* article focused on a third explanation: changes in the U.S. Environmental Protection Agency's (EPA's) water treatment rules, specifically the "Lead and Copper Rule [LCR]" calling for lower levels of natural organic matter (NOM), lead, and copper in drinking water.² Those espousing this school of thought cited earlier laboratory experiments demonstrating that NOM acts as an anodic inhibitor to copper and that metals corrode very differently in waters with NOM than in those without it. The main drawback to this theory, however, was that there was no known case in which anyone had replicated the copper pitting



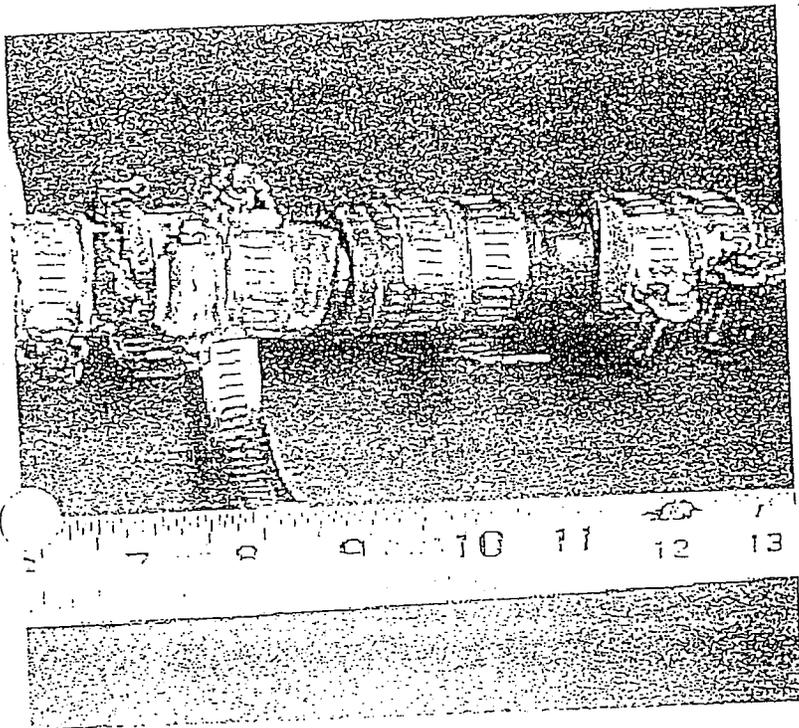
phenomenon as it actually occurs. The corrosion engineer interviewed in the article has informed *MP* that this barrier has been overcome.

A Turning Point

This was the first experiment to replicate pitting of copper to be in the U.S. and as it occurs in practice," says Marc Edwards, professor of Civil and Environmental Engineering at the Virginia Polytechnic Institute and State University (Virginia Tech) (Blacksburg, Virginia). "During the test, eight holes were formed in a 1-ft [0.3-m] section of pipe in just 1 year using a potable water chemistry that anyone can synthesize in the laboratory. The solution was based on the water chemistry associated with outbreaks of copper pitting that have occurred in Maryland, Washington, D.C., and other areas of the country."

Edwards explains that the experiment showed that the water chemistry produced partly as a result of the LCR—a combination of aluminum-free chlorine and pH greater than 8.0—minimize

May Explain Copper Tubing



This copper pipe, from the D.C. WASA system, is largely hidden by clamps placed over individual pinhole leaks. There have been cases in which such leaks have occurred at a frequency greater than one per inch of copper tube, and hundreds of leaks have occurred in individual buildings. New research from Virginia Tech concludes that aggressive water, partly an effect of federal water quality regulations, causes this problem. Photo courtesy of Marc Edwards, Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, Virginia.

pitting. "We have proven that the combination of higher pH, low organic matter, aluminum, and free chlorine in water can cause pinhole leaks of copper," he says. In addition, the research found that aluminum in water catalyzed the cathodic reaction between copper and chlorine after being deposited on the copper surface. "This is a major driver of the copper pitting attack," he says, adding that the findings were confirmed in a subsequent third-party study funded by the Copper Development Association (CDA) (New York, New York). It should be noted that previous studies have identified aluminum (in hot water) as the cause of pitting in copper.

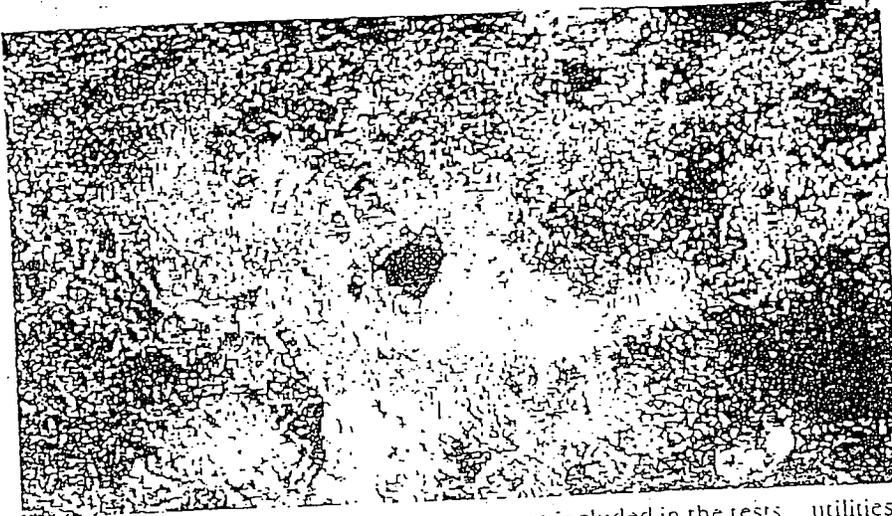
Edwards also suspects that using chloramines instead of free chlorine with elevated pH, low NOM, and aluminum can cause copper pitting as well, he cautions, however, that this has not yet been confirmed. Chloramines are produced by combining chlorine and ammonia (NH_3). A weaker yet more stable than chlorine, chloramines re-

than chlorine; the disinfectants thus enhance protection from bacteria, viruses, and other organisms. Because they are not as reactive to organic material in water as chlorine, chloramines produce substantially lower concentrations of disinfection byproducts in the distribution system. "Chloramines are less reactive with organic matter and more persis-

tent—two characteristics that are presently deemed highly desirable in the context of modern disinfection," says Edwards.

According to Edwards, the study proves that water alone can be the cause of pinhole leaks. "Our experiments used copper tube as purchased, as well as copper tube that had been polished to a mirror-like finish to remove all surface contaminants," he says. The composition of the water used in the laboratory was based on the makeup of treated water Edwards and his colleagues collected from areas that were experiencing pitting problems. "[The chlorine and aluminum levels] were only 25 to 50% higher than measured directly in field testing," explains Edwards. "Given the fact that outbreaks of pitting have occurred in many areas when this combination of water factors is present, we strongly feel these factors were already strongly implicated based on the sampling data—even before they were proven with certainty in the lab."

Edwards adds that it is important to consider



This photo shows a MIC-induced through-wall pit in copper pipe on a solar panel. Photo courtesy of Paula Scott, CARIAD Consultants, Heraklion, Greece.

the elements that were not included in the tests. "Other factors thought by some to cause pitting—including flux, joints, poor plumbing practice, electrical grounding, or lightning—were not present," he says. "Simply exposing the tube to the water in question caused the eight leaks per foot in 1 year. The surface that had been pitted to a mirror-like finish was also severely pitted. We had only two factors: the water and the pipe. There is no other explanation."

The Aluminum Anomaly

Andrew J. Kireta, Jr., CDA's National Program Manager for Tube, Pipe, and Fittings, contends that Edwards' conclusion is significant for two reasons. "This finding stresses the importance of considering the impact of water treatment and water chemistry changes and regulations on the distribution systems and materials," he says. Kireta finds equally important Edwards' observation that the aluminum in the water plays a significant role in the pinhole leaks. "Aluminum could be considered the major contributing factor in this situation because it is the anomaly," he says.

"Chlorine, which exists in some level in virtually all publicly delivered water supplies in the U.S., was shown not to cause the corrosion concern on its own," says Kireta. "Only in the presence of aluminum did the pinhole leaks develop. This is significant because, while aluminum is present in many treated waters at low levels, never before was it found in significant levels where pinhole leaks had occurred in cold water systems." In fact, Kireta says that CDA's copper tubing research only began to show significant levels of aluminum on corrosion pits and in water deposits on a regular basis in the late 1990s. "Before this time, aluminum was rarely detected," he says. "This indicates that changes being made to water chemistry are causing an increase in the amount of aluminum delivered to the home wa-

ter distribution system, either through aluminum being added to the treated water or, more likely, the leaching of aluminum from the public water distribution system."

Kireta believes that the study is ground-breaking because it finally provides the evidence explaining why "epidemics" of corrosion are occurring in domestic water systems in the District of Columbia and elsewhere in the U.S. "Prior to this work, it was commonly assumed that the only link between corrosion outbreaks in different

utilities was the fact that copper tube was involved and suffering pinhole leaks," he says. "This would inevitably lead to what has now been proven to be a mistaken conclusion that the problem was due to faulty or inferior copper tube or a result of something in the manufacturing process that made the tube unsuitable for use. This work proved beyond a shadow of a doubt that the only significant factor in causing the corrosion was the chemistry of the water—and that the copper tube surfaces were not the cause of the failures."

What About MIC?

Another known cause of pitting in copper tubing is MIC. The inherent toxicity of copper ions initially led some to assume that copper alloys could ward off MIC. However, copper-tolerant strains of bacteria now are known to exist. "The mechanisms of corrosion by bacteria are numerous," says Paula Scott, Principal of CARIAD Consultants (Heraklion, Greece) and an internationally recognized expert on biological corrosion. "They differ in copper only in that, since copper is naturally microbiocidal, bacteria protect themselves by typically producing copious amounts of a gelatinous goo called 'extracellular polymeric substances.'"

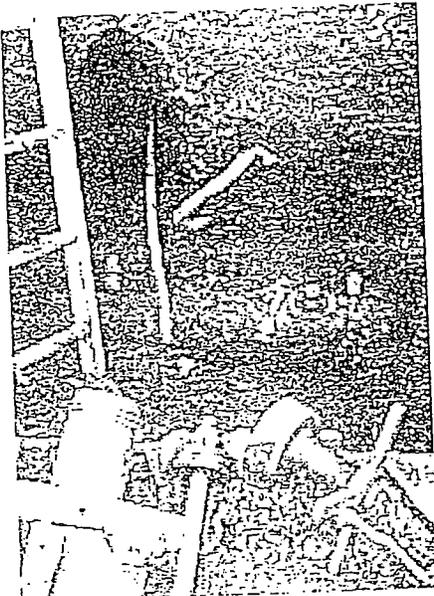
Scott notes that MIC can occur wherever bacteria are present. Like all living organisms, bacteria need free water to survive. They also typically live in temperatures below 176°F (80°C), but Scott points out that some thermophilic bacteria can endure higher temperatures. Chlorine and other biocides most often are used to control MIC.

"The more we look, the more we find it," says Scott of MIC-induced pitting in copper tubing. "MIC of copper has been found in several countries so far and is probably more widespread than we think, especially where water treatment is inadequate." Nevertheless, copper pitting is still con-

The Lead and Copper Connection

Some ascribe the unusually high number of copper pitting cases in suburban Washington, D.C., and elsewhere to the water chemistry required by the EPA's LCR. Could the treatment practices mandated by the rule also attack other metallic components of plumbing and cause lead to leach from residential service lines into drinking water?

In recent months, the detection of high levels of lead in tap water supplied to older Washington, D.C., residences has received considerable public scrutiny. The LCR requires a water system to use certain treatment techniques when samples reach an EPA-defined "action level" of 15 ppb of lead. The EPA, which is responsible for ensuring the safety



Aggressive water can attack metallic plumbing materials other than copper. Exceedances of the EPA's lead action limit can force utilities to replace existing lead pipe. This photo shows a pit dug to partly replace an existing publicly owned lead pipe with a copper pipe. Photo courtesy of Marc Edwards, Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, Virginia.

of drinking water in the District of Columbia, received a report in July 2001 from the District of Columbia Water and Sewer Authority (WASA) showing elevated concentrations of lead in water samples taken from residences in the WASA service area. A subsequent report, for the period July 1, 2001, to June 30, 2002, revealed that lead levels exceeded the action level in 26 of the 53 samples taken for that monitoring period. A test conducted in the summer of 2003 revealed that two-thirds of 6,188 District homes had water that exceeded the lead action level. Moreover, WASA found that more than 150 of these homes had lead levels exceeding 300 ppb—more than 20 times EPA's action level. It has been reported that a few samples even had lead levels in the thousands of parts per billion, exceeding thresholds for hazardous waste. Because an estimated 23,000 homes in the city utilize lead service lines, some believe that the number of "exceedances" is much greater. At this writing, however, approximately three-fourths of the homes have not been tested—nearly 3 years after the first reports of elevated lead levels. Moreover, the matter did not begin receiving widespread publicity until early this year when it was the subject of a *Washington Post* article.⁸

What is causing this rapid increase in lead exceedances? No conclusive answer was available at press time. However, it is clear that the corrosivity of the District's drinking water increased when the U.S. Army Corps of Engineers in November 2000 switched to chloramines (from free chlorine) as a secondary disinfection agent at the Washington Aqueduct. Testifying at a recent congressional oversight hearing, Washington Aqueduct General Manager Thomas P. Jacobus stated that the disinfection practices were modified in order to comply with EPA regulations.⁹

In an EPA-commissioned study and in his testimony to Congress, Edwards reported that in some circumstances chloramines dramatically increased lead leaching to water from pure lead pipe or leaded brass. Edwards' report, submitted to the EPA in October 2003, also suggests that introducing orthophosphates or other phosphate-based corrosion inhibitors could stop the leaching.

The detected and undetected instances of lead leaching notwithstanding, the turn of events in Washington, D.C., has generated a separate controversy among various government officials. D.C. city leaders as well as members of Congress have criticized WASA and the D.C. Department of Health for responding slowly to the crisis. The agencies have since begun distributing water filters to homes with lead service lines. EPA officials also have charged WASA with violating federal law. In addition, the chairman of the U.S. House subcommittee that has prime jurisdiction over the Safe Drinking Water Act recently asked the General Accounting Office (GAO) (Washington, D.C.) to conduct an independent inquiry into the operation of WASA and the Washington Aqueduct and the manner in which the public was notified about test results showing elevated lead levels. "My prime concern is that the public health is protected," U.S. Representative Paul Gillmor (R-Ohio), Chairman of the House Energy and Commerce Subcommittee on Environment and Hazardous Materials, noted in a written statement. "We need to gather all the information before making any final decisions or conclusions, but with some of D.C.'s lead levels approaching 20 times the safe limit set by the EPA, I am inclined to believe this was not so much a failure of law, but of persons."

For updates on this ongoing story, access the following Web sites:

- D.C. WASA: www.dcwasa.com
- D.C. Department of Health: dchealth.dc.gov/index.asp
- U.S. EPA Region 3: www.epa.gov/region3
- U.S. House Committee on Government Reform: reform.house.gov/GovReform/Hearings/EventSingle.aspx?EventID=797
- U.S. House Energy and Commerce Subcommittee on Environment and Hazardous Materials: energycommerce.house.gov/108/subcommittees/Environment_and_Hazardous_Materials.htm

sidered a rare phenomenon in industrialized countries.⁵ Furthermore, it is important to remember that more advanced water treatment systems do have a built-in defense against MIC. "One of the reasons that piping is not corroded more in potable water systems is that in most places, water treated for human consumption is pretty well free of bacteria," says Scott. She does point out, however, that what is considered "potable" in one locale may not be so in another. "It would be very naïve to think that all potable waters are adequately treated around the world," she says.

Acknowledging his own research that sulfate-reducing bacteria can likely cause copper pitting corrosion, Edwards echoes Scott's point that potable water is not typically an ideal place for MIC-causing bacteria to flourish. "If you look at many previous studies examining MIC, the researchers almost always utilized nutrient media that are highly unrepresentative of drinking water," he says. "For instance, it is not uncommon for MIC researchers to use levels of orthophosphate [as a nutrient source or buffer], grams per liter of organic matter such as glucose [C₆H₁₂O₆], and even grams per liter concentrations of ammonia in MIC experiments. In such cases, the 'water' is more representative of concentrated raw sewage than drinking water, and I strongly argue that results from such studies cannot be readily extrapolated to potable water systems. The fact of the matter is that despite years of study, MIC corrosion has never been proven to cause fully penetrating copper pitting as it occurs in real systems. In drinking water systems, addition of even 1 mg/L orthophosphate completely alters copper corrosion, 0.1 mg/L of biodegradable organic matter is a concern for bacterial regrowth, and ammonia concentrations rarely exceed a few milligrams per liter."

Contrasting the above MIC research scenario with his own copper pitting study, Edwards says that he and his colleagues limited bacterial activity with a relatively high pH of 9.2, continuous high free chlorine disinfectant residuals (greater than 3 mg/L), and the omission of any added organic matter, NH₃, or phosphate. "Not surprisingly, measurements of bacterial activity were negligible," he says. "Yet extreme pitting did occur."

Scott points out that MIC corrosion indeed has been shown to cause fully penetrating copper pitting in actual water systems. In a NACE International book that she recently coauthored with Michael Davies, her partner at CARLAD, Scott cites instances in which MIC caused through-wall pitting in real systems.⁶ "That doesn't mean it is the cause in all cases, but it certainly is in some," she says. "There is room for more than one mechanism of corrosion in copper tubing. Prob-

ably, some corrosion mechanisms, not involving bacteria, operate at high biocide levels. MIC operates... in systems where the water treatment is inadequate."

Scott also notes that carbon dioxide (CO₂) contributes to copper pitting in cold potable water. It has been stated that dissolved CO₂ in aggressive water initiates pits and depolarizes the cathodes on the copper when the metal is exposed to cold water.⁷ Space limitations preclude a more detailed discussion of the role of CO₂ in copper pitting in this article.

Not the Last Word?

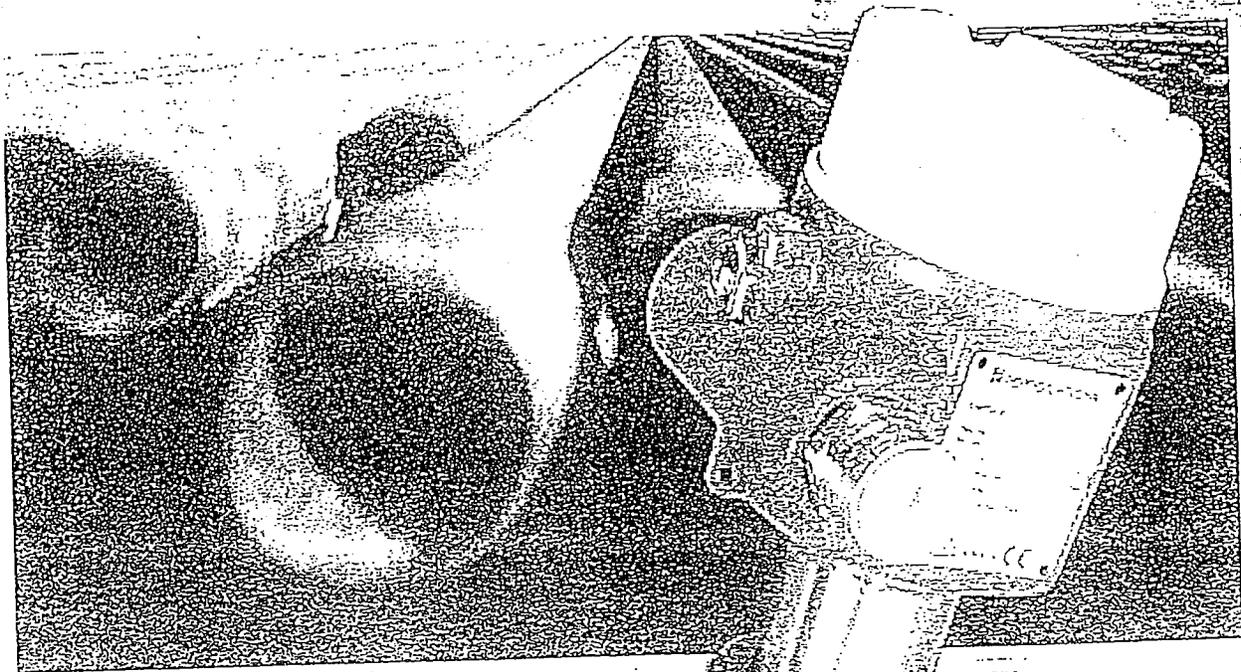
Edwards points out that the level of scientific understanding of copper pitting remains very limited, and he dispels any inference that the Virginia Tech study presents the sole explanation for the phenomenon. "I believe it is likely that there are other situations that will create aggressive water that can eat holes in a copper pipe, and with further testing we can identify those that are most important and how to prevent them" he says. "It is important to note that pitting can be mitigated by either preventing production of aggressive water in the first place or by addition of corrosion inhibitor."

According to Edwards, his own 12 years of study into "most of the other purported causes" never found that these factors cause pitting by themselves. "This does not mean that someone might someday do the test slightly differently and prove they do cause pitting," he concludes. "But the onus is now on others to develop that proof, because our team has now shown that it can be caused by the water alone."

References

1. M.V. Veazey, "Insidious Leaks Plague Homeowners," MP 41, 12 (2002), p. 16.
2. U.S. Code of Federal Regulations (CFR) Title 40, "Control of Lead and Copper," Parts 141 and 142 (Washington, D.C.: Office of the Federal Register, 1991).
3. A. Cohen, J.R. Myers, "Water Treatment to Mitigate Corrosion of Copper Plumbing Systems," MP 32, 8 (1995), p. 45.
4. "Use of chloramines in drinking water," EPA Region 9, February 23, 2004. <http://www.epa.gov/region09/water/chloramine.html> (April 6, 2004).
5. M.P.H. Brongers, "Drinking Water and Sewer Systems," in Corrosion Costs and Preventive Strategies in the United States, Federal Highway Administration Final Report, FHWA-RD-01-156, January 2002.
6. M. Davies, P.J.B. Scott, Guide to the Use of Materials in Water (Houston, TX: NACE Press, 2003).
7. J.R. Myers, A. Cohen, "Pitting Corrosion of Copper in Cold Potable Water Systems," MP 34, 10 (1995), p. 61.
8. D. Nakamura, "Water in D.C. Exceeds EPA Lead Limit," Washington Post, January 31, 2004. <http://www.washingtonpost.com/ac2/wp-dyn-A64766-2004-01-30?language=printer> (Feb. 3, 2004).
9. House Committee on Government Reform, The Federal Role in Ensuring Safe Drinking Water in the District of Columbia: Oversight Hearing, 108th Cong., 2nd sess., 2004. *NP*

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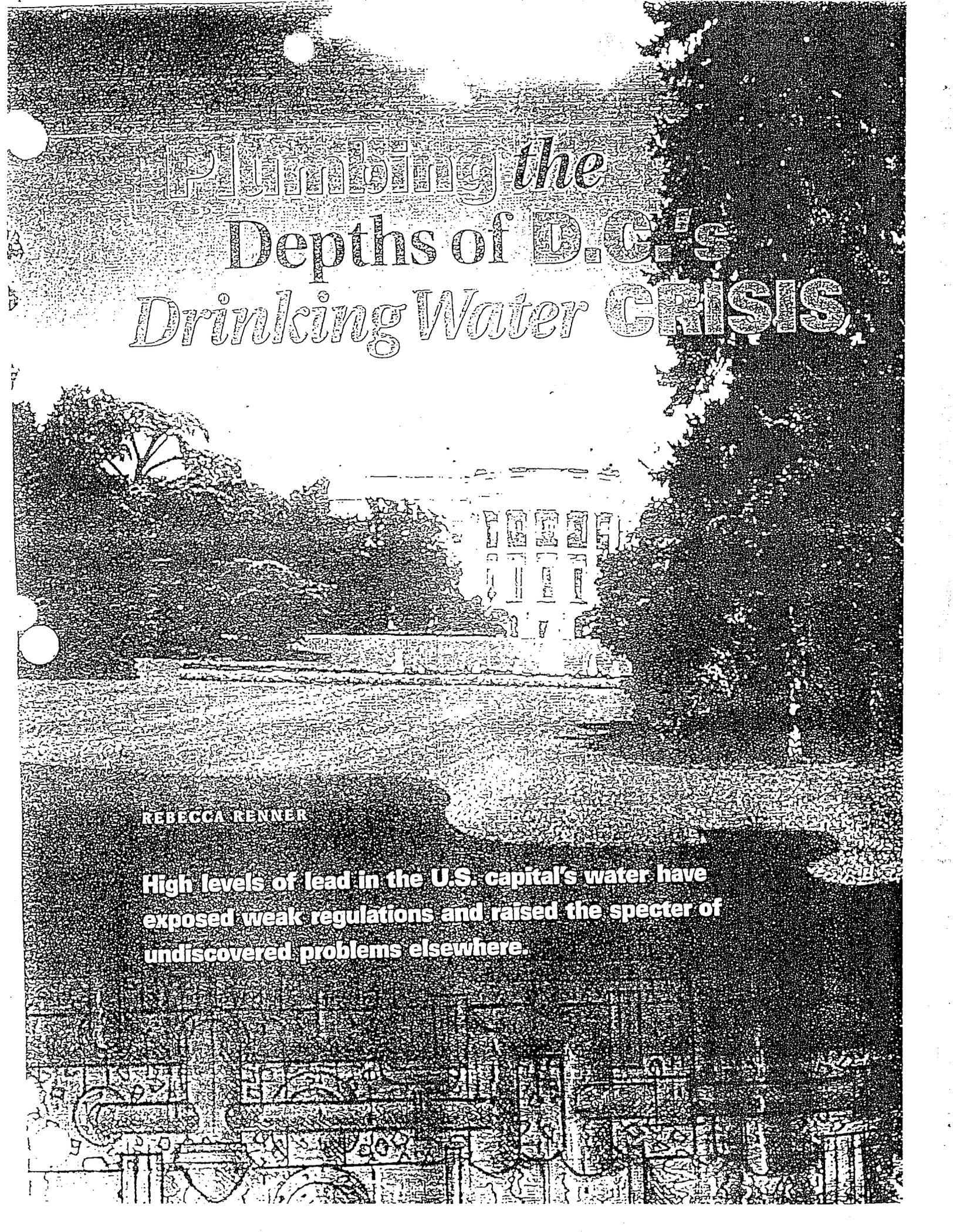
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Plumbing the Depths of D.C.'s Drinking Water **CRISIS**

REBECCA RENNER

High levels of lead in the U.S. capital's water have exposed weak regulations and raised the specter of undiscovered problems elsewhere.

As scientists dig more deeply into the murky origins of the high levels of lead in the District of Columbia's drinking water, they are beginning to suspect that they have uncovered a problem that could affect many water systems. In addition to exposing weaknesses and conflicts in U.S. drinking water regulations, the investigation is revealing a dearth of knowledge about what happens to drinking water during its long trip from the water treatment plant to the household tap.

The reported magnitude of Washington's lead problem has grown steadily since the public first learned about it in February. To date, officials have documented that tap water from more than 157 houses contained lead levels exceeding 300 parts per billion (ppb), and thousands more have exceeded the U.S. EPA's 15-ppb action level. The D.C. Water and Sewer Authority, which distributes the water; the Army Corps of Engineers, which manages the Potomac River water treatment plant; and EPA Region 3, which is the regulator, have faced heavy criticism for their handling of the problem. The public has received contradictory advice about the severity of the problem, how long to run the tap before using the water, and whether replacement of lead service lines will solve the problem. Congress has already conducted an investigation, and the head of the D.C. Health Department has been fired, in part, for not adequately responding to the problem. A citizens' lawsuit is in the works, and an expert committee is searching for the source of the problem.

Officials in Washington have not yet determined the cause of the lead contamination, but they believe it results from a 2000 decision by the water treatment plant, known as the Washington Aqueduct, to modify its disinfection program from chlorine to chloramines—a mixture of chlorine and ammonia. The change was initiated to comply with the 1998 Disinfection Byproducts Rule (DBR), which restricts disinfection byproducts in water.

ES&T's investigation of the problem suggests that the change in chemistry coupled with old lead pipes in Washington, D.C.'s, water system was the source of the crisis. In fact, corrosion expert Marc Edwards at Virginia Tech in Blacksburg has been warning EPA and the water industry for years that changes in drinking water treatment are likely to cause problems in home plumbing systems and tap water. As a consultant to EPA Region 3, Edwards is the scientist who first called attention to the D.C. problem. An alleged conflict of interest prevents him from being officially involved in further studying the D.C. problem.

Warring regulations

Scientists who study corrosion in drinking water systems are a relatively small group. As the parties involved in the D.C. water crisis scramble for expert advice, it has become difficult to find corrosion experts who will speak on the record. However, many were

eager to talk to ES&T on the condition that their names not be published in this story.

What Edwards and many of the other corrosion scientists contend is that lead problems may lurk in other cities, both with and without lead service lines. Moreover, many agree that it is time to revamp the 1991 Lead and Copper Rule (LCR), which regulates lead and copper in drinking water. In their view, the law does not work. Among the inadequacies of the lead rule are the lack of an enforceable regulatory standard, delegation of oversight to states ill-equipped to handle technical and scientific complexities, weak public notification requirements, insufficient monitoring information to assess the rule's effectiveness, gaps in controlling corrosion and lead leaching, and poorly designed lead sampling and testing programs.

The situation has already led EPA's acting Assistant Administrator for Water, Benjamin Grumbles, to acknowledge that the LCR may need an overhaul. On March 5, he told the U.S. House of Representatives' Government Reform Committee that EPA has decided to review the LCR.

Leaders of the House committee wasted little time in taking him up on the offer. In a seven-page, March 17 letter to Grumbles, they castigated the agency's regulations as "weak" and called for changes in the way local jurisdictions are required to test for lead, control pipe corrosion, and notify residents of contamination problems.

However, scientists most familiar with the problem say that the situation requires more action. "We are nowhere close to researching important lead and copper treatment chemistry issues," says one scientist. "The Disinfection Byproduct Rule and the Lead and Copper Rule are warring regulations. There is an urgent need for EPA to harmonize the rules. But EPA management has just avoided the issue," the scientist says.

The crux of the issue is that utilities must take very different and often opposing actions to comply with the DBR and the LCR. To combat the byproducts that form when disinfectants, particularly chlorine, react with natural organic and inorganic matter in source water and distribution systems, the DBR directs water companies to make sure that levels of these compounds stay below a certain regulatory standard or Maximum Contaminant Level (MCL). Although many of the hundreds of byproducts that can form as the result of disinfection have been shown to cause cancer and reproductive and developmental effects in laboratory animals, the rule focuses on representative compounds that serve as surrogates.

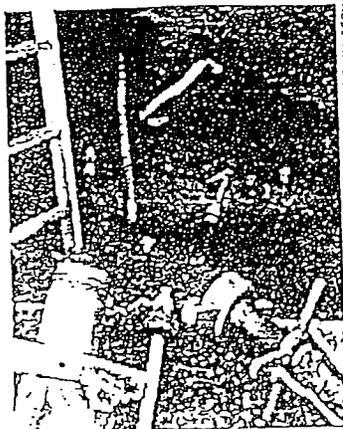
The LCR, on the other hand, sets an "action level" of 15 ppb. This means that if 10% of the samples collected from homes thought to be at highest risk contain more than 15 ppb lead, then a water company must take additional action. But exactly what actions should be taken (e.g., informing the public) is open to interpretation. Washington's water provider, for instance, first mentioned the high lead levels in a small section of a larger report that mainly trumpeted its

water quality and compliance with all EPA regulations.

Water companies take MCLs more seriously than action levels, says one Midwest water consultant. "If you violate an MCL, the water company's name is in the paper, people get concerned, and there's an uproar. If you exceed an action level, things stay quiet and you just start to 'take steps'."

For example, says Edwards, a water system can get its lead below the 15-ppb action level by raising the pH to 9, but taking that action leads to a violation of the MCL for disinfection byproducts. To avoid exceeding the DBR MCL, the system can be granted permission to continue exceeding the lead action limit indefinitely. Some systems in the United States have never regularly achieved the lead action limit, he contends.

"People warned that the DBR could have nasty potential secondary implications for corrosion," says one university water chemist. EPA acknowledged this possibility in a 1999 compliance guidance manual for the DBR, which discusses the potential for corrosion problems in a general way but offers little specific procedural advice, says the scientist. As a result, some savvy proactive water companies have had the sense to bench-test new water treatment technologies so they don't wind up with nasty surprises in the pipe, he says. But the scientists and consultants agree that such tests are time-consuming and expensive, so that without some sort of requirement, most water companies are unlikely to conduct them. D.C.'s Washington Aqueduct treatment plant never conducted such testing, according to Edwards.



An old, publicly owned lead pipe is replaced with a copper one.

MARC EDWARDS, VIRGINIA TECH

laboratory in Cincinnati. No one has really investigated what happens when chloramines are added to the already complex water chemistry that occurs in the new and old pipes that modern distribution systems comprise. What is clear is that replacing free chlorine with chloramines changes the chemistry by typically raising the pH and lowering the water's oxidizing potential, according to water chemist Phillip Singer at the University of North Carolina, Chapel Hill.

Schock's lead scale surprise

Lead can get into tap water from various sources. Many of the smaller service pipes that branch out from the main water line still contain lead (photo at top). These pipes are often coated with scales made of thin layers of lead-bearing minerals and other minerals that often keep lead out of drinking water.

"Lead-free" brass can also be a source of lead because it can contain up to 8% lead, in accordance with a definition set by Congress as part of the Safe Drinking Water Act. Brass is used in meters and household fixtures such as water taps. Edwards and Schock recently presented data that show that current leaching tests for brass are not very reliable. Lead is also found in the solder used to weld pipes together.

Many corrosion experts believe that the lead in D.C.'s water is probably coming from several sources. Schock has been studying the thin shells of mineral scales that coat the insides of many water pipes. In the process, he is amassing evidence of one possible explanation for the lead in Washington tap water.

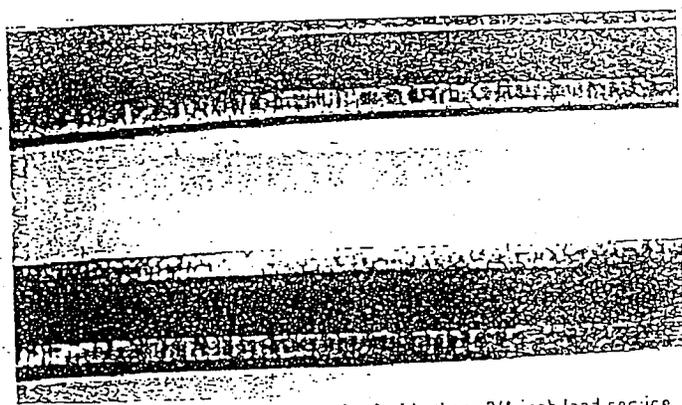
Conventional water chemistry holds that in natural water lead exists as Pb^{2+} . So, minerals that occur in water pipe scales are generally assumed to be Pb^{2+} minerals. But Schock has discovered that in drinking water, which is often highly oxidizing, Pb^{4+} minerals, most importantly PbO_2 , can form. In fact, Schock found PbO_2 in pipe scales when he examined Washington, D.C., service lines (photo at left).

Schock has constructed a stability diagram for lead that relates stable lead compounds and species to pH and redox conditions. The diagram shows that PbO_2 is likely to be stable in highly oxidizing water over the pH range of treated drinking water. This means that when Washington used large amounts of chlorine in the past, the highly oxidizing water fostered development of PbO_2 scales. As long as the drinking water remained highly oxidizing, those scales were stable and insoluble.

With the shift to chloramines, the oxidizing potential of D.C.'s water was lowered. Thus, Schock's research shows that after the change, D.C. water might well dissolve the PbO_2 scale, which would then raise the contaminant levels.

Many scientists say that Schock's research currently offers one of the most promising clues to the very high lead levels. "Schock's work is excellent, groundbreaking research that has very important implications for water quality," Singer says.

Are there other water utilities with pipes coated



The red-brown coating inside these 3/4-inch lead service lines is PbO_2 .

One of the most common ways to comply with the DBR is to use chloramines (a mixture of chlorine and ammonia) instead of chlorine. Some 37% of major U.S. water companies are currently making this substitution and the proportion is likely to grow as limits on disinfection byproducts are tightened.

However, the consequences of adding chloramines aren't fully understood, says chemist Michael Schindler with EPA's National Risk Management Research Lab

MARC EDWARDS, VIRGINIA TECH

with stable PbO_2 scales that might dissolve if chloramines or some other water treatment technology is introduced? No one knows.

Edwards' shocking results

The scale-dissolution theory based on Schock's research may explain why homes with lead service lines have contaminated water. But in Washington, many houses without lead service lines also have high lead concentrations in their tap water; Edwards' work on chloramines and galvanic corrosion may well provide the best explanation.

In an October 2003 consultant's report to EPA Region 3, Edwards noted that several papers indicated that chloramines might be particularly prone to mobilizing lead from brass, which his preliminary experiments confirmed directly. He also discovered that galvanic corrosion was enhanced by chloramines.

Galvanic corrosion is basic electrochemistry—it occurs when two dissimilar metals are connected, causing one metal (the anode) to be sacrificed and the other (the cathode) to be protected. Hot water heater tanks, for instance, incorporate an aluminum anode rod to protect the steel lining.

As with the lead speciation, conventional water chemistry wisdom appears to be misleading in the Washington case. In some situations, when brass is connected to copper, it is cathodic relative to copper. This means that the brass pieces are protected from corrosion and thus lead is not released into the drinking water. The copper is sacrificed, but the effect is minor because there is so much copper plumbing and hence so much copper surface area.

But Edwards' group recently discovered that in the presence of chloramines, the brass is highly anodic and the copper is cathodic (Figure 1). Moreover, the galvanic corrosion current persists for days, even without a new supply of water. These forces conspire to accelerate the release of any lead from brass pipes. "The problem is that... the entire copper plumbing network in the house can act to accelerate the corrosion of the brass right at the faucet where the water is collected. Lead leaching can increase 4 to 100 times faster than normal," he says.

The tip of the iceberg?

At this point, the studies and theories put forward by Schock and Edwards need to be corroborated with further testing. No one knows for sure why D.C.'s change to chloramines caused the lead levels in tap water to rise. But if their work is corroborated, it hints at serious weaknesses in the federal testing program and conflicts between the DBR and the LCR. In addition, it raises the prospect that other cities also have—or may soon have—problems, according to many scientists and engineers.

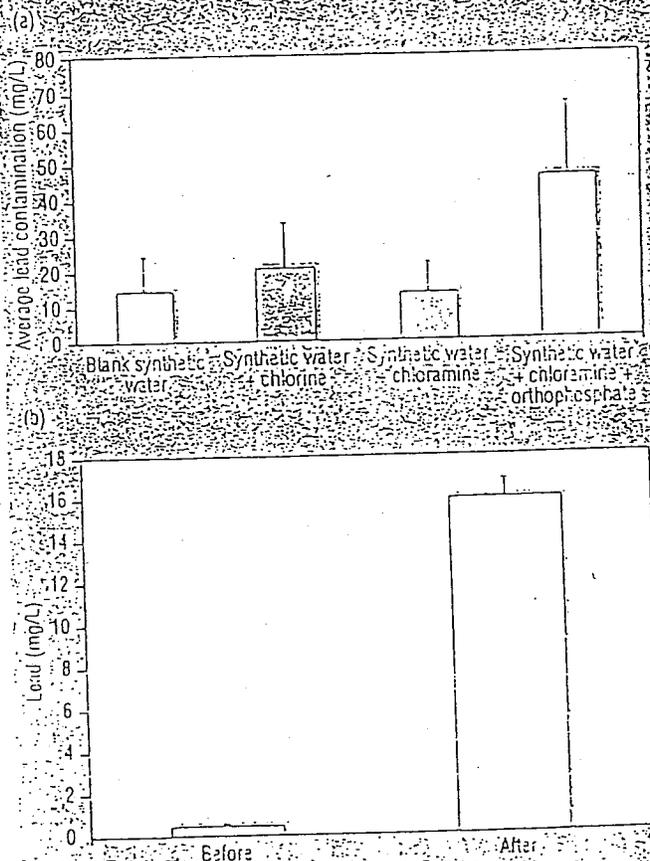
"We are learning hard lessons [in D.C.] about corrosion control in full-scale testing of real systems while consumers are drinking the water," Edwards testified at the Congressional hearings on March 5, 2004. Who knows what further lessons could be in store?

Drinking water system corrosion has been a neglected area of research, but perhaps the Washington crisis will change that. The National Science Founda-

FIGURE 1

Lead can leach from old lead pipes even when they are connected to newer copper lines

(a) In lab experiments, Marc Edwards' group documented lead levels as high as 45,000 ppb by galvanically connecting an old lead pipe to one made of new copper under varying conditions. The figure shows average lead levels measured after eight hours of galvanic connection. Similarly high levels have been measured in D.C. tap water. (b) In another experiment, switching from free chlorine (before) to chloramines (after) with the same water promoted attack on lead materials, including brass faucets. Experiment time was two months and the pH was 8.5. The magnitude of this effect depends on the type of brass, pipe age, and other water quality factors. Error bars mark 95% confidence limits.



Source: Marc Edwards, Virginia Tech.

tion, newly alert to the public health significance of corrosion, recently expressed an interest in funding some aspect of corrosion research, according to Schock.

EPA has just begun "a thorough review" of the LCR, and if the agency enacts the Congressional committee's March 17 recommendations, utilities in every state likely would be required to increase testing and report more data on lead, copper, and other indicators of water chemistry. More explicit public notification about potential health risks and incentives for replacing private portions of lead service lines might also be required.

Perhaps the District's current misery will benefit us all in the long run.

Elisabeth Rosenthal is a contributing editor for E&T.

Drinking Water
Symposium

An educational discussion on
the negative health effects that
copper plumbing pipes can have
on your health.

Meet our Speakers:

Larry Sparks, Ph.D.
Senior Scientist & Head of the Roberts Lab of Neurodegenerative Research
Sun Health Research Institute

Larry Sparks, Ph.D., is the Senior Scientist & Head of the Roberts Lab of Neurodegenerative Research at the Sun Health Research Institute in Sun City, Arizona, where he is responsible for many advancements in the field of Alzheimer's disease. Further, he has done research in aging and development of the central nervous system, neuropathology of heart disease, hypertension and sudden infant death syndrome, neurodegeneration and correlative neurochemical alterations, Alzheimer's disease and other disorders.

Prior to joining Sun Health he was and remains the Chief Biochemical Consultant for the Kentucky State Medical Examiner's Program, Justice Cabinet. He has been the principal investigator of many ongoing and completed clinical trials & research projects over the last 3 years. He has spoken both nationally and internationally on the negative health effects of copper and has been rated among the top 30 Alzheimer's disease scientists in the world. He has appeared in Newsweek, USA Today, and has been interviewed by many TV and Radio stations.

Dr. Sparks is also the principle investigator for the Alzheimer's disease Cholesterol-lowering Treatment Trial sponsored by Pfizer. On Nov 17, 2004, the results of Dr. Sparks' study were featured on Peoria's Independent and Sun City's Independent news stories. These stories featured the exciting results of this trial and a new one that is currently underway. Dr. Sparks also received national (appeared on NBC news) and local visibility for his test results that indicate statin drugs such as Lipitor[®] may help delay the progression of Alzheimer's disease.

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Trace amounts of copper in water induce β -amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease

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Despite the crucial role played by cholesterol and copper in nutrition and normal brain function, recent evidence indicates that they may both be important factors in the etiology of Alzheimer's disease (AD). Here we provide critical evidence for the role of cholesterol and copper in AD by showing that the addition of trace amounts of copper (0.12 ppm) to water given to cholesterol-fed rabbits can induce β -amyloid ($A\beta$) accumulation, including senile plaque-like structures in the hippocampus and temporal lobe, and can significantly retard the ability of rabbits to learn a difficult trace conditioning task. The $A\beta$ deposits do not affect the ability of rabbits to detect or respond to the training stimuli nor to learn a simpler delay conditioning task. Trace amounts of copper in drinking water may influence clearance of $A\beta$ from the brain at the level of the interface between the blood and cerebrovasculature and combined with high cholesterol may be a key component to the accumulation of $A\beta$ in the brain, having a significant impact on learning and memory. Cholesterol-fed rabbits have at least 12 pathological markers seen in AD, suggesting that the cholesterol-fed rabbit is a good animal model for studying AD.

Cholesterol is important for cell function, neurotransmission, and synaptic plasticity (1, 2), but it is also an important risk factor for atherosclerosis and Alzheimer's disease (AD) (3, 4). Recent evidence suggests that the cholesterol-lowering statins may affect AD by reducing the deposition of β -amyloid ($A\beta$) (5, 6). Copper is an essential nutrient (7) but has also been implicated as an important factor in AD (8–11). Indeed, $A\beta$ has been hypothesized to act as a bioflocculant that binds trace metals such as copper and zinc (12), and up-regulation of the $A\beta$ precursor protein (APP) has been shown to suppress elevated copper levels (11). Consequently, chelation of copper has been suggested as a potential therapy for AD (9, 13–16).

Substantial evidence has accrued that cholesterol-fed rabbits develop a considerable number of pathological indices of AD, including accumulation of $A\beta$ in the hippocampus and temporal lobe (17–27). We have recently found that there are between-laboratory differences in the levels of $A\beta$ that accumulate in cholesterol-fed rabbits and that these differences are traceable to the drinking water (28). Facilities that used distilled water had cholesterol-fed rabbits with lower levels of $A\beta$ than those that used tap water. Analysis of the water from locales where tap water produced this differential effect indicated that, among likely trace metal candidates implicated in AD pathology (8, 29), copper levels ranging from 0.06 to 0.21 ppm were present in the drinking water. We initiated studies to assess the biological and behavioral effects of copper by supplementing distilled drinking water given to cholesterol-fed rabbits with copper sulfate at levels within this range (0.12 ppm copper) but at one-tenth the Environmental Protection Agency maximum allowable contaminant level for copper in municipal drinking water (1.3 ppm) (30).

Materials and Methods

Subjects. A total of 68 male New Zealand White rabbits (*Oryctolagus cuniculus*) 3–4 months of age and weighing \approx 2.2 kg were

housed individually, with free access to Purina rabbit chow and water, maintained on a 12-h light/12-h dark cycle and treated following National Institutes of Health guidelines. Each of these rabbits received a combination of food and water comprising normal chow or normal chow plus 2% cholesterol and tap water, distilled water, or distilled water plus copper. The number of subjects assigned to each condition is shown in Table 1. Briefly, 19 of the 68 rabbits were fed normal chow and 49 were fed the normal chow plus 2% cholesterol. Of the 19 rabbits fed normal chow, 9 received tap water (Tap) and 10 received distilled water (dH_2O). Of the 49 rabbits fed cholesterol, 16 received tap water (Tap/Chol), 21 received distilled water (dH_2O /Chol), and 12 received copper in their distilled water (dH_2O /Chol/Cu). Cholesterol-fed rabbits given copper received copper sulfate in their distilled drinking water with a final copper concentration of 0.12 ppm (0.12 mg/liter). All rabbits were maintained on their respective food and water regimes for a total of 10 weeks before euthanasia and subsequent histological analysis.

Behavioral Procedures. The behavioral procedures and equipment have been described (31). After 8 weeks on their respective diets, six of the cholesterol-fed rabbits given distilled water (dH_2O /Chol) and six of the cholesterol-fed rabbits given distilled water supplemented with 0.12 ppm copper (dH_2O /Chol/Cu) received 1 day of adaptation; one 60-trial session of air puff testing (pretest) to assess sensitivity to air puff; eight daily sessions of trace classical conditioning to assess their ability to learn a difficult conditioning task; a 60-trial session of air puff testing (posttest) to test for any changes in sensitivity to air puff; four daily sessions of delay classical conditioning to test for their ability to learn a simple conditioning task; and 2 days of tone intensity testing during trace conditioning to assess their hearing. Each air puff test trial pretest and posttest involved the presentation of 1 of 15 possible combinations of stimulus intensity (0.5, 1.0, 2.0, 4.0, or 8.0 psi) and stimulus duration (25, 50, or 100 ms). Each trace conditioning trial consisted of a 100-ms, 1-kHz, 82-dB tone that was followed by a 500-ms trace interval of no stimulation and then a 100-ms, 4-psi air puff. Each delay conditioning trial consisted of a 400-ms, 1-kHz, 82-dB tone that coterminated with a 100-ms, 4-psi air puff. Tone intensity testing trials consisted of one of seven, 100-ms tone intensities (60, 65, 70, 75, 80, 85, or 90 dB) or a zero intensity (0 dB) followed by a 200-ms trace interval and then a 100-ms, 4-psi air puff. The tone intensities were presented as a random sequence eight times. All trials were delivered, on average, every 60 s (50–70 s range). Repeated-measures ANOVA was used to compare conditioned

This paper was submitted directly Track II to the PNAS office.

Abbreviations: $A\beta$, β -amyloid; APP, $A\beta$ precursor protein; CP, ceruloplasmin; CR, conditioned response; GPx, glutathione peroxidase; SOD, superoxide dismutase; SP, senile plaque.

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Table 1: Number of A β immunoreactive neurons in differing regions of rabbit brain

| Treatment | Hippocampus | Hilus (center) | Temporal cortex | Parietal cortex | Superior cortex |
|---------------------------|------------------|----------------|-----------------|-----------------|-----------------|
| Tap | 43.9 \pm 12.6 | 15 \pm 4 | 42 \pm 8 | 18 \pm 5 | 26 \pm 5 |
| Tap/Chol | 49.4 \pm 8.6 | 19 \pm 5 | 72 \pm 9* | 28 \pm 5 | 59 \pm 5* |
| dH ₂ O | 31.2 \pm 5.8 | 10 \pm 3 | 29 \pm 4 | 11 \pm 3 | 27 \pm 4 |
| dH ₂ O/Chol | 48.3 \pm 7.8* | 12 \pm 3 | 58 \pm 6* | 28 \pm 6* | 49 \pm 4* |
| dH ₂ O/Chol/Cu | 64.9 \pm 16.3* | 21 \pm 4** | 87 \pm 11** | 27 \pm 5 | 88 \pm 14** |

Tap, tap water and normal chow (n = 9); Tap/Chol, tap water and 2% cholesterol diet (n = 16); dH₂O, distilled water and normal chow (n = 10); dH₂O/Chol, distilled water and 2% cholesterol diet (n = 21); dH₂O/Chol/Cu, distilled water with 2% cholesterol diet and 0.12 ppm Cu as CuSO₄ (n = 12). *, P < 0.05; **, P < 0.01 (Tap/Chol vs. dH₂O; dH₂O/Chol vs. dH₂O; dH₂O/Chol/Cu vs. dH₂O).

responses (CR) and unconditioned responses between the two groups.

Histology. Rabbits were anesthetized deeply with a mixture of ketamine (35 mg/kg) and xylazine (5 mg/kg), blood was drawn from the heart, and the rabbits were perfused transcardially with 0.5% paraformaldehyde. Brains were extracted and postfixed for 14 days in 4% paraformaldehyde. Fifty-micrometer vibratome sections of hippocampus and hippocampal cortex of the brain were immunostained with an antibody to A β (10D5; provided by Dale Schenk of Elan Pharmaceuticals, San Diego) by using published peroxidase-antiperoxidase immunohistochemical methods (17).

Although we consider 10D5 immunohistochemistry to be the most consistent and reliable method of quantifying A β accumulation, a number of alternative labeling methods have confirmed that we are observing A β accumulation in the cholesterol-fed rabbits. In addition to 10D5, which recognizes the first 16 aa of the N-terminal sequence of A β , the 2H3 antibody (Athena Neurosciences, San Francisco) as well as appropriate negative controls have been used to study A β immunoreactivity in cholesterol-fed rabbits with copper in their drinking water (23). The antibody 2H3 recognizes the first 12 aa of the N-terminal sequence of A β as well as the last 12 aa of the C-terminal sequence of secreted APP. This antibody was compared with 8E5 (Athena Neurosciences), which is specific for amino acids 444–592 of the N-terminal sequence of secreted APP. The immunohistochemistry showed significant immunoreactivity to 2H3 in the hippocampus and temporal cortex of cholesterol-fed rabbits with copper in the drinking water, and no detectable levels of 8E5. Thus, the staining observed in the brain tissue was of A β and not secreted APP (23). The 2H3 staining of A β reported by D.L.S. (23) was indistinguishable from previous and present 10D5 staining of A β . D.L.S. (25) reported europium immunoassays for A β 1–42 with polyclonal antibodies performed on fresh-frozen brain tissue from cholesterol-fed rabbits with copper in their drinking water. The immunoassays found elevated concentrations of total A β in the temporal cortex compared with normal-diet controls. Finally, both immunohistochemistry with the 6E10 monoclonal antibody for A β and quantification of A β with a europium immunoassay have appeared in an independent report (26), further corroborating the accumulation of A β in cholesterol-fed rabbit brain.

Immunoreactive neurons were counted as described (28). In brief, the number of A β immunoreactive cells within 10 randomly chosen 0.5 \times 0.5-mm square fields were counted at \times 20 magnification and averaged by an experimenter blind to the treatment of the rabbits. The average numbers of cells for all rabbits were subjected to ANOVA and then to follow-up pairwise comparisons, which were performed between the distilled water normal chow (dH₂O) control condition and the separate treatment groups.

Blood samples were assayed for superoxide dismutase (SOD; a copper/zinc free radical scavenger enzyme) and glutathione

peroxidase (GpX; a peroxide detoxifying enzyme) as described (24), and ceruloplasmin (CP; a copper chaperone) was measured according to published methods (32). The average levels of SOD, GpX, and CP for all rabbits were subjected to ANOVA and then to follow-up pairwise comparisons, which were performed between the distilled water normal chow (dH₂O) control condition and the separate treatment groups.

SOD and glutathione levels have been reported for fresh-frozen brain tissue taken from cholesterol-fed rabbits with copper in their drinking water (23). There was a significant increase in SOD in the hippocampus and a suggestion of an increase in the temporal cortex of cholesterol-fed rabbits with copper in their drinking water relative to controls. Although there was a suggestion of a reduction in the levels of glutathione in cholesterol-fed rabbits with copper in their drinking water, there was no significant difference in either area (23).

Results

Copper Induces A β Accumulation and Senile Plaque (SP)-Like Structures. The brains of rabbits were analyzed by using the 10D5 antibody, which shows A β immunoreactivity (33, 34). Cholesterol-fed rabbits with copper (0.12 ppm) added to their distilled drinking water (dH₂O/Chol/Cu) revealed a nearly 50% increase in the number of A β immunoreactive neurons compared with animals on a cholesterol diet and unaltered distilled water (dH₂O/Chol) (Table 1). The hippocampus, temporal cortex, and superior parietal cortex were preferentially affected compared with lateral parietal cortex, an observation that is similar to the regional distribution of SPs in AD. The superior temporal gyrus, also severely affected in AD, demonstrated a laminated distribution of A β immunoreactive neurons (Fig. 1B). This propensity for A β immunoreactive neurons to occur in layers II and IV–V is reminiscent of the laminar distribution of SPs in layers II and IV–V of the AD temporal cortex (24, 35). We also noted an increased A β immunoreactive staining on the meningeal surface of the brain in rabbits supplemented with copper (Fig. 1A Right and B Right), consistent with observations in the AD brain (35). Perhaps most significantly, cholesterol-fed rabbits also showed extracellular SP-like deposits of A β immunoreactive material (Fig. 1A and C). These SP-like deposits were not found among animals given distilled water (dH₂O/Chol), and rarely occur among animals on tap water (Tap/Chol, <10%; one or two per section in 1 of 16 animals). Strikingly, 75% of the cholesterol-fed animals on distilled water supplemented with 0.12 ppm copper (9 of 12) displayed SP-like deposits, and three of these rabbits had >10 SP-like deposits per histological section (Fig. 1A).

Copper and Cholesterol Increase SOD and Decrease GpX. We sought to find a bloodborne marker related to elevated circulating cholesterol or altered copper levels that might co-vary with A β immunoreactive neurons and/or SP-like deposits in the cholesterol-fed rabbit. Free radicals and associated changes of SOD and GpX activities are known to accompany increased circulating cholesterol (37), and CP can vary with copper levels in the

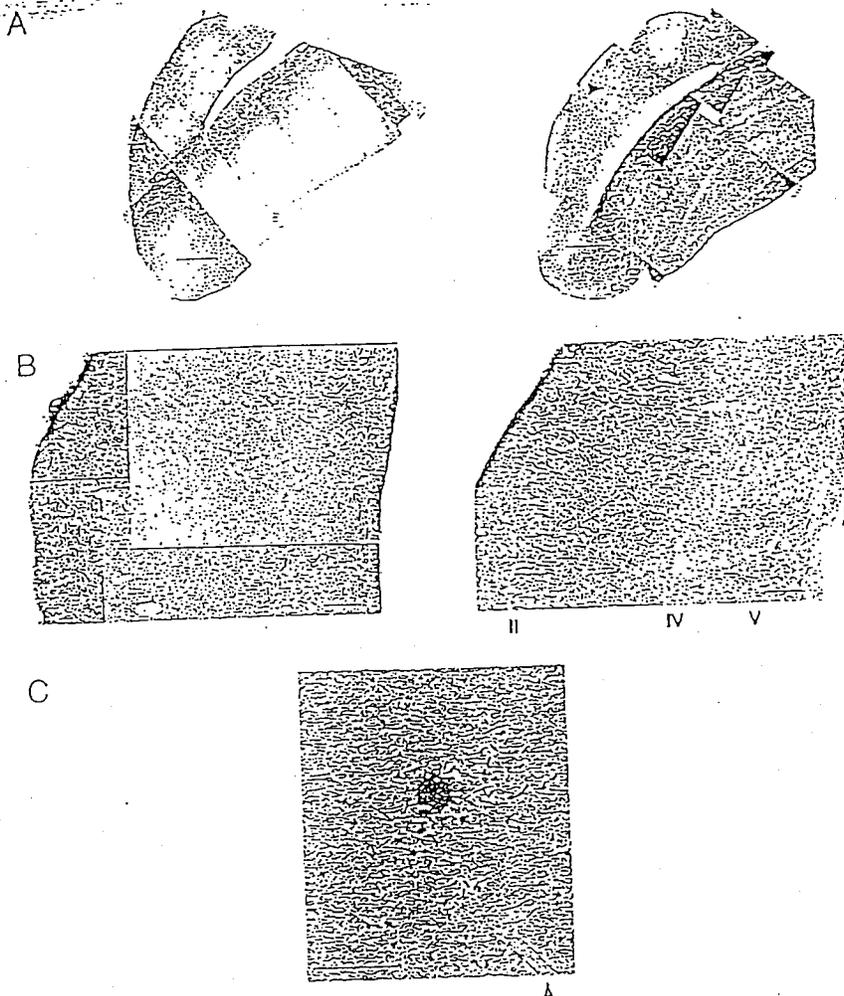


Fig. 1. Trace amounts of copper induce A β immunoreactive neurons and SP-like structures in the brain of the cholesterol-fed rabbit. (A) Photo montage of temporal lobe and hippocampus for a cholesterol-fed rabbit given distilled water (Left) and distilled water supplemented with 0.12 ppm copper (Right) (scale bar, 1 mm). Note the numerous dark spots in the copper-supplemented rabbit; these are SP-like structures. An example is indicated by the arrowhead and is magnified in C. (B) Photo montage of superior temporal gyrus for distilled water (Left) and copper-supplemented rabbit (Right) with banding of A β immunoreactive neurons in layers II and IV-V (scale bar, 100 μ m). (C) SP-like structure surrounded by A β immunoreactive neurons. Note that the plaque-like structure is somewhat removed from a blood vessel indicated by the arrowhead (scale bar, 100 μ m).

blood (38). Using rabbits on distilled water as the reference control (dH₂O), the activity of SOD in rabbits fed normal chow and given tap water (Tap) was marginally increased in RBCs and unchanged in the plasma (Table 2). Addition of cholesterol to the diet of animals on distilled water (dH₂O/Chol) produced a 3-fold increase in SOD activity in the plasma, and the addition of 0.12 ppm copper (dH₂O/Chol/Cu) produced an almost 4-fold increase in SOD activity (Table 2). Among the cholesterol-fed rabbits given copper sulfate in their distilled water, the activity of GpX decreased significantly compared with cholesterol-fed animals given distilled water. Although there was a suggested increase in CP, there was no significant effect of circulating cholesterol levels, concentration of copper in the drinking water, and levels of a CP in rabbit blood.

Copper-Induced A β Retards Trace Conditioning but Not Delay Conditioning. To assess the effects of copper-induced A β accumulation on learning, memory, and sensory processing, we devised and

administered a battery of behavioral tests to a subset of the cholesterol-fed rabbits. The tests included a measure of sensitivity and responsiveness to air puff, a difficult learning task, an easy learning task, and a task designed to measure their hearing. We chose trace classical conditioning of the rabbit nictitating membrane response as the difficult learning task because it engages the hippocampus and temporal lobe (39, 40), areas in which we noted the highest levels of A β accumulation. Measures of the average percent CR to the tone across 8 days of hippocampally dependent tone-air puff trace conditioning showed that 0.12 ppm copper had a profound effect on the ability of cholesterol-fed rabbits to acquire CR (Fig. 24). In fact, rabbits with copper in their drinking water (dH₂O/Chol/Cu) did not exceed an average level of 15% CR, whereas rabbits on distilled water (dH₂O/Chol) acquired CR approaching average levels of 60%. In strong contrast, when rabbits given copper were shifted to a simpler, hippocampally independent, short-delay condition-

Table 2. Rabbit blood levels of SOD, GpX, and CP

| Treatment | RBC SOD | Plasma SOD | RBC GpX | Plasma GpX | Plasma CP |
|---------------------------|--------------|------------------|------------------|----------------|-------------|
| Tap | 53 \pm 4.6 | 0.57 \pm 0.03 | 1.73 \pm 0.17 | 7.4 \pm 0.55 | 21 \pm 1 |
| Tap/Chol | 52 \pm 3.7 | 1.87 \pm 0.23 | 1.73 \pm 0.19 | 9.3 \pm 0.71 | 82 \pm 42 |
| dH ₂ O | 49 \pm 3.5 | 0.55 \pm 0.05 | 1.97 \pm 0.17 | 8.3 \pm 0.57 | 30 \pm 8 |
| dH ₂ O/Chol | 43 \pm 4.5 | 1.69 \pm 0.25 | 1.64 \pm 0.25 | 8.8 \pm 0.77 | 47 \pm 17 |
| dH ₂ O/Chol/Cu | 40 \pm 5.9 | 2.05 \pm 0.49* | 0.68 \pm 0.06* | 4.9 \pm 1.2* | 78 \pm 34 |

* $P < 0.05$ (dH₂O/Chol/Cu vs. dH₂O).

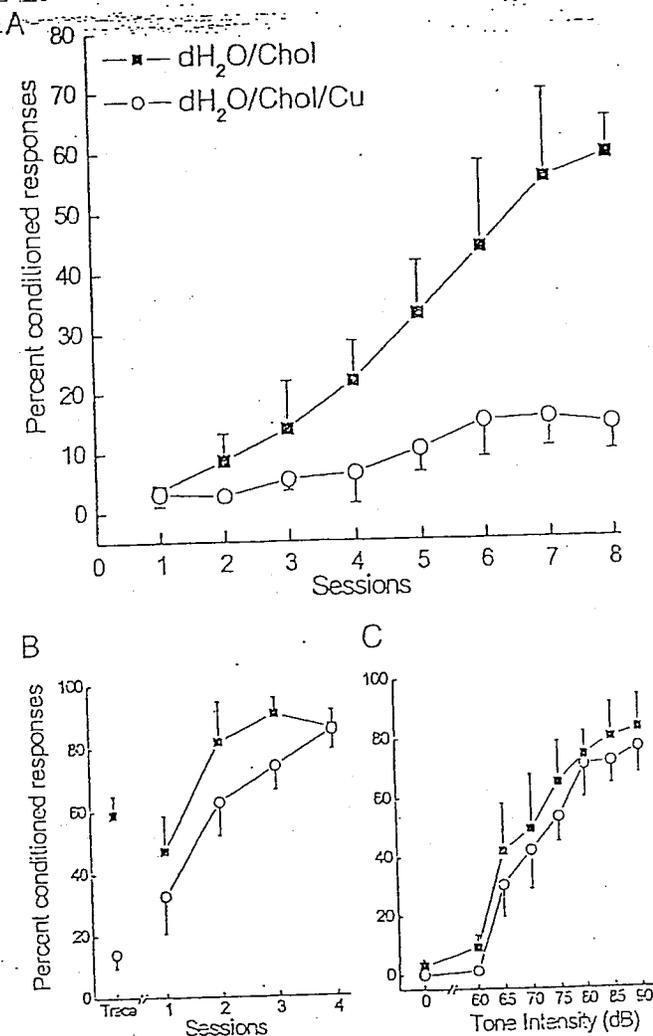


Fig. 2. Effects of trace amounts of copper (0.12 ppm) induce deficits in trace but do not delay classical conditioning of the rabbit nictitating membrane response. (A) Trace conditioning. Mean (\pm SEM) percent CR for cholesterol-fed rabbits with distilled water (dH₂O/Chol) and with copper sulfate (dH₂O/Chol/Cu) added to their distilled water revealed a significant overall copper-induced learning deficit as a main effect of groups [$F(1,9) = 7.37, P < 0.05$] and a highly significant groups-sessions interaction [$F(7,63) = 4.61, P < 0.001$]. (B) Delay conditioning of the rabbit nictitating membrane response showed that there was no difference in rate or level of learning of a simpler task between rabbits given copper in their distilled water or distilled water alone. (C) Tone intensity testing after delay conditioning showed no differences in the ability of rabbits given copper in their distilled water or distilled water alone to hear the tone used during trace conditioning.

ing paradigm, they were able to acquire CR to levels approaching 80% (Fig. 2B). Equally important, all rabbits showed comparable levels of sensitivity and responsiveness to the air puff and to the tone. Specifically, there were no significant differences between the copper-supplemented and distilled water groups in any of the dependent variables used to assess sensitivity and responsiveness to air puff, including response frequency, amplitude, latency, or area (P values > 0.07). An analysis of hearing measured as percent CR to seven different tone intensities (60–90 dB) indicated that there were no between-group differences in the levels of responding to the tone intensities (P values > 0.49) (Fig. 2C).

Discussion

Copper and AD. The present data provide strong support for the suggestion that copper is implicated in the accumulation of A β

(8, 10). A recent report described APP as an exporting system for copper and suggested that elevated levels of APP in the brain may remove increased copper (11). Indeed, as we have already noted, chelation of copper that would prevent A β accumulation has been suggested as a potential therapy for AD (9, 13–15). Clioquinol, a copper–zinc chelator, is in phase I (13) and phase II clinical trials (16). In contrast to A β 's potential neuronal role in clearing copper, copper may influence clearance of A β from the brain at the level of the interface between the blood and cerebrovasculature.

The Environmental Protection Agency maximum contaminant level goal (MCLG) for copper in drinking water is set at 1.3 ppm (1.3 mg/liter) copper or corrosives liberating 1.3 ppm copper, and treatment plants that exceed this MCLG are required to monitor the water and implement techniques that will reduce its corrosiveness (30). The MCLG is based on the lowest observed adverse health effect level (LOAEL) of 5.3 mg/day, a level which may induce gastrointestinal distress (30). Interestingly, although the dietary reference intake [DRI, formerly called recommended daily allowance (RDA)] for copper in adults is only 0.9 mg/day, the normal tolerable upper limit is considered to be 10 mg/day (7). Cholesterol-fed rabbits in the present experiment were allowed 0.12 ppm or 0.12 mg/liter, and given that rabbits drink ≈ 300 –600 ml of water per day, they consumed between 0.04 and 0.03 mg of copper per day. The present data show that this level of copper consumption was sufficient to induce significant A β accumulation and the formation of significant SP-like structures in the cholesterol-fed rabbit. Although we can only speculate about how the effects of copper consumption in cholesterol-fed rabbits relate to those in humans, it is of note that the levels of copper in the cholesterol-fed rabbit drinking water that induced A β and SP-like structures are well below those considered safe for humans.

A Cholesterol-Fed Rabbit Model of AD. Unlike transgenic mouse models, which have failed to produce more than one or two signs of AD pathology (41), the cholesterol-fed rabbit shows pathologies consistent with at least 12 different features of the AD brain. These include the currently described neuronal accumulation of A β immunoreactivity (17, 19), extracellular A β plaques (23), and meningeal A β immunoreactivity (36), as well as previously noted apolipoprotein E immunoreactivity (18, 22), cathepsin D immunoreactivity (20, 23), SOD immunoreactivity (23), microgliosis (21, 23), apoptosis (21), vascular activation of SOD (24), mouse endothelial cell antigen (MECA-32) immunoreactivity (25), breaches of the blood brain barrier (25), elevated brain cholesterol (23), and elevated A β concentration (24). To these indices of pathology we can now add a deficit in the ability to learn a difficult task that depends on the temporal cortex and hippocampus.

Conclusions

The addition of trace amounts of copper to the drinking water of cholesterol-fed rabbits induces accumulation of A β , formation of SP-like structures, reduction of GpX activity, increases in SOD activity, and retardation of the rabbit's ability to learn a difficult task. Thus, there may be a relationship between the diminished ability to inactivate peroxide due to reduced GpX activity in the blood and increased neuronal accumulation of A β immunoreactivity, formation of SP-like structures in the neuropil, and observed deficits in complex memory acquisition. Overall, we would suggest that cholesterol entering the brain from the circulation of cholesterol-fed rabbits induces the neuronal accumulation of A β , and that copper influences clearance of A β from the brain at the level of the interface between the blood and cerebrovasculature.

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1. Goritz, C., Mauch, D. H., Nagler, K. & Pirieger, F. W. (2002) *J. Physiol. (Paris)* 96, 257-263.
2. Koudinov, A. R. & Koudinova, N. V. (2001) *FASEB J.* 15, 1858-1860.
3. Sparks, D. L., Martin, T. A., Gross, D. R. & Hunsaker, J. C., III (2000) *Microsc. Res. Tech.* 50, 287-290.
4. Hartmann, T. (2001) *Trends Neurosci.* 11, S45-S48.
5. Buxbaum, J. D., Cullen, E. I. & Friedhoff, L. T. (2002) *Front. Biosci.* 7, a50-a59.
6. Fassbender, K., Stroick, M., Bertsch, T., Ragoschke, A., Kuehl, S., Walter, S., Walter, J., Brechtel, K., Muehlhauser, F., von Bergmann, K., et al. (2002) *Neurology* 59, 1257-1258.
7. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (2001) *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc: A Report of the Panel of Micronutrients, Subcommittees on Upper Reference Levels of Nutrients* (Natl. Acad. Press, Washington, DC).
8. Atwood, C. S., Moir, R. D., Huang, X., Scarpa, R. C., Bacarra, N. E., Romano, D. M., Harshorn, M. A., Tanzi, R. E. & Bush, A. I. (1998) *J. Biol. Chem.* 273, 12317-12326.
9. Bush, A. I. & Tanzi, R. E. (2002) *Proc. Natl. Acad. Sci. USA* 99, 7317-7319.
10. White, A. R., Multhaup, G., Galatis, D., McKinstry, W. J., Parker, M. W., Pipkorn, R., Beyreuther, K., Masters, C. L. & Caputo, C. (2002) *J. Neurosci.* 22, 365-376.
11. Maynard, C. L., Cappia, R., Volitakis, I., Cherny, R. A., White, A. R., Beyreuther, K., Masters, C. L., Bush, A. I. & Li, Q.-X. (2002) *J. Biol. Chem.* 277, 44570-44676.
12. Robinson, S. R. & Bishop, G. M. (2002) *Neurobiol. Aging* 23, 1051-1072.
13. Regland, B., Lehmann, W., Abedini, I., Blennow, K., Joansson, M., Karlsson, I., Sjogren, M., Wallin, A., Xilinas, M. & Gottfries, C. G. (2001) *Dement. Geriatr. Cognit. Disord.* 12, 408-414.
14. Cherny, R. A., Atwood, C. S., Xilinas, M. E., Gray, D. N., Jones, W. D., McLean, C. A., Barnham, K. J., Volitakis, I., Fraser, F. W., Kim, Y.-S., et al. (2001) *Neuron* 30, 665-676.
15. Opazo, C., Barria, M. I., Ruiz, F. H. & Inestrosa, N. C. (2003) *Biometals* 16, 91-99.
16. Bush, A. I. (2003) *Trends Neurosci.* 26, 207-214.
17. Sparks, D. L., Scheff, S. W., Hunsaker, J. C., III, Liu, H., Landers, T. & Gross, D. R. (1994) *Exp. Neurol.* 126, 83-94.
18. Sparks, D. L., Liu, H., Gross, D. R. & Scheff, S. (1995) *Neurosci. Lett.* 187, 142-144.
19. Sparks, D. L. (1996) *Neurobiol. Aging* 17, 291-299.
20. Haas, U. & Sparks, D. L. (1996) *Mol. Chem. Neuropathol.* 29, 1-14.
21. Stritz, W. J. & Sparks, D. L. (1997) *J. Mol. Med.* 72, 130-138.
22. Sparks, D. L. (1997) *Ann. N.Y. Acad. Sci.* 826, 123-146.
23. Sparks, D. L. (1997) *Nutr. Metab. Cardiovasc. Dis.* 7, 255-266.
24. Sparks, D. L. (1999) in *Cerebral Cortex*, eds. Peters, A. & Morrison, J. H. (Kluwer Academic, New York), pp. 733-772.
25. Sparks, D. L., Kuo, Y.-M., Rober, A. E. & Martin, T. A. (2000) *Ann. N.Y. Acad. Sci.* 903, 335-344.
26. Wu, C.-W., Liao, P.-C., Lin, C., Kuo, C.-J., Chen, S.-T., Chen, H.-I. & Kuo, Y.-M. (2003) *J. Neural Transm.* 110, 641-649.
27. Zatta, P., Zambenedetti, P., Stella, M. P. & Licastro, F. (2002) *J. Alzheimer's Dis.* 4, 1-9.
28. Sparks, D. L., Lochhead, J., Horstman, D., Wagoner, T. & Martin, T. (2002) *J. Alzheimer's Dis.* 4, 519-525.
29. Lovell, M. A., Robertson, J. D., Tesdale, W. J., Campbell, J. J. & Markesbery, W. R. (1998) *J. Neurol. Sci.* 158, 47-52.
30. U.S. Environmental Protection Agency (1994) *Federal Register* 59, 33850-33864.
31. Buck, D. L., Seager, M. A. & Schreurs, B. G. (2001) *Behav. Neurosci.* 115, 1039-1047.
32. Schosinsky, K. H., Lehmann, H. P. & Beiler, M. F. (1974) *Clin. Chem.* 20, 1556-1563.
33. Parvizi, J., Van Hoesen, G. W. & Damasio, A. R. (2001) *Ann. Neurol.* 49, 53-66.
34. Bacskai, B. J., Kajdasz, S. T., McLellan, M. E., Games, D., Scubert, P. & Schenk, D. (2002) *J. Neurosci.* 22, 7373-7378.
35. Beach, T. G. & McGreer, E. G. (1992) *Acta Neuropathol.* 83, 292-299.
36. Sbinikai, Y., Yoshimura, M., Ito, Y., Ohtaka, A., Suzuki, N., Yanagisawa, K. & Ihara, Y. (1995) *Ann. Neurol.* 38, 421-423.
37. Erdineler, D. S., Seven, A., Inci, F., Beger, T. & Candan, G. (1997) *Clin. Chim. Acta* 265, 77-84.
38. Araya, M., Olivares, M., Pizarro, F., Gonzalez, M., Speisky, H. & Uauy, R. (2003) *Biometals* 16, 199-204.
39. Moyer, J. R., Jr., Deyo, R. A. & Distenfeld, J. F. (1990) *Behav. Neurosci.* 104, 243-252.
40. Manns, J. P., Clark, R. E. & Squire, L. R. (2000) *Learn. Mem.* 7, 257-272.
41. Bishop, G. A. & Robinson, S. R. (2002) *Neurobiol. Aging* 23, 1101-1105.

LIPID-LOWERING THERAPIES

Cholesterol, Copper, and Accumulation of Thioflavine S-Reactive Alzheimer's-Like Amyloid β in Rabbit Brain

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Abstract

Accumulation of β -amyloid (A β) in the Alzheimer's disease (AD) brain is considered to be causally related to the behavioral symptoms of the disorder. Transgenic mouse models of AD exhibit accumulation of A β in the brain and simultaneous memory deficits, and A β accumulation is enhanced if dietary cholesterol is administered. Likewise, dietary cholesterol induces neuronal accumulation of A β in New Zealand white rabbits. The cholesterol-induced accumulation of A β in rabbit brain is increased when distilled drinking water is supplemented with 0.12 ppm copper ion (as copper sulfate) compared to the cholesterol-induced accumulation of A β in rabbit brain of animals given unaltered distilled water. The numbers of affected neurons and the intensity of neuronal A β immunoreactivity is consistently increased among animals administered the copper ion in their drinking water. A copper-induced decrease in the clearance of overproduced A β from the brain is proposed as the mechanism causing A β accumulation and resulting in the observed memory deficits. Current studies reveal that intensely immunoreactive neurons, extracellular deposits of A β , and brain vessels in cholesterol-fed rabbits given copper-supplemented water were stained by thioflavine S. Thioflavine S-reactive features were not observed in cholesterol-fed rabbits given unaltered distilled drinking water. The data suggest that there is an accumulation of fibrillar A β induced in the brains of rabbits fed a cholesterol diet and administered trace levels of copper ion in their drinking water.

Index Entries: Alzheimer's pathology; cholesterol; copper; water quality; amyloid.

Introduction

The clinical impression of Alzheimer's disease (AD) is confirmed only after neuropathologic exam, based on the occurrence of characteristic lesions for the disorder to the exclusion of other dementing conditions. Such hallmark neuropathologic features of AD are senile plaques (SPs) and neurofibrillary tangles (NFTs). A sufficient number—set by convention—of both SPs and NFTs is required by most neuropathologists to affix the diagnosis of AD. In distinct contrast, there are two seemingly mutually exclusive camps of investigators, each of which consider the mechanism and genetic influences leading

to the formation of either SPs or NFTs as integral to the etiology of AD.

The predominantly investigated component of SPs is the β -amyloid (A β) peptide, but a wealth of other compounds also occurs in SPs, including cholesterol and its chaperone in the CNS, apolipoprotein E. The A β peptide is an aberrant metabolic by-product of a larger amyloid precursor protein (APP). Under normal conditions APP spans the cell membrane, with the A β portion embedded in the membrane, the N-terminal portion of the protein residing in the extracellular space, and the C-terminal portion remaining within the cell. After synthesis, APP is packaged in a vesicle, where the future extracellular domain is

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protected from premature normal proteolytic cleavage (α -secretase) in the lumen. The vesicle is transported to, and docks with, the internal membrane surface, allowing insertion of APP in the appropriate orientation.

Genetic mutations of the APP gene have been associated with production and accumulation of A β as SPs in the brains of individuals with familial AD. Accumulation of A β is presumed to be a result of genetically induced overproduction, but reduced clearance of the peptide is a likely contributing factor. Investigators have capitalized on these observations in familial AD by isolating and inserting human genetic material containing such human APP mutations into the mouse genome. Investigating the mechanism of SP formation has been facilitated by the inception of these transgenic mouse models, as well as mouse models containing co-introduced genetic information also thought to be associated with overproduction of A β (presenilin). Memory deficits and accumulation of SP-like deposits of A β occur with increasing age in transgenic mouse models of AD. A role for reduced clearance as a reason for the accumulation of A β in these transgenic mouse models comes from recent immunotherapy studies (DeMattos et al., 2001, 2002a). Introduction of A β antibodies into the blood, which need not enter the CNS proper, assist in the removal of A β from the brains of such transgenic mice. It is also proposed that as A β is cleared from the brain, some peptide is trapped in the cerebrovasculature as it exits the brain to the blood.

The effect of cholesterol on production and accumulation of Alzheimer's-like A β in the brain has gained considerable prominence in recent years. Transgenic mouse models of AD have been shown to accumulate A β earlier or in greater abundance, or both, if administered excess cholesterol in the diet (Durham et al., 1998; Li et al., 1999; Bales et al., 2000; Refolo et al., 2000; Shie et al., 2002). Culture studies have demonstrated that cholesterol is capable of shifting normal metabolism of A β precursor protein (A β PP) to production of amyloidogenic peptides and predominantly of newly synthesized A β PP (Racchi et al., 1997; Simons et al., 1998; Frears et al., 1999; Austen et al., 2000, 2003; Beyreuther 2000; Galbete et al., 2000). Analogous culture studies have also shown that inhibition of the rate-limiting step of cholesterol synthesis with a statin (β -hydroxy- β -methylglutaryl-coenzyme A reductase inhibitors) overcomes the effect of exogenous administration of cholesterol and reduces the level of A β produced

(Simons et al., 1998; Frears et al., 1999; Austen et al., 2000; Bergmann et al., 2000; Fassbender et al., 2001; Refolo et al., 2001). Similar to culture studies, experiments in which coadministration of a cholesterol-lowering drug to cholesterol-fed AD transgenic mice show reduced levels of A β in the brain, often below levels observed with the transgene alone (control diet) (Refolo et al., 2001).

Studies performed in cholesterol-fed New Zealand white rabbits as an animal model of human coronary artery disease (CAD)—based on the finding of SPs in the brains of nondemented individuals with CAD—indicate that dietary cholesterol in animals induces a pronounced accumulation of neuronal A β compared to that fed to animals on normal chow diet (Sparks et al., 1994). Further studies in the cholesterol-fed rabbit revealed at least a dozen features similar to the pathology observed in AD brain (Sparks et al., 1995, 2000; Sparks, 1996, 1997, 1999; Streit and Sparks, 1997). Recent studies suggested that the accumulation of A β in the brains of cholesterol-fed rabbits was dependent on the quality of the water the animals were administered. Animals given tap water accumulated considerably more A β in the brain than animals administered distilled water (Sparks et al., 2002). Pilot studies indicated that transgenic mice with the 695-Swedish mutation did not develop the level of A β pathology routinely observed in other investigators' hands if allowed distilled water. The neuronal accumulation of A β in cholesterol-fed rabbits was significantly affected by the quality of the water they drank. Both the intensity of the immunoreactivity observed and the number of neurons affected was greatly diminished in animals allowed distilled water. Morphologically, the neurons with A β immunoreactivity often appeared shrunken in size among the cholesterol-fed rabbits on tap water compared with animals on distilled water. Investigation of A β levels in cholesterol-fed rabbits administered tap water showed that A β levels in the blood were lower suggesting A β accumulated in the brain because of reduced clearance (Sparks et al., 2002). This is analogous to findings in mouse models of AD, where the equilibrium of A β in the brain and blood might vary with the level of its deposition in the brain (DeMattos et al., 2002a,b).

Investigation of the trace metal content in the tap water used in our experiments excluded certain agents thought to be possible culprits in the etiology of AD, including zinc (Bush et al., 1993, 1994; Cornett et al., 1998; Molina et al., 1998; Moir et al., 1999; Huang et al., 2000), aluminum, and mercury

(Fung et al., 1996; Cornett et al., 1998), but copper ion was not one of the trace metals excluded. Copper ion has gained attention because it might play a role in promoting AD (Moir et al., 1999; White et al., 1999; Atwood et al., 2000; Chen et al., 2000; Cuajungco et al., 2000; Huang et al., 2000), as chelation of CNS copper and zinc reduces levels of A β in the brains of transgenic mouse models of AD (Cherny et al., 2001). It has also been shown that A β PP and A β bind copper (White et al., 1999; Chen et al., 2000); this binding of copper produces more soluble aggregates of A β (Moir et al., 1999), which might promote hydrogen peroxide formation (Huang et al., 2000) and the neurotoxicity of A β (Cuajungco et al., 2000).

Based on the premise that copper ion in tap water promoted accumulation of A β in the cholesterol-fed rabbit brain, we determined the effect of adding trace levels of copper ion to distilled water compared to cholesterol-fed animals given unaltered distilled water. As observed in cholesterol-fed animals given tap water (containing copper ion) compared with animals administered distilled water, addition of copper ion to distilled water promoted the neuronal accumulation of A β (Sparks and Schreurs, 2003). This neuronal A β accumulation occurred in a regional and a cortical laminar distribution similar to that observed for SPs in humans with AD. Although rare among animals administered tap water, extracellular SP-like deposits were common in cholesterol-fed animals given copper-supplemented distilled drinking water. Initial assessment of the cholesterol-fed rabbits' ability to acquire complex memory using the eye-blink behavioral paradigm yielded conflicting results. Cholesterol-fed rabbits, allowed local tap water (Morgantown, WV), actually performed somewhat better than animals on normal chow (diet without cholesterol) (Schreurs et al., 2003). These animals exhibited limited neuronal accumulation and no extracellular deposition of A β (Schreurs et al., 2003), similar to cholesterol-fed animals administered distilled drinking water. Determinations revealed negligible levels of copper ion in Morgantown tap water. Subsequent studies indicated that introduction of copper ion into the drinking water of cholesterol-fed rabbits produced an 80% deficit in the ability of animals to acquire complex memory compared with animals fed the same diet and allowed unaltered distilled drinking water (Sparks and Schreurs, 2003). As noted above, a significant difference in the neuronal accumulation and extracellular deposition of A β accompanied memory deficits in cholesterol-fed animals administered

copper ion in drinking water compared with cholesterol-fed animals given unaltered distilled water. Furthermore, as noted in AD, increased levels of the copper binding protein ceruloplasmin were observed in the blood of cholesterol-fed animals on drinking water supplemented with copper ion. As with tap water, it was suggested that copper ion in the drinking water led to the inhibition of A β clearance from the brain, overproduced as a result of elevated cholesterol, thus leading to its accumulation and subsequent memory deficits.

We now report a difference in thioflavine S-reactive features in the brain and cerebrovasculature of cholesterol-fed rabbits administered distilled water supplemented with trace levels of copper ion (0.12 ppm) compared with cholesterol-fed animals given unaltered distilled drinking water.

Methods

Adolescent male New Zealand white rabbits (3000–4000 g) were housed in the rabbit facility at SHRI operating under the guidelines of the USDA with a 12:12-h light cycle, at $67 \pm 7^\circ$ F, and 45–50% humidity. Animals were randomly assigned to one of three groups as a subset of a larger IACUC-approved experimental protocol. One group of animals was administered normal chow and allowed distilled water *ad libitum* ($n = 6$). One experimental group of animals was administered a cholesterol diet (2%) and allowed distilled water supplemented with 0.12 ppm copper ion (as copper sulfate) *ad libitum* ($n = 8$; Arrowhead distilled drinking water). The other experimental group of animals was administered a 2% cholesterol diet and allowed unaltered distilled drinking water *ad libitum* ($n = 8$). Control and cholesterol diets were commercially obtained from Purina Mills, Inc. (Laboratory Rabbit Diet with and without 2% cholesterol). Dietary food intake was limited to one cup per day (8 oz.), and *ad libitum* water consumption varied between 32 and 40 oz./d.

Animals were sacrificed 10 wk after initiating the experimental dietary (food and water) protocol. Animals were administered a cocktail of ketamine and xylazine (im; 45–75 mg/kg and 5–10 mg/kg, respectively) on the day of sacrifice, and the brain was perfused via the heart under pressure with 120 mL of 4% paraformaldehyde at a constant rate of 5 mL/min using a constant pressure pump. The brain was removed and further fixed by immersion in 4% paraformaldehyde for 2 wk before sectioning.

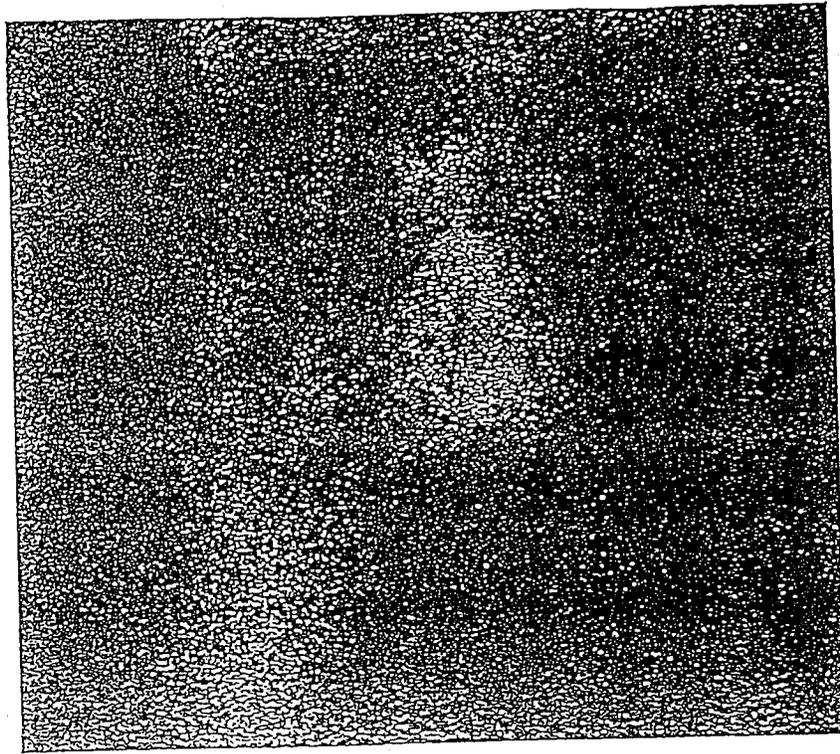


Fig. 1. A thioflavine S-reactive neuron in the temporal cortex of a rabbit fed a cholesterol diet and given distilled water supplemented with 0.12 ppm copper as sulfate. The photomicrograph width is approx 100 μ m.

Fifty-micron vibratome sections of hippocampus and hippocampal cortex of the brain were stained using standard thioflavine-S methods. Thioflavine S-stained sections were viewed and photographed using fluorescence microscopy.

Results

Thioflavine S-reactive features were only observed in cholesterol-fed rabbit administered distilled drinking water supplemented with 0.12 ppm copper ion as copper sulfate. Neurons exhibiting thioflavine S reactivity were prominent in regions of brain where there was an abundance of intensely stained A β immunoreactive neurons. Even in highly affected regions of brain, not all A β immunoreactive neurons demonstrated thioflavine S reactivity. Thioflavine S staining in affected neurons appeared granular, filling the cell body, and clearly excluded the nuclear envelope (Fig. 1).

As SP-like deposits of A β immunoreactivity occurred only among cholesterol-fed animals allowed copper-supplemented distilled drinking water, only this group of rabbits exhibited extracellular deposits of thioflavine S-reactive material (Fig. 2). Not all

SP-like deposits of A β immunoreactivity were stained by thioflavine S.

Thioflavine S-reactive blood vessels in the cortex of the copper/cholesterol rabbits were also found (Fig. 3). The observed vascular thioflavine S staining was clearly not an artifact, because the edges of the vessels were not prominently highlighted (edge artifact). Thioflavine S vascular staining occurred in medium (approx 60 μ m)- and small (approx 20 μ m)-bore vessels. The staining was not uniform throughout the vessel and seemed to occur in parallel with the length of the vessel and not around the circumference.

Discussion

We have found that some neurons exhibiting intense A β immunoreactivity also show thioflavine S staining, but only among those rabbits administered a 2% cholesterol diet and given distilled water supplemented with 0.12 ppm copper ion as sulfate. This was compared with animals provided a cholesterol diet and unaltered distilled drinking water, where enhanced A β immunoreactivity occurred but not with the intensity or numbers seen among animals with copper added to the water. In most cases, specific

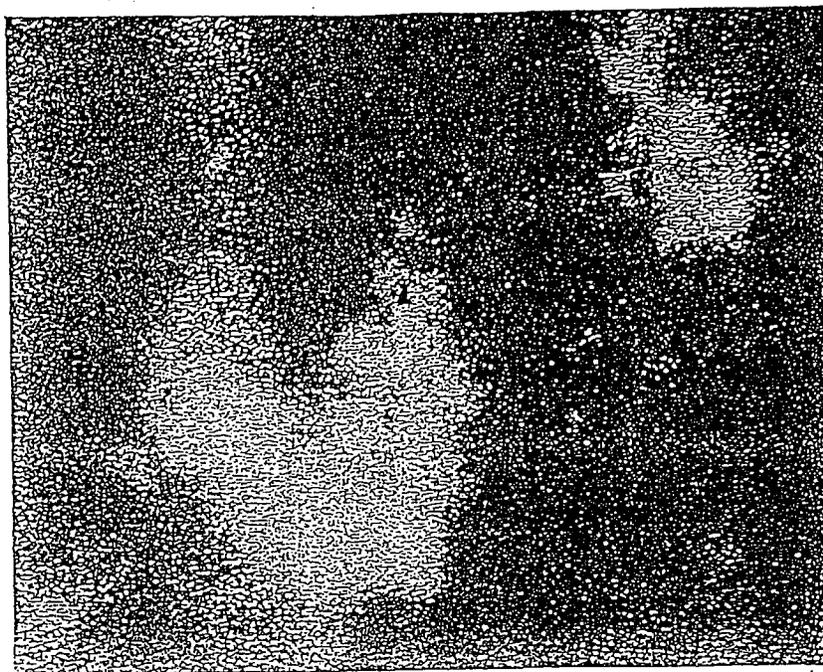


Fig. 2. A thioflavine S-reactive SP-like structure in the temporal cortex of a rabbit fed a cholesterol diet and given distilled water supplemented with 0.12 ppm copper as sulfate. The photomicrograph width is approx 100 μ m.

features of thioflavine S staining could not be discerned, as the fluorescence was too intense. In isolated cases (Fig. 1), uniform granular thioflavine S fluorescence was confined to the cytoplasm and excluded from the nucleus. The presence of thioflavine S staining, indicative of sufficient β -pleating of the A β peptide, suggests an early stage of fibril formation. Two possible reasons for the aggregation of the observed prefibrillar A β might stem from the mechanism of action of the copper ion in the setting of elevated circulating cholesterol.

One likely mechanism for the copper-induced aggregation of A β comes from the evidence that there is a copper binding site on the peptide (White et al., 1999; Chen et al., 2000) and that binding of copper produces aggregates of A β that are more soluble (Moir et al., 1999) and thus possibly more toxic (Cuajungco et al., 2000). Another hypothesis suggests that binding of copper by A β is a scavenger mechanism by which the peptide aggregates with or flocculates copper, thus inactivating the metal ion (Robinson and Bishop, 2002). Regardless of these disparate hypothesized mechanisms, chelation of CNS copper has been shown to reduce the levels of A β in the brains of transgenic mouse models of AD (Cherny et al., 2001), and the copper/zinc chelator clioquinol and the copper chelator n-penicillamine

are in clinical trials to test their benefit in the treatment of AD (Regland et al., 2001; Squitti et al., 2002; Finefrock et al., 2003). Whether there are increased concentrations of copper in the brain because of dietary exposure, as there is for cholesterol, is under active investigation. Isolated breaches of the blood-brain barrier have been noted in the cholesterol-fed rabbit brain (Sparks et al., 2000). Eventual observation of increased or no difference in CNS copper content among animals on a cholesterol/copper diet compared with those animals on normal chow/copper and cholesterol/distilled water will add support for this hypothesis or the following alternate, respectively.

The other likely mechanism for copper-induced aggregation of A β is that copper ions attenuate the effect of cholesterol-induced overproduced A β from the brain. Studies in cholesterol-fed rabbits, comparing the effect of distilled drinking water with tap water (containing trace levels of copper ion), support attenuated clearance of A β (Sparks et al., 2002). Among animals fed cholesterol and given tap water there was a significant increase of A β in the brain with only minor increments in circulation; this was contrasted with animals fed cholesterol and given distilled water where minor A β accumulations in the brain were accompanied by increased



Fig. 3. A thioflavine S-reactive blood vessel in the temporal cortex of a rabbit fed a cholesterol diet and given distilled water supplemented with 0.12 ppm copper as sulfate. The photomicrograph width is approx 400 μ m.

circulating levels. Under these conditions, as toxic levels of A β accumulate in the brain because of reduced clearance, concentration effects or failsafe metabolic pathways lead to fibrillarized aggregates within neurons and extracellular deposition if the neuron continues to be capable of ridding itself of the toxin.

The occurrence of plaque-like A β deposits in the neuropil was rare among cholesterol-fed rabbits given tap water (Sparks et al., 2002) but was a common feature in cholesterol-fed rabbits given distilled water supplemented with 0.12 ppm copper ion as sulfate (Sparks and Schreurs, 2003). Some of these plaque-like structures, but not all, showed thioflavine S staining (Fig. 2). It might be that the concentration of A β occurring in an extracellular deposit determines whether the pathologic feature

exhibits thioflavine S reactivity, or once A β is in the neuropil with efflux inhibited that it is processed into plaque-like structures that eventually exhibit thioflavine S reactivity with maturation. Concomitant microglia in the cholesterol-fed rabbit brain might support the latter possibility (Sparks, 1996; Streit and Sparks, 1997).

It was unexpected to find thioflavine S-reactive blood vessels in the cortex of copper/cholesterol rabbits (Fig. 3) because previously there has been no A β immunoreactivity observed in the cerebrovasculature of the cholesterol-fed rabbit brain. Finding such vascular thioflavine S reactivity is consistent with observations in AD brain and might support a lack of A β clearance associated with the trapping of some peptide in the blood vessels during the attempted efflux process.

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References

- Atwood C. S., Scarpa R. C., Huang X., Moiré R. D., Jones W. D., Fairly D. P., et al. (2000) Characterization of copper interactions with Alzheimer amyloid beta peptides: Identification of an attomolar-affinity copper binding site on amyloid beta1-42. *J. Neurochem.* 75, 1219-1233.
- Austen B. M., Frears E. R., and Davies H. (2000) Cholesterol upregulates production of Abeta 1-40 and 1-42 in transfected cells. *Neurobiol. Aging* 21, S254.
- Austen B. M., Sidera C., Liu C., and Frears E. (2003) The role of intracellular cholesterol on the processing of the B-amyloid precursor protein. *J. Nutr. Health Aging* 7, 31-36.
- Bales K. R., Fishman C., DeLong C., Du Y., Jordan W., and Paul S. M. (2000) Diet-induced hyperlipidemia accelerates amyloid deposition in the APPv717f transgenic mouse model of Alzheimer's disease. *Neurobiol. Aging* 21, S139.
- Bergmann C., Runz H., Jakala P., and Hartmann T. (2000) Diversification of gamma-secretase versus beta-secretase inhibition by cholesterol depletion. *Neurobiol. Aging* 21, S278.
- Bayreuther K. (2000) Physiological function of APP processing. *Neurobiol. Aging* 21, S69.
- Bush A. I., Multhaup G., Moir R. D., Williamson T. G., Small D. H., Rumble B., et al. (1993) A novel zinc (II) binding site modulates the function of the beta A4 amyloid protein precursor of Alzheimer's disease. *J. Biol. Chem.* 268, 16109-16112.
- Bush A. I., Pettingell W. H., Jr., Paradis M. D., and Tanzi R. E. (1994) Modulation of A beta adhesiveness and secretase site cleavage by zinc. *J. Biol. Chem.* 269, 12152-12158.
- Chen M., Durr J., and Fernandez H. L. (2000) Possible role of calpain in normal processing of beta-amyloid precursor protein in human platelets. *Biochem. Biophys. Res. Commun.* 273, 170-175.
- Cherny R. A., Atwood C. S., Xilinas M. E., Gray D. N., Jones W. D., McLean C. A., et al. (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 30, 665-676.
- Cornett C. R., Markesbery W. R., and Ehmann W. D. (1998) Imbalances of trace elements related to oxidative damage in Alzheimer's disease brain. *Neurotoxicology* 19, 339-345.
- Cuajungco M. P., Goldstein L. E., Nunomura A., Smith M. A., Lim J. T., Atwood C. S., et al. (2000) Evidence that the beta-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of a beta by zinc. *J. Biol. Chem.* 275, 19439-19442.
- DeMattos R. B., Bales K. R., Cummins D. J., Dodart J. C., Paul S. M., and Holtzman D. M. (2001) Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 98, 8850-8855.
- DeMattos R. B., Bales K. R., Cummins D. J., Paul S. M., and Holtzman D. M. (2002a) Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science* 295, 2264-2267.
- DeMattos R. B., Bales K. R., Parsadanian M., O'Dell M. A., Foss E. M., Paul S. M., and Holtzman D. M. (2002b) Plaque-associated disruption of CSF and plasma amyloid-beta (Abeta) equilibrium in a mouse model of Alzheimer's disease. *J. Neurochem.* 81, 229-236.
- Durham R. A., Parker C. A., Emmerling M. R., Bisgaier C. L., and Walker L. C. (1995) Effect of age and diet on the expression of beta-amyloid 1-40 and 1-42 in the brains of apolipoprotein-E-deficient mice. *Neurobiol. Aging* 19, S281.
- Fassbender K., Simons M., Bergmann C., Stroick M., Jutojohann D., Keller P., et al. (2001) Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 98, 5371-5373.
- Finefrock A. E., Bush A. I., and Doraiswamy P. M. (2003) Current status of metals as therapeutic targets in Alzheimer's disease. *J. Am. Geriatr. Soc.* 51, 1143-1148.
- Frears E. R., Stephens D. J., Walters C. E., Davies H., and Austen B. M. (1999) The role of cholesterol in the biosynthesis of beta-amyloid. *NeuroReport* 10, 1699-1705.
- Fung Y. K., Meade A. G., Rack E. P., Blotcky A. J., Claassen J. P., Beatty M. W., and Durham T. (1996) Mercury determination in nursing home patients with Alzheimer's disease. *Gen. Dent.* 44, 74-75.
- Galbete J. L., Martin T. R., Perassini E., Modena F., Bianchi R., and Forloni G. (2000) Cholesterol decreases secretion of the secreted form of amyloid precursor protein by interfering with glycosylation in the protein secretory pathway. *Biochem. J.* 345, 307-313.
- Huang X., Cuajungco M. P., Atwood C. S., Moir R. D., Tanzi R. E., and Bush A. I. (2000) Alzheimer's disease, beta-amyloid protein and zinc. *J. Nutr.* 130, 1438S-1492S.
- Li L., Zeigler S., Lindsey R. J., and Fukuchi K. (1999) Effects of an atherogenic diet on amyloidosis in transgenic mice overexpressing the C-terminal portion of b-amyloid precursor protein. *Soc. Neurosci.* 25, 1859.
- Moir R. D., Atwood C. S., Romano D. M., Laurans M. H., Huang X., Bush A. I., et al. (1999) Differential effects of apolipoprotein E isoforms on metal-induced

- aggregation of A beta using physiological concentrations. *Biochemistry* 38, 4595-4603.
- Molina J. A., Jimenez-Jimenez F. J., Aguilar M. V., Mesenguer I., Mateos-Vega C. J., Conzalez-Munoz M. J., et al. (1998) Cerebrospinal fluid levels of transition metals in patients with Alzheimer's disease. *J. Neural Transm.* 105, 479-488.
- Racchi M., Baetta R., Salviotti N., Ianna P., Franceschini G., Paoletti R., et al. (1997) Secretory processing of amyloid precursor protein is inhibited by increase in cellular cholesterol content. *Biochem J.* 322, 893-898.
- Refolo L. M., Pappolla M. A., LaFrancois J., Malester B., Schmidt S. D., Thomas-Bryant T., et al. (2001) A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* 5, 890-899.
- Refolo L. M., Pappolla M. A., Malester B., LaFrancois J., Bryant-Thomas Wang R., et al. (2000) Hypercholesterolemia accelerates Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol. Dis.* 7, 321-331.
- Regland B., Lehmann W., Abedini I., Blennow K., Jonsson M., Karlsson I., et al. (2001) Treatment of Alzheimer's disease with Clioquinol. *Dement. Geriatr. Cogn. Disord.* 12, 408-414.
- Robinson S. R. and Bishop G. M. (2002) Ab as a bioflocculant: Implications for the amyloid hypothesis of Alzheimer's disease. *Neurobiol. Aging* 23, 1051-1072.
- Schreurs B. G., Smith-Bell C. A., Lochhead J., and Sparks D. L. (2003) Cholesterol modifies classical conditioning of the rabbit nictitating membrane response. *Behav. Neurosci.* 117, 1220-1232.
- Shie F.-G., Jin L.-W., Cook D. G., Leverenz J. B., and LeBoeul R. C. (2002) Diet-induced hypercholesterolemia enhances brain Ab accumulation in transgenic mice. *NeuroReport* 13, 455-459.
- Simons M., Keller P., De Strooper B., Beyreuther K., Dotti C. G., and Simons K. (1998) Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6460-6464.
- Sparks D. L. (1996) Intraneuronal beta-amyloid immunoreactivity in the CNS. *Neurobiol. Aging* 17, 291-299.
- Sparks D. L. (1997) Dietary cholesterol induces Alzheimer-like beta-amyloid immunoreactivity in rabbit brain. *Nutr. Metab. Cardiovasc. Dis.* 7, 255-266.
- Sparks D. L. (1999) Neuropathologic links between Alzheimer's disease and vascular disease, in *Alzheimer's Disease and Related Disorders*, Iqbal K., Swaab D. F., Winblad B., Wisniewski H. M., eds., John Wiley, Chichester, West Sussex, England, pp. 153-163.
- Sparks D. L. and Schreurs B. G. (2003) Trace amounts of copper in water induce beta-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1065-1069.
- Sparks D. L., Kou Y.-M., Roher A., Martin T. A., and Lukas R. J. (2000) Alterations of Alzheimer's disease in the cholesterol-fed rabbit, including vascular inflammation. Preliminary observations. *Ann. N. Y. Acad. Sci.* 903, 335-344.
- Sparks D. L., Liu H., Gross D. R., and Scheff S. W. (1995) Increased density of cortical Apolipoprotein E immunoreactive neurons in rabbit brain after dietary administration of cholesterol. *Neurosci. Lett.* 187, 142-144.
- Sparks D. L., Lochhead J., Horstman D., Wagoner T., and Martin T. (2002) Water quality has a pronounced effect on cholesterol-induced accumulation of Alzheimer amyloid b (Ab) in rabbit brain. *J. Alzheimer Dis.* 4, 523-529.
- Sparks D. L., Scheff S. W., Hunsaker J. C. III, Liu H., Landers T., and Gross D. R. (1994) Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp. Neurol.* 126, 88-94.
- Squitti R., Rossini P. M., Cassetta E., Mofia F., Pasqualetti P., Cortesi M., et al. (2002) D-penicillamine reduces serum oxidative stress in Alzheimer's disease patients. *Eur. J. Clin. Invest.* 32, 51-59.
- Streit W. J. and Sparks D. L. (1997) Activation of microglia in the brains of humans with heart disease and hypercholesterolemic rabbits. *J. Mol. Med.* 75, 130-133.
- White A. R., Reyes R., Mercer J. F., Camakaris J., Zheng H., Bush A. I., et al. (1999) Copper levels are increased in the cerebral cortex and liver of APP and APLP2 knockout mice. *Brain Res.* 842, 439-444.

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Mental Block

Maverick Scientist Is Winning Converts On Alzheimer's

Dr. Bush Was Widely Derided
When He Said Zinc, Copper
Played Role in Disease

Tainted by Aluminum Brush

By BERNARD WYSOCKI JR.

BOSTON—Ashley Bush, a 44-year-old researcher at Harvard Medical School, was pilloried after he put forth a radical theory of Alzheimer's disease in 1994.

"Worthless," wrote one scientific critic. Others have described his style as brash, his content as flimsy, and his ideas unworthy of being published. At worst, Dr. Bush recalled, it felt like "nate mail."

Over the years, he submitted 30 scientific papers that were rejected by scientific journals. Eight times, his grant applications were spurned by the National Institutes of Health.

Dr. Bush's theory is that the real culprit in Alzheimer's is a copper and zinc buildup in the brain—an idea few scientists have looked at. He believes the accumulated metals mix ab-

normally with a protein called beta amyloid in the brain, oxidizing—literally rusting—and destroying nerve cells. Published in the prestigious journal Science, his hypothesis swiftly drew criticism because it ran counter to the leading theory that Alzheimer's disease is caused mainly by the protein clumps themselves. And by highlighting metals as the



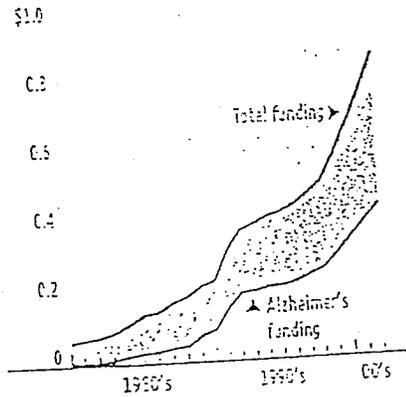
Ashley Bush

Now scientists are giving Dr. Bush more credence. He has a five-year grant from the NIH and this year won an American Academy of Neurology prize for Alzheimer's disease research.

One big reason: He is on the trail of a drug that absorbs his culprits—the ex-

Growing Share

Total funding for the National Institute on Aging and amount dedicated to Alzheimer's disease, in billions



Source: NA Budget Office

cess copper and zinc—and dissolves the protein clumps in the brains of experimental animals. Dr. Bush has found a potential Alzheimer's treatment in a 70-year-old dysentery drug with a history of toxic side effects. What's more, he and his colleagues this month published their first human clinical trial showing the drug's promise. "It's like Drano," he says. "It blows them away."

The small trial's results are "significant" and "innovative," says Roger Rosenberg, a neurologist at the University of Texas Southwestern Medical Center and editor of the Archives of Neurology, which published the research.

Dr. Bush's odyssey shows how rejects in the world of science can sometimes re-emerge as important figures. The history of science in the last 50 years could be written with papers rejected by prestigious journals, observed

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Scientist Who Converts to Alzheimer's Theory

Continued From First Page

Paul Lauterbur of the University of Illinois after he won the 2003 Nobel Prize for medicine. His original paper on his prizewinning achievement, magnetic resonance imaging (MRI), was initially rejected in 1973 by *Nature*, which later ran it.

It's far from clear whether Dr. Bush has unearthed a new key to Alzheimer's that yields a treatment, or just a dry hole. Nobody knows if his drug will work any better than the handful of therapies on the market or the dozens more in the pipeline.

Alzheimer's is a degenerative brain disease, affecting about four million Americans in the U.S. alone. Although not considered part of normal aging, Alzheimer's attacks mostly elderly people. About 1% of those age 65 show symptoms, a rate that surges to nearly 50% by age 85. Its main feature is memory loss, but in advanced stages, the disease erodes personality, judgment, powers of speech and the ability to perform the functions of daily living.

Abnormal Proteins

The brains of Alzheimer's victims, when examined at autopsy, appear speckled with two kinds of abnormal protein. One is beta amyloid clumps between brain cells, known as plaques. The other is neurofibrillary tangles, or protein strands that look like knotted skeins of yarn, inside cells. Nobody knows for sure whether these clumps and strands are causes or merely byproducts of the disease. But to many mainstream researchers, the amyloid proteins are the leading suspects.

This is where Dr. Bush breaks from the crowd. He believes that amyloid clumps aren't the ultimate villain, but more of an accomplice in the relentless destruction wrought by the disease. "The classic amyloid cascade hypothesis is wrong," he insists.

In Dr. Bush's view, amyloid protein plays a helpful role in the brain: absorbing metals like a sponge. But in Alzheimer's victims, he contends, the metals overwhelm the protein. He believes that copper mixes abnormally with amyloid, releasing hydrogen peroxide and other toxic chemicals that damage the nearby cells. Some of that protein breaks free, becomes "rogue" amyloid, and mixes with zinc to form clumps that leak more hydrogen peroxide. Thus he indicts metals as the real culprits. This theory is still controversial.

Some critics see his metals theory as mere speculation. "Based on science, there is no substantiation for what Ashley says," says Bruce Yankner, professor of neurology at Harvard. "Ashley's ideas are interesting. But that's what they are—interesting."

One problem in verifying Dr. Bush's

als exist in the brain, but Dr. Bush contends that excessive amounts build up in some aged people. Among unanswered questions is where the metal buildup comes from. Dr. Bush doesn't claim to know.

Among Alzheimer's baffling aspects is the fact that it might have multiple causes. A small fraction of the population carries rare mutant forms of certain genes that increase the risk of Alzheimer's. At the same time, many researchers believe that such things as nutrition, exercise and mental stimulation may also play a role in keeping dementia at bay.

In recent years, a handful of drugs have hit the market to treat Alzheimer's, from Pfizer Inc., Johnson & Johnson and other companies, but these offer limited relief. The big challenge is to bring drugs to market that attack the underlying cause of the disease. Because the ultimate cause of Alzheimer's is unknown, different theories have fueled the pursuit of different kinds of drugs. Some aim to prevent the "snipping" of amyloid protein into fragments that form plaques. Dr. Bush considers this misguided, since he believes it's the metals interacting with the protein that do the damage.

The son of Jewish refugees from Poland and Palestine who fled their homes for Australia during World War II, Dr. Bush grew up in Melbourne. As a medical student at the University of Melbourne, he started training as a surgeon, then switched to psychiatry.

He was drawn to Alzheimer's after working in the mid-1980s as a young physician at a mental institution, where he saw 700 patients with dementia who were given little in the way of diagnosis or treatment. He started studying the possible role of zinc while in Melbourne. He was inspired by a University of Texas researcher, Christopher Frederickson, who detailed the presence of zinc traces in the brain. Dr. Bush saw that the zinc and the amyloid were in the exact same spots, giving him a clue that they were somehow inter-related. He stayed on the trail when he moved to Harvard and the affiliated Massachusetts General Hospital in 1992.

Working in the lab of neurologist Rudolph Tanzi, he found that by adding zinc to dead brain tissue in test tubes, the amyloid suddenly formed clumps that looked like Alzheimer's plaques. This was a possible clue that the metals also played a role in triggering the plaques in the living brain as well. Dr. Bush submitted the paper to the prestigious journal *Science*. Dr. Tanzi warned he was shooting for the moon. But the paper was published in 1994.

"He started out extremely lucky," says Dr. Tanzi, his co-author on this and other papers. More luck followed. He ob-

co-founded in 1997.

But Dr. Bush's luck soon soured. His work was "bucking conventional wisdom," says Dr. Tanzi. He drew attacks from mainstream Alzheimer's researchers who were asked to review his submissions to peer-reviewed journals. One of the reviewers, who are traditionally shielded by anonymity, was especially savage.

"I do not think the manuscript is worthy of being published in *Nature* or elsewhere," the reviewer wrote, blasting the manuscript as "worthless." Another critic chastised him for muddying the already turbid waters of Alzheimer's research.

Rising Frustration

By 1999, his frustration rose as grant funds ran low. Like many researchers in academia who lack endowed chairs, Dr. Bush isn't paid by Harvard. He runs his lab and pays salaries out of grants and other such funds.

He had hoped a grant from the National Institute on Aging, part of the NIH, would solve his woes. But Dr. Tanzi judged some of his protegee's proposals as flimsy and ill-crafted. While he admires Dr. Bush's originality and experiments, Dr. Tanzi says he remains dubious about some of his theories. Dr. Bush, he jokes, is considered the "wacky uncle" around the lab.

Meanwhile at closed-door NIH meetings, grant reviewers weren't so jocular. They issued a pointed challenge to his work. Dr. Bush recalls. He says these outside experts asked: "If you are so sure this is the cause of Alzheimer's disease, where is your drug?"

"Why am I having such a difficult time?" Dr. Bush recalls asking NIH after his rejections.

But it turned out that Dr. Bush had an ally at NIH. He was Stephen Snyder, a Ph.D. in pathology at the National Institute on Aging. Dr. Snyder oversees grant applications dealing with the origins of Alzheimer's. He heard reviewers complain because Dr. Bush's applications were long on brain chemistry and short on biology. Dr. Snyder passed all this along to Dr. Bush, and vowed to help Dr. Bush improve his applications.

It also turned out that Dr. Bush was indeed pursuing a treatment. Working with mice given a gene for Alzheimer's, Dr. Bush tested oral doses of a 70-year-old drug called clioquinol, versus placebos. When Dr. Snyder learned of this, he quickly asked to see the data. After nine weeks, the treated mice had a 49% reduction of beta amyloid deposits.

"Holy Finoki!" Dr. Bush e-mailed Dr. Tanzi.

In late 1999, Dr. Bush sent a photographic slide of the results to the aging institute. Dr. Snyder remembers think-

plaques. The treated mice had brains as clean as the day they were born." He recalls deciding, "I'm going to go to the wall for this."

Dr. Bush crafted his ninth NIH grant proposal. The review committee gave it a score in the top third—not great, but enough to get a \$750,000, five-year grant.

"The mice came along at the right time," Dr. Snyder says. The journal *Neuron* published the mouse study. In Melbourne, Prana, the biotech company Dr. Bush co-founded, prepared to launch human clinical trials.

But there was a problem: Cloquinol had a disastrous history. It was introduced in the 1930s by Swiss drug giant Ciba-Geigy AG, as a treatment for amoebic dysentery, a potentially deadly intestinal ailment. The drug was later promoted in Japan for all types of stomach trouble. By 1970, however, nearly 10,000 people who had been treated with the drug, mostly in Japan, developed paralysis or blindness.

These days, some scientists believe the adverse effects might have been influenced by a vitamin B-12 deficiency in the postwar Japanese diet. So Prana added vitamin B-12 supplements to the cloquinol in the Alzheimer's study. That did the trick, the company says.

Prana's randomized double-blind clinical trial was launched in 2000 and completed by 32 volunteers in 2002. Half of them got cloquinol; half got a placebo. In spring of 2002, Colin Masters, chairman of Prana's scientific board, gave the first peek at the results, declaring Alzheimer's disease was slowed by the drug.

This month the *Archives of Neurology* published the full report. The results: Volunteers on placebo showed a "substantial worsening" of the disease based upon cognitive tests, while people on cloquinol experienced "minimal deterioration." In addition, blood levels of beta amyloid protein in the blood declined among those taking the drug but increased in those on placebo. As for side effects, the drug was "well-tolerated," wrote Dr. Bush and his co-authors from the U.S., Europe and Australia. One participant, who had a history of hypertension and glaucoma, suffered impaired vision during the trial. But the symptoms disappeared when the trial ended.

Now scientists in the U.K., Japan and the U.S. are pushing forward with research on cloquinol or drugs like it. Doctors at Duke and Thomas Jefferson Universities are planning large-scale clinical trials. There are some glitches. Prana is in a patent dispute with onetime collaborators at a Greek pharmaceutical company, PN Gerolymatos SA. (It didn't respond to requests for comment.) Meantime, Prana is working on a drug similar to cloquinol.

In retrospect, Dr. Bush concedes he was treated fairly by the NIH. "I just chose to pursue a subject that defied fashion," he says. "A good scientist needs a

Grounding Can Affect Water Quality

S. J. Duranceau, G. E. C. Bell, M. J. Schiff, R. M. Powell, and R. L. Bianchetti



The practice of using water piping as part of the grounding system of the building has been common place for more than 80 years¹. The practice was predicated on the assumption that with grounding there was little or no effect of alternating current (ac), as compared to direct current (dc), on the corrosion behavior of metals¹. When electrical transformers serve multiple buildings and customers, this results in the water service piping and distribution piping acting as both grounding electrodes and transformer neutral return paths for the shared electrical systems^{1,2}. The National Electrical Code has mandated since 1923 that the external buried water service piping be used as all or part of the ground electrode system^{1,3}.

From the beginning, the water utility industry had concerns regarding the effect of grounding currents on water quality. Early studies on the effects of grounding on water quality, including Warren⁴, Ellassen⁷, American Research Committee on Grounding⁵, and Ellassen and Goldsmith⁶. These studies were primarily concerned with the effects of grounding on water quality in terms of odor and taste. Alternating and direct current studies were conducted along lengths of electrically continuous metallic water service piping. Changes in metal content of the water were measured using the analytical techniques available at the time. These early studies concluded that the presence of ac and dc current on electrically continuous metallic water service piping did not effect water quality.

The promulgation of the Safe Drinking Water Act Lead and Copper Rule in 1991 again raised concerns about the effect of grounding currents on water quality. A recent study by the Orlando Utilities Commission¹⁰ was performed using a model house system. The study concluded that grounding currents flowing on electrically continuous potable water pipes do not cause characteristic and distinct increases in metal concentrations for the conditions investigated. However, post-testing metallographic examination of the pipe surfaces indicated changes in protective oxide films, suggesting a depolarization effect of ac current. Further, increases in metal concentrations were measured during lightning storms. Similar results regarding effects of lightning on internal corrosion and metal release were reported by Guererra¹¹.

As part of a research project for the American Water Works Association Research Foundation entitled *The Effects of Electrical Grounding on Pipe Integrity and Shock Hazard*¹², the increasing use of plastic service piping and other dielectric or electrically insulating connections in water services became apparent. In addition, field research found that rubber gasketed water meter connections can become resistive over time and effectively act as electrical insulators. Electrical resistance or insulation in the normally conductive pipe will force some current to flow through the parallel internal water and external soil paths. Discharge of current on the inside of the pipe increases metal concentrations in the service line, while discharge of current on the exterior of the pipe could increase service line failures.

Previous studies on the effects of grounding on water quality and metal release did not investigate the effects of resistance or dielectric fitting on water quality. These earlier studies^{3,4} applied either ac or dc or both ac and dc to an electrically continuous section of pipe and measured the change in metal ion concentration over a period of time. Without significant resistance in the metal pipe wall, electrical current continues to flow in the much more conductive pipe wall¹³. No current is discharged on the inside or the outside of pipe and it is not surprising that no effect on water quality was previously found

when electrically continuous pipe sections were tested. In this study, the effects of dielectric fittings on water quality and metal release was studied.

EBMUD and PCU Studies

EBMUD and Pinellas County Utilities (PCU) agreed to participate in the water quality study. The objectives of the additional studies were:

- To repeat and confirm preliminary screening results from field and lab tests performed as part of the pipe integrity study¹²;
- To investigate the effects of short sections of plastic pipe as insulators on water quality; and,
- To evaluate the effect of lead solder surfaces and solder joints on metal (lead, copper and zinc) release.

Test Conditions And Test Article Design

Four voltages (120, 25, 10, and 5 volts-ac) were intended to be investigated in the follow-on testing. Three different types of test articles ("A", "B" and "C") were used in the testing for three lengths of time (24, 72, and 168 hours). Each type of test article had an associated control with no applied voltage for 168 hours. Test articles "A" investigated dielectric unions on lead-free soldered copper pipe systems. Test articles "B" investigated the use of 6 in. of PVC as an insulating method. Test articles "C" investigated the effects of dielectric unions on systems where lead solder was used to make joints with copper. The test articles were wired in parallel so that the applied voltage across all the test articles were equal. A 10-ohm resistor ($\pm 2\%$) on the neutral side of the test articles acted as a current measuring shunt for each test article.

Data Collection Procedures

At start-up of a test series (i.e. 120 V-ac, 25V-ac and 5V-ac) measurements of ac voltage and 10 ohm resistor voltage drop (10 millivolts = 1 milliamperes) every 5 minutes were made for the first half hour. After this initial start-up period, at least three daily measurements (morning, noon, and end of day) of ac voltage across insulator or PVC tubing along with current flowing in each 10 ohm resistor were made. It is recommended that measurements should be made with properly rated test leads and meters by personnel trained with respect to electrical safety and familiar with the hazards related to the tests.

At the end of the test period for each test article (24, 72 and 168 H), the power was turned off and the test article water emptied into a labeled container. The power was then turned back on and the start and stop times for the power were recorded. The temperature, pH and dissolved oxygen of the water sample were measured on site per EPA approved method. Laboratory analysis of the test article water samples and control samples per EPA approved methods for TDS and/or conductivity, total and calcium hardness, total alkalinity, lead, copper, and zinc were performed.

Results

Tests were identified by the test article, applied voltage, and exposure time. For example, A-005-024 designates the test conducted with test article type "A" with 5 Vac applied across the insulator for 24 h. Unfortunately, PCU was unable to perform tests at 120V due to high currents which caused shorting across one of the dielectric unions and increased water temperature above 60°C. Measured resistances were about four times lower at PCU ground water as compared with the EBMUD snow melt water. PCU conducted tests with a maximum of 50 Vac applied voltage instead of the 120 Vac.

Figures 1 through 3 depict the dependence of metal pick up of the water as a function of the product of the average milli-amperes of ac and the contact time (ac-ma*h) for Cu, Zn and Pb for some of the testing performed at EBMUD and PCU.

For the A-series test articles, which consisted of copper tubing separated by a dielectric union fabricated with lead-free solder, copper, zinc and lead increased with increasing ac-ma*h for both the EBMUD and PCU data. The level of metal release was less for PCU as compared to EBMUD, despite the fact higher currents were present at PCU as compared to EBMUD. Total metal concentrations were substantially lower for both PCU and EBMUD for the B-series, which consisted of copper tubing separated by 6 inches of PVC pipes as an insulating connector. The lower metal concentrations are directly attributable to the higher resistance and, thus, lower currents present in the test systems. EBMUD showed significant copper pick-up with very little zinc increase. Lead release in the EBMUD-B series was sporadic and in some cases significant.

The source of the lead in the EBMUD tests was probably brass plugs in the test articles. PCU data for B-series test articles showed low copper pick-up but significant zinc release. The source of zinc is not clear and may be related to solder fluxes used during fabrication. Lead levels were low for the PCU B-series tests when plastic plugs were used in place of brass plugs to seal the end of the test article.

For the C-series test articles, which consisted of copper tubing separated by a dielectric union with 50:50 lead:tin solder surfaces exposed to the water, copper, zinc and lead increased with increasing ac-ma*h for both the EBMUD and PCU data. The level of copper releases was less for PCU as compared to EBMUD, despite the fact higher currents were present at PCU as compared to EBMUD. Levels of zinc were somewhat higher for EBMUD, and lead increases for PCU were higher than EBMUD.

Discussion

Copper, lead and zinc release were significant and in many cases exceeded the action levels for primary or secondary drinking water standards. Differences in metal release behavior between the PCU and EBMUD are obvious and indicate that the differences are due to the different sources and treatment of water at the two utilities.

The mechanism of ac corrosion has been postulated to be caused by rectification of alternating current to direct current by "metallic" rectifiers at the metal/oxide interface 3, 15, 17. In metallic rectifiers such as copper/copper oxide the rectifying junction is between the oxide semiconductor and the metal surface. The direction of the current is from the semiconductor to the metal. For copper surfaces, copper oxides act as the semiconductor on the copper metal surface and

produces a half wave rectifying circuit which results in net direct current.

PCU is a groundwater source while EBMUD is a surface water source utility. As such, PCU has much lower dissolved oxygen (DO = -2 mg/l) in their make-up water as compared to EBMUD (DO = -10 mg/l). Since the formation of copper oxide is related to the amount of oxygen present in the make up water

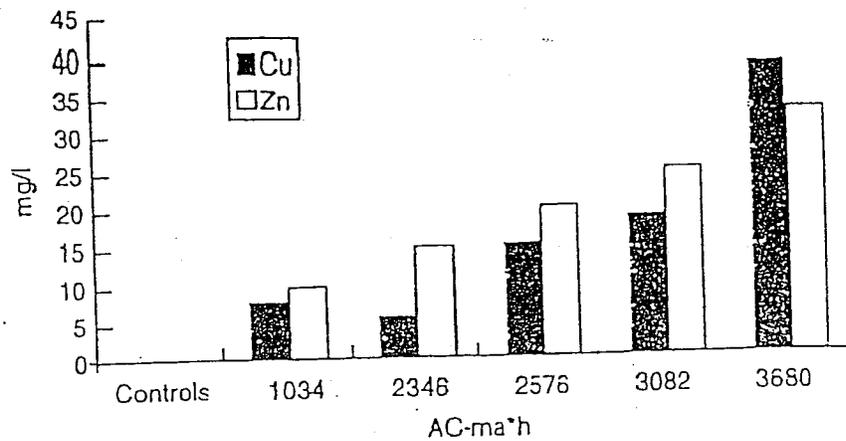


Figure 1 Copper and zinc concentrations as a function of AC-ma*h for initial scoping tests at EBMUD with 123 VAC applied

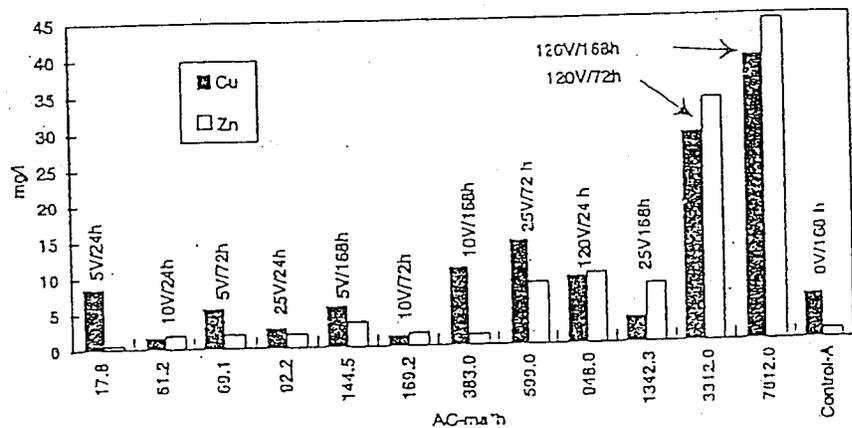


Figure 2 Copper and zinc concentrations as a function of AC-ma*h for follow-on tests at EBMUD with Type "A" test articles

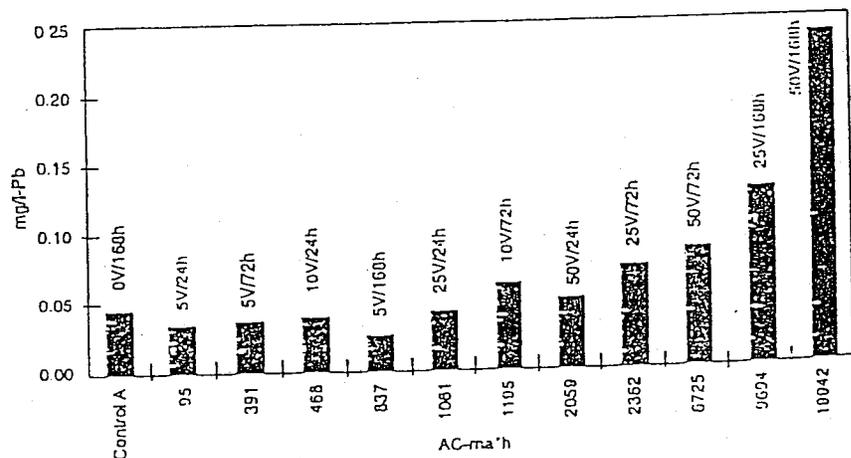


Figure 3 Lead concentration as a function of AC-ma*h for follow-on tests at PCU with Type "A" test articles

and dissolved oxygen would be consumed in corrosion reactions which are causing metal release, the reduction in DO was correlated with the increase in metal ion equivalents in the solution.

For EBMUD conditions of higher initial DO, there is good correlation between increased metal ion content of the water and the reduction in DO. The correlation does not hold for the lower initial DO PCU data. This suggests that DO plays a role in the magnitude of the release of metal. In addition to the differences in DO, PCU utilizes a blended phosphate corrosion inhibitor to control copper corrosion. EBMUD does not use a corrosion inhibitor. The presence of the inhibitor probably contributes to differences in metal release with respect to copper and zinc, between the two utilities.

In addition, the literature and data collected in the EBMUD and PCU lab testing show that voltages and currents which increase metal release on the interior of the copper piping with insulators in the water service can also increase soil-side corrosion on the exterior of underground copper water services. Whether or not this increase in corrosion rate due to ac is significant over the life of the building is unknown.

Summary And Conclusions

A series of tests were conducted to investigate the effect of electrically insulating unions on water quality in the presence of applied alternating current voltages of 123 and 50 volts. The following conclusions can be drawn from the data presented:

1) The data indicate that there was a pronounced effect of ac voltage on copper release for both the 123 and 50 volt-ac tests. Copper and lead contents measured after 24 hours of exposure at the lower applied voltage exceeded EPA Lead and Copper action levels, 0.015 mg/l and 1.3 mg/l for lead and copper, respectively.

2) Metal release generally increased with charge transfer, in accordance with Faraday's law.

3) Based on the metal release data collected in these limited tests, the ac corrosion rate is calculated as 0.14% of the corresponding dc rate for the conditions investigated here.

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References

1. Steffan, R. F. "Grounding of Electric Circuits to Water Services: National Electric Code Versus AWWA Policy." *JAWWA*, p. 82, (February 1980).
2. Kennedy, R. C. Ground-Wire Attachments To Water Pipes. *JAWWA*, 44(5): 383-386 (1952).
3. Waters, F. D. A. C. Corrosion. *Materials Protection*, 1, (March):26-32 (1962).
4. Hertzberg, L. B. "The Water Utilities Look At Electrical Grounding." *IEEE Transactions*, IGA-6(3): 278-281 (1970).
5. National Electrical Code. Batterymarch Park, MA: National Fire Protection Association- 1993.
6. Warren, H. S. Electrical Grounds on Water Pipes. *J. New England Water Works Association* 48(September):350-371 (1934).

7. Ellassen, R. Program For Research On The Effects of Electrical Grounding On Water Pipes. *JAWWA*, 33(9): 1541 - 1552 (1941).
8. American Research Committee on Grounding. Interim Report of Investigations. *JAWWA*, 36(April): 383-405 (1944).
9. Ellassen, R. and P. Goldsmith. "Effects of Grounding on Water Pipe", *JAWWA*, 36, 563, (May 1944).
10. Orlando Utilities Commission and CH2M Hill, *The Evaluation of the Effects of Electrical Grounding on Water Quality*. AWWARF, Denver, CO. (1994).
11. Guerra, A. A. Experience With Grounding of Electrical Circuits To Water Pipe. In Prog. 1979 AWWA Annual Conference, San Francisco, CA: AWWA (1979).
12. Bell, G.E.C., M.J. Schiff and S.J. Duranceau. "Observation of the Effects of Grounding on Water Piping." *CORROSION/95*, Paper No.95603, March 28-31, 1995, Orlando, FL.
13. Rossun, J.R. "Corrosion from Improper Grounding Questioned." In Letters to the Editor, *JAWWA*, 73:1:38 (1981).
14. Hamlin, A. W. "Alternating Current Corrosion." *Materials Performance*, 25(1): 55-58(1986).
15. Kulman, F.E. Effects of Alternating Currents Causing Corrosion. *Corrosion*, 1(3): 34-35 (1961).
16. Gallimberti, C.E. Corrosion of Lead By Alternating Current. *Corrosion*, 20(5): 150t-157t (1964).
17. Bruckner, W. H. "Soil Corrosion of Steel By Alternating Currents." In Prog. 19th Annual Conference of National Association of Corrosion Engineers. Houston, TX: NACE International (1963).
18. Williams, J. F. "Corrosion of Metals Under the Influence of Alternating Current." *Materials Protection*, 5(2): 52-53(1966).

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SUMMARY OF LITERATURE SEARCH ON COPPER LEACHING INTO DRINKING WATER FROM COPPER PIPE

The following summary has three components:

- A. Known and Suspected Toxicity of Copper to Humans and Other Living Organism.
- B. Pipes Leaching Copper and Other Contaminants Into The Drinking Water.
- C. Scientific Community Recommendations on Copper in Drinking Water.

A Known and Suspected Toxicity of Copper to Humans and Other Living Organism

- 1. From CalEPA's Office of Environmental Health Hazard Assessment:

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHHA) to adopt Public Health Goal (PHGs) for contaminants in drinking water based exclusively on public health considerations. The PHG technical support document provides information on health effects from contaminants in drinking water. The PHG describes concentrations of contaminants at which adverse health effects would not be expected to occur, even over a lifetime of exposure. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

A PHG of 170 ppb has been developed for copper in drinking water.

Copper does not appear to be carcinogenic in animals or humans, therefore the PHG is based on noncarcinogenic effects. The PHG is based on gastrointestinal effects in children, the sensitive group for this chemical. In one case report of a Vermont family that consumed drinking water with a copper concentration of 7.8 mg/L, a seven-year-old girl experienced abdominal pain and a five-year-old girl experienced episodes of vomiting and abdominal pain after drinking the water. To calculate the lowest-observed-adverse-effect-level (LOAEL) the water consumption of the two girls was estimated at one liter per day. An uncertainty factor of 10 was employed to extrapolate from an LOAEL to a no-observed-adverse-effect-level (NOAEL), and a relative source contribution of 80% was assumed. Based on these assumptions, OEHHHA calculates a PHG of 0.17 mg/L (170 ppb) for copper in drinking water.

2. The following health and environmental problems have been identified by various health organizations and groups as potential toxic problems associated with copper in drinking water. The below comes from the Environmental Defense and have hyperlinks attached.

| <u>Human Health Hazards</u> | <u>Reference(s)</u> |
|---|-----------------------------------|
| <u>Cardiovascular or Blood Toxicant</u> | <u>KLAA</u> |
| <u>Developmental Toxicant</u> | <u>EPA-SARA</u> |
| <u>Gastrointestinal or Liver Toxicant</u> | <u>ATSDR DOSS KLAA RTECS ZIMM</u> |
| <u>Kidney Toxicant</u> | <u>MERCK</u> |
| <u>Reproductive Toxicant</u> | <u>EPA-SARA FRAZIER</u> |
| <u>Respiratory Toxicant</u> | |

3. The National Research Council (NRC)¹ stated that the current health level established by U.S. EPA for Cu is for acute exposure and is not suitable for establishing a health base level for MCLG (maximum contaminant level goal). The need to develop a chronic exposure level is necessary and will be much lower concentration.
4. The average absorption of Cu by the body is controlled by the liver function and is 30% to 40% and is influenced by age and genetic background. (see NRC 2000 Report)
5. Infants fed formula with tap water are much more sensitive to elevated copper in water because they have a higher absorption rate and reduced capacity to excrete copper as those of an older age. (see NRC 2000 Report)
6. There is the potential role of genetics that underlie infant and childhood copper toxicosis. (see NRC 2000 Report)
7. Evidence suggests that when the bodies ability to regulate Cu is surpassed by excess Cu, a large amount of Cu is released into the bloodstream damaging red blood cells and causing acute hemolytic anemia. (see NRC 2000 Report)
8. Because reproductive and development effects are affected by small amounts of Cu intrauterine devices by preventing embryogenesis by blocking implantation and blastocyst development that Cu exposure during the early postnatal period requires additional study to determine teratology during pregnancy. (see NRC 2000 Report)
9. The ingestion of Cu should be cautioned against because of the possibility of the hepatic (liver) susceptibility. (see NRC 2000 Report)

¹ At the direction of Congress, U.S. EPA asked the National Research Council (NRC) to review independently the scientific and technical basis for U.S. EPA's health level for copper in drinking water. The Committee members were from the fields of toxicology, epidemiology, pathology, pharmacology, genetics, physiology, medicine, public health, exposure assessment, nutrition, chemistry, biostatistics, and risk assessment. The Committee reviewed available toxicological, epidemiological, and exposure data and made specific recommendations in their 2000 published report titled "Copper in Drinking Water," Committee on Copper in Drinking Water, Board of Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council.

10. There is an association between liver toxicity and copper in sensitive population (Wilson Disease-2% of population). (see NRC 2000 Report)
11. A Wisconsin Department of Health and Social Service's Division of Health study stated Health studies have found that copper in drinking water can add 4 to 45 percent more copper to a person's diet than what is in food sources.
12. In sensitive human populations, the majority target of chronic copper toxicity is the liver and neurological toxicity with those with Wilson disease. (see NRC 2000 Report)
13. The liver and brain are targets of copper toxicity in patients with Wilson disease. (see NRC 2000 Report)
14. Excess digestion of copper in drinking water can cause nausea, diarrhea, vomiting, and intestinal cramps. Severe cases of copper poisoning have lead to anemia and to the disruption of liver and kidney functions. They also sated that individuals with Wilson's and Menke's disease (genetic disorders resulting in abnormal copper absorption and metabolism) are at a higher risk from copper exposure than the general public and can have serious health problems. (see NRC 2000 Report)
15. Chronic exposure to excess copper causes liver toxicity and a number of chronic cases of liver toxicity have been reported. (see NRC 2000 Report)
16. Dr Lewis Mehl-Madrona, M.D., Ph.D, Program Director, Center for Health and Healing, Beth Israel Hospital/Albert Einstein School of Medicine reported that studies suggest that environmental factors associated with learning disabilities such as Attention-Deficit/Hyperactivity Disorder (ADHA) have found correlations between certain toxic agents such as copper accumulating in brain tissue.
17. Dr. William Walsh, Ph.D., Co-Founder and Senior Scientist for "The Health Institute and Pfeiffer Treatment Center" suggested that studies point to a potential correlation for Autism Disorder and copper injuston that could impair neuronal development, especially in the first 30 months of life, which could result in incomplete maturation of the G.I. track and brain.
18. Research is being carried out by Ashley Bush, Harvard Medical School and the University of Melbourne and the PRANA Biotech School Melbourne that studies the a link of copper accumulation in the brain that causes a buildup of hydrogen peroxide which induces amaloid plaques in the brain i.e., Alzheimer's disease.
19. An old antibiotic, Clioquinoline, is now being tested on 50 Alzheimer's patients according to Dr. Ashley Bush of Massachusetts General Hospital and Harvard Medical School. The drug was effective in mice experiments because it removed copper and zinc in amaloid plaques in the brain that are a major feature of alzheimer's. There was a 51 percent reduction in the plaques in the mice and the hope is that for humans it will aid the brain to "heal" itself to "clear out the mess" causing Alzheimer's.
20. In 1992 a 6 week old girl was diagnosed with methemoglobinemia induced by simultaneous exposures of copper at levels close to the federal drinking water standards. The study stated that drinking water that stands overnight in copper pipes may often contain copper levels that exceed federal drinking water standards and that the water should be flushed prior to drinking water that stands overnight in copper pipes according to the investigation by the Wisconsin Department of Health and Social Service's Division of Health.
21. Two Material Safety Data Sheet states that copper may cause anemia and other blood cell abnormalities and copper accumulates in various tissues and may result

- in liver, kidney, and brain damage. It has also been reported that copper poisoning has lead to hemolytic anemia and accelerates arteriosclerosis.
22. The Medical Toxicology Unit from Guy's and St. Thomas' Hospital stated that "Chronic poisoning with copper leads to gross hepatic copper overload with severe liver disease in young children. Indian childhood cirrhosis have reports of poisoning in young children as a result of high copper content in well water
 23. The NRDC cited a study (Sidhu, K. 1995²) in its report that recommended decreasing the federal maximum allowed amount of Cu in drinking water in order to adequately protect children³. This study found that infants and children up to ten years of age have greater sensitivity due to the presence of normally high concentrations of copper in the liver during early life and the lack of a fully developed physiological mechanism for regulating levels of copper in the body. One study, recognizing this difference, recommended decreasing the federal maximum allowed amount of Cu in drinking water in order to adequately protect children.^{4,5}
 24. In a report by the International Programme of Chemical Safety titled "Environmental Health Criteria 200 Copper the following findings were made:
 - (1) Ingestion of excess copper is infrequent in humans and is usually a consequence of the contamination of beverages (including drinking-water) or from accidental or deliberate ingestion of high quantities of copper salts. Effects which occur at lowest levels are those on the gastrointestinal tract; for example, nausea, vomiting and diarrhoea. Doses which induce such effects have not been well characterized and confounders such as microbiological quality of water supplies or other potential causes of the symptoms have not been adequately considered. On the basis of available data, gastrointestinal illness appears to be associated with consumption of drinking-water containing several mg/litre of copper, but it is not possible to provide a precise number. Symptoms disappear following a change of water supply.
 - (2) Human health risks are risks associated with low intakes as well as high intakes of essential elements. The relationship between intake/exposure level and risk therefore has a U-shaped curve, wit risks from deficiency at low intakes and risk of toxicity at high intakes. There is a need to define an intake range that prevents both deficiency and toxicity for the general population. The range of acceptable intakes to meet the biological requirement, as well as prevent risk of toxicity, may be extremely narrow. A balanced and comparable scientific approach to assess risk from deficit as well as excess is needed when evaluating essential elements such as copper.
 - (3) When copper homeostatic control is defective and/or copper intake is excessive, copper toxicity may occur.
 - (4) People with Menkes and Wilson disease are at risk with copper contained drinking water.
 - (5) Menkes disease is an X-linked recessive disorder of copper metabolism that occurs in approximately 1 in 200 000 live births. Clinically the condition resembles a copper deficiency state and is characterized by skeletal abnormalities, severe mental

² Sidhu, K. et al., "Need to Revise the National Drinking Water Regulation for Copper," *Regulatory Toxicology and Pharmacology* 22, August 1995, pp. 95-100.

³ From NRDC's "Toxic Chemicals & Health: Kids' Health: In Depth: Report: Our Children At Risk: The 5 Worst Environmental Threats To Their Health. Chapter 7, Drinking Water Contamination.

retardation, neurological degeneration and death in early childhood. The symptoms of Menkes disease result from a deficiency of copper and its effects on the function of copper-dependent enzymes.

- (6) Wilson disease is the most extensively described inherited disorder of copper metabolism. The gene is distributed worldwide, having been demonstrated in virtually all races. Current global estimates indicate that the incidence rate of the disease is approximately 1 in 30 000 live births, with prevalence ranging from 15 to 30 per million. The gene frequency varies between 0.3 and 0.7%, corresponding to a h Copper (EHC 200, 1998)
- (7) Idiopathic copper toxicosis, or non-Indian childhood cirrhosis Scattered reports of early childhood cirrhosis similar to ICC, referred to as copper-associated idiopathic copper toxicosis (ICT) have appeared from some Western countries (Walker-Smith & Blomfield, 1973; Müller-Höcker et al., 1987; Adamson et al., 1992; Gormally et al., 1994). Copper (EHC 200, 1993)
- (8) In England a correlation study, with measurements made after diagnosis of coronary heart disease, has shown higher serum copper levels in cardiovascular disease patients (Punsar et al., 1975).
- (9) Available data in humans and animals are inadequate to assess the reproductive/developmental effects of copper compounds on humans..
- (10) Bioaccumulation of copper by microorganisms, plants or animals from their surrounding environment can be adverse and must be studied further.
- (11) Copper exhibits significant toxicity to some aquatic organisms, although the degree of toxicity is highly variable and the bioavailability of copper dictates its toxicity to a large extent.

B. Copper Water Pipes Leaching Copper and Other Contaminants Into The Drinking Water

1. The California Department of Health Services (DHS), Drinking Water Program, has a Water Quality CD available which is all of the historical drinking water data beginning in the late 1980's through the present. The data is the mandated sampling, testing and reporting of drinking water by water systems, primarily large water systems (more than 200 connections), drawn at designated delivery/sample locations before delivery to households. DHS was unaware of any governmental agency that had data on water samples taken from the tap in households.
2. According to the U.S. EPA⁴ and the National Research Council (NRC), metallic copper is unstable and subject to corrosion when in contact with water and it is a "mistake to" assume that copper metal (Cu) and its alloys "do not leach into the drinking water." This includes water termed non-corrosive or water treated to make it less corrosive. Copper occurs in drinking water primarily due to its use in plumbing materials. Copper leaching continues from installation until about 10 year of service.
3. A review of the Associated Laboratories data developed from July 17 through July 25, 2002 at the Murrieta Ranchos Development in the City of Murrieta, California showed copper concentrations from 22 homes ranging from a low of

⁴ USEPA Ground Water and Drinking Water Technical Fact Sheet on Copper.

146 ppb to a high of 2,400 ppb. The Public Health Goal for copper in drinking water as established by CalEPA's Office of Environmental Health Hazard Assessments is 170 ppb. 21 of these homes have exceeded this PHG level. The average pH of the water tested was 7.4 which is slightly basic not acidic.

4. A 1975 AWWA Journal reported that corrosion of household Cu plumbing
5. Several states have measured CU concentrations in drinking water from Cu pipes that exceeds U.S. EPA MCLG levels. (See NRC 2000 Report)
6. Water softeners using ion exchange are likely to have increase Cu contamination levels in the drinking water. (See NRC 2000 Report)
was a major source of Cu metal contamination in U.S. drinking water.
7. The Agency for Toxic Substances and Disease Registry (ATSDR)⁵ created the "Public Health Statement for Copper" which states that you may be exposed to high levels of soluble copper in your drinking water, especially if your water is corrosive and you have copper plumbing and brass water fixtures. The average concentration of copper in tap water ranges from 20 to 75 parts per billion (ppb). However, many households have copper concentrations of over 1,000 ppb (near the upper limit of U.S. EPA's Maximum Contaminant Level. This is because copper is picked up from copper pipes and brass faucets when the water sits in the pipes overnight. After the water is allowed to run for 15-30 seconds, the concentration of copper in the water decreases.
8. Copper pollution in the Town of Discovery Bay, California has lead to a NPDES permit issued by the California Regional Water Quality Control Board requiring the removal of Cu from the water supply through a "Pollution Prevention Plan." The major cause of copper pollution is from the use of water softeners that are installed on copper piping systems in homes of Discovery Bay. (see letter from Virgil Koehne, General Manager of the Town of discovery Bay, August 22, 2003.)
9. In February 1997, the Office of Drinking Water for the U.S. EPA Environmental Criteria and Assessment Office reported that a majority of copper present in drinking water appeared to come from copper pipes and they were unable to estimate the number of individuals who regularly consume water that exceed safe MCL (Maximum Contaminant Level) levels for copper.
10. An article published a recent issue of the Wisconsin Medical Journal detailed two separate cases in Wisconsin reported that ingestion of copper-contaminated drinking water resulted in numerous reports of nausea, vomiting and abdominal discomfort because of new copper piping systems. Samples analyzed showed copper level exceeding Federal MCL levels. In the following weeks, 251 families submitted first drawn water samples (after sitting overnight) and 48 had copper levels that exceeded federal limits. The homes were built in the past 10 years had the highest copper levels in the water. .
11. Washington State Department of Health stated that most Cu in drinking water comes from household plumbing and that copper contamination can accumulate overnight (called "first flush") and recommends that households flush their water before use for the first 30 to 45 seconds.

⁵ ATSDR is an agency of the U.S. Department of Health and Human Services whose purpose is to serve the public by using the best science, taking responsive public health actions, and providing trusted health information to prevent harmful exposures and disease related to toxic substances.

12. A Wisconsin Department of Health and Social Service's Division of Health study stated that "first flush" drinking water in Cu pipes often contain Cu levels that exceed federal drinking water standards and should be flushed prior to drinking.
13. Each year thousands of pounds of copper enter the San Francisco Bay, and the accumulation is harming aquatic life. Research shows that corrosion from newly installed copper pipes is 5 times higher than that from older systems. A Copper Action Plan for the NPDES permit for Palo Alto whose receiving waters are the San Francisco Bay estimated in 2002 that corrosion accounts for 60% of the estimated Cu sources. The Regional Water Quality Control Plant's discharge permit (Order No. 00-109) requires outreach to plumbers and designers to reduce corrosion of copper pipe via better design and installation.
14. Test carried out in Victoria in 1976 by the State Water Supply commission indicated that fluoride is involved in the corrosion of the copper pipes, which causes more leaching into the water. Leaving fluorinated water standing in the copper pipes for longer periods of times allows for more corrosion. Countries such as Switzerland, Belgium, Holland, Germany, and Sweden have terminated the use of fluoride due to its potential health hazard.
15. The presence of acidic water in household plumbing systems resulted in levels of copper or zinc up to 9,000 ppb being recorded in water from cold taps, and up to 22,500 ppb from hot taps. (IRC 2000, Section R324)
16. In the City of Highland, the Richmond Creek subdivision experienced 175-200 pin hole leaks in the 67 homes (Letter to Department from Richmond American Homes of California, March 1, 1993)
17. The initiation of pits in copper water tubing has been correlated to carbon films left on the surface during manufacturing, flux residues from soldering, debris left in tubes during installation and water chemistry parameters (Chester Neff, P.E. Chemist, July 31, 1991 Letter to DEC Consultants in San Diego)

C. Scientific Community Recommendations on Copper in Drinking Water

1. Office of Environmental Health Hazard Assessment (OEHHHA) established in 1997 the current Public Health Goal of 0.17 mg/L (ppb) for copper which was based on acute gastrointestinal effects in children. OEHHHA is currently reviewing the 1997 standard and staff has suggested that a target level of copper in drinking water, measured at the tap, should be in the range of 0.1 to 0.3 mg/L (ppb), i.e., at about the level of the current PHG.
2. Given the potential risk for toxicity in humans, quantification of copper toxicity should be undertaken and the MCLG for copper be re-evaluated. NRC stated the current health level established by U.S. EPA for copper is based on acute exposure to copper and is not suitable for establishing a MCLG (maximum contaminant level goal). A chronic exposure level is necessary for the MCLG.
3. The National Research Council (NRC)⁶ stated that because reproductive and development effects are affected by small amounts of Cu intrauterine devices by preventing embryogenesis by blocking implantation and blastocyst development that Cu exposure during the early postnatal period requires additional study to determine teratogenicity during pregnancy. The reproductive and development effects of excess copper is not well known other than small amounts of copper

⁶ See footnote #1

- from intrauterine devices can prevent embryogenesis by blocking implantation and blastocyst development. The committee commended that copper exposure during the early postnatal period requires additional study to determine teratogenicity during pregnancy. (see NRC 2000 report)
4. The NRC recommended that increase in the ingestion of Cu should be cautioned against until the hepatic (liver) susceptibility is clearly identified.
 5. The NRC recommended that studies be conducted to characterize the potential role of genetics that underlie infant and childhood copper toxicosis.
 6. The NRDC cited a study (Sidhu, K. 1995⁷) in its report⁸ that recommended decreasing the federal maximum allowed amount of Cu in drinking water in order to adequately protect children. This study found that infants and children up to ten years of age have greater sensitivity due to the presence of normally high concentrations of copper in the liver during early life and the lack of a fully developed physiological mechanism for regulating levels of copper in the body. One study, recognizing this difference, recommended decreasing the federal maximum allowed amount of Cu in drinking water in order to adequately protect children¹⁰.
 7. The Agency for Toxic Substances and Disease Registry⁹ which created the "Public Health Statement for Copper" stated that because many households have copper concentrations of "first drawn" water over 1,000 ppb (near the upper limit of U.S. EPA's Maximum Contaminant Level that after the water is allowed to run for 15-30 seconds, the concentration of copper in the water decreases.
 8. A Wisconsin Department of Health and Social Service's Division of Health study stated that drinking water that stands overnight in copper pipes may often contain copper levels that exceed federal drinking water standards and that the water should be flushed prior to drinking water that stands overnight in copper pipes according to the investigation.
 9. A Washington State Department of Health and Social Division of Health Study stated that most copper in drinking water comes from household plumbing and that copper contamination can accumulate overnight (called "first flush") and recommends that households flush their water before use for the first 30 to 45 seconds.
 10. The Nebraska Health and Human Services recommends flushing household water supply prior to use if it has stood in pipes for six hours or more and this water should not be used for drinking or cooking. If you live in an apartment complex, flushing may not be as effective for reducing copper levels. Water from the hot water tap shouldn't be used for drinking or cooking. If the results of water testing show elevated copper levels, flushing may not be adequate for children or infants and an alternate source of water may be needed.
 11. The Regional Water Quality Control Plant's discharge permit (order No. 00-109) requires outreach to plumbers and designers to reduce corrosion of copper pipe.
 12. Known Copper pollution in the Town of Discovery Bay, in Danville, CA that has a California Regional Water Quality Control Board NPDES permit with a

⁷ Sidhu, K. et al., "Need to Revise the National Drinking Water Regulation for Copper," *Regulatory Toxicology and Pharmacology* 22, August 1995, pp. 95-100.

⁸ From NRDC's "Toxic Chemicals & Health: Kids' Health: In Depth: Report: Our Children At Risk: The 5 Worst Environmental Threats To Their Health; Chapter 7; Drinking Water Contamination.

⁹ ATSDR is an agency of the U.S. Department of Health and Human Services whose purpose is to serve the public by using the best science, taking responsive public health actions, and providing trusted health information to prevent harmful exposures and disease related to toxic substances.

requirement for a Pollution Prevention Plan to remove copper from the water supply. The recommendation will be to replace the copper pipe for new homes with alternative plastic pipe.

13. The NRC recommended genetic animal models be used to determine the associations between liver toxicity and Cu in sensitive population (Wilson Disease-2% of population).
14. The NRC recommended that epidemiological studies of population who have been chronically exposed to elevated copper should be carried out to determine the nature and frequency of chronic effects, especially in sensitive populations.
15. Determine the bioavailability of dietary copper, particularly in vegetarian diets. Copper (EHC 200, 1998)
16. In human populations develop the methodology for identifying adverse effects of marginal copper deficiency and of intakes in excess of recommended levels. This should include an evaluation of stable isotope technology to define bioavailability and body stores of copper. Copper (EHC 200, 1998)
17. Determine the concentrations of copper and the other quality parameters of drinking-water that produce toxicity from single and chronic exposures (e.g. gastrointestinal effects). Copper (EHC 200, 1998)
18. Characterize the mechanisms that influence copper homeostasis including placental transfer of copper. Copper (EHC 200, 1998)