THESIS

COGNITIVE EFFECTS OF EXPOSURE TO MANGANESE IN DRINKING WATER IN CALIFORNIA SCHOOL CHILDREN

Submitted by

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ABSTRACT OF THESIS

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The hypothesis tested in this study was that low level exposure to manganese in children through drinking water is associated with impaired cognitive performance on standardized tests of intellectual function. The study was based on the pathological and toxicological effects of exposure to manganese in laboratory animals and recent epidemiologic evidence showing an association between exposures to manganese in drinking water and decreased intellectual function (IQ) as well as hyperactive behaviors in children. A clear analogy with lead exists. California has a statewide monitoring system for drinking water manganese which was used to identify school districts for water sampling and analysis. Target school districts were identified for sampling from the California Department of Public Health Drinking Water Program. 100 schools within those cities were identified with manganese concentrations ranging from 20 µg /L to over 900 µg /L. Water samples were collected (n=81), analyzed for manganese concentration (ppb), and grouped into three exposure categories High (>30µg/L), Low (2 -29µg/L), and ND (< 2µg/L). Cognitive assessment was determined from standardized test score data for 3rd, 4th, and 5th grade children from the California Standardized Testing and

Reporting Program (STAR) for each school. Analysis of Variance, Analysis of Covariance, and Mixed Effect General Linear Regression analyses were used to analyze the data and adjusted for covariates including age, gender, ethnicity, parental education and economic status. The results of this study did not suggest mean test scores to be significantly different between high, low, and non-detect manganese exposure groups when adjusted for confounders. Mean test scores were not highest in the non-detect exposure group and lowest in the high exposure group as predicted.

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Dedicated to
the memory of Brit E.Todd,
boss, mentor, friend

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CHAPTER 1

Introduction

Background and Significance

Manganese is a transition metal that occurs naturally in the environment. It is also an essential element for humans required for important cellular enzymes. Like many other essential metals, excessive exposure has been associated with adverse health effects, in this case, neurotoxicity. Occupational studies have, almost exclusively, studied the effects of inhalation exposure to manganese. These studies have identified manganese as an important neurotoxicant, impairing cognitive and motor function as well as inducing a Parkinson's Disease like syndrome (Bouchard et al., 2005; Rom et al., 1998; Antonini et al., 2006; Bowler et al 2007; Bowler et al., 2006). However, studies examining other routes of exposure to manganese are sparse. Recent evidence from epidemiological studies (Wasserman et al., 2006; Bouchard et al., 2007) suggests that exposure to moderately elevated levels of manganese in drinking water leads to impaired cognitive function in children. It is estimated that heredity accounts for up to half of the variance of cognitive, behavioral, and personality traits among individuals. It may then be assumed that the other half may result from environmental influences (Plomin, 1994). Because children are still developing, exposure to toxic metals can cause permanent damage to

very sensitive biological systems, especially neurological functions. If this association is validated in further studies, the public health consequences would be substantial, as has been clearly demonstrated for low level exposure of children to lead.

Several methodological weaknesses limit the interpretation of these studies and they are not adequate to establish causality between ingestion of excess manganese and preclinical neurological effects in children. Nonetheless, they strongly suggest exposures to manganese via drinking water may affect neurobehavioral function and intellectual development. The U.S. EPA recently issued a drinking health advisory for manganese with a lifetime level of 300 µg/L (Cheng et al., 2004). It is estimated that approximately 6 percent of private wells in the U.S. have manganese levels > 300 µg/L (Wasserman et al., 2006) Thus the relationship between manganese and children's neurologic and intellectual function needs further study.

Hypothesis and Specific Aims

This study was designed to test the hypothesis that low level exposure to manganese in children through drinking water is associated with impaired cognitive performance on standardized tests of intellectual function.

To test the hypothesis, the following three primary study objectives were formulated:

1. Identify school districts in California supplied by municipal water systems containing varying levels of manganese by water sampling and analysis for manganese.

2. Collect test score data from the California Standardized Testing and Reporting Program for schools in the exposure analysis sample.

3. Conduct multivariate analyses of the relationship between test scores and water manganese concentration using multiple linear and logistic regression analyses

CHAPTER 2

Literature Review

Properties of Manganese

Manganese is the twenty fifth element in the periodic table. It can exist in eleven oxidation states; manganese^{+2} and manganese^{+4} are the states most commonly taken in by the body (Aschner, 2006). In nature, manganese is found in more than 100 minerals such as oxides, carbonates, and silicates, because it does not occur in its natural state as a base metal (Nordberg 2007). In most cases, manganese is considered a major metal because compared to trace elements, it is found to have relatively high concentration in waters and soil. Other metals found abundantly in water are magnesium, iron, zinc, and copper (Hashemi, 2000). Manganese is also commonly found in most iron ores. Because of the environmental persistence of these metals, water samples (especially those with very low concentrations) can be easily contaminated. Special care must be taken in sample handling, pre-treatment and analysis.

Significant exposure to manganese can occur through ingesting contaminated drinking water because some manganese compounds are readily soluble. Water can become contaminated with manganese from discharge from industrial facilities or as leachate

from landfills and soil (Nordberg, 2007). The characteristics of available anions, oxidation-reduction potential, and pH of the water determine the solubility of the specific form of manganese present, thus controlling the transport and partitioning of manganese (Nordberg, 2007).

Adequate daily intake range suggestions vary by study. For example, Freeland-Graves et al. recommend a recommended dietary allowance (RDA) range of 3.5-7mg a day (1994) Other studies have suggested a range of 0.7-10.9 mg of manganese per day (WHO, 2004). RDA is defined as "the levels of intake of essential nutrients, that on the basis of scientific knowledge, are judged by the Food and Nutrition Board to be adequate to meet the known nutrient needs of all healthy persons" (National Research Council, 1989). Establishing RDAs for manganese has proven difficult because it is necessary for maintaining proper health as an essential element, but can be toxic with chronic exposure to high levels. Deficiency in manganese can result in ataxia, impaired growth, skeletal abnormalities, and reproductive deficits (WHO, 2004). Factors affecting the ability to ascertain an accurate and more precise RDA include absorption rates, uneven distribution in food, age, health status and the absence of good biomarkers for manganese. Based on different diets, individuals will ingest different amounts of manganese per day (EPA, 2003). As of 1989, the EPA set the Estimated Safe and Adequate Daily Dietary Intake range at 2.0-5.0mg manganese/d (Greger, 1997). However, the current No Observed Adverse Effect Level, set at 11mg/day, is based on calculated values rather than actual measurements (Ljung, 2007). The absorption of manganese in the gastrointestinal tract is less than five percent but this rate is increased by iron deficiency (Casarett et al., 1996).

The EPA has estimated that an intake of 10 mg manganese/day (assuming a body weight of 70kg) in the diet is safe for a lifetime of exposure based on dietary information from WHO (1973), Schroeder et al., (1966), and NRC (1989).

Currently in the US there are no enforceable standards for manganese levels in drinking water. National Primary Drinking Water Regulations, set by the USEPA, are mandatory water quality standards for drinking water contaminants. Maximum contaminant levels (MCLs) are enforceable standards established to protect the public against consumption of drinking water contaminants that present a risk to human health by setting the maximum allowable amount of a contaminant in drinking water that is delivered to the consumer. Some contaminants are not considered a threat to human health but are still monitored for aesthetic considerations such as taste, color, and odor. These contaminants have secondary maximum contaminant levels (SMCLs) which are tested by public water systems on a voluntary basis (USEPA, 1992). Currently, the USEPA has established a secondary maximum contaminant level (SMCL) of 50 µg/L. At levels above this range manganese causes drinking water to become a black to brown color; black staining of fixtures, laundry, fixtures; and a bitter metallic taste (USEPA, 1992).

Mechanisms of Toxicity

Absorption of manganese through the gastrointestinal tract depends on the amount ingested. Humans, on average, absorb approximately 3-5% of ingested manganese (Davidsson et al., 1989). When absorbed in the divalent form from the gut via the portal

blood, normal organ concentration is maintained and removed by the liver (Aschner, 2002). The main route of excretion for manganese from the body is through the feces with only a small proportion being eliminated in the urine (Davis and Greger, 1992).

Iron deficiency can enhance absorption of divalent metals such as lead, cadmium, aluminum and manganese. Iron intake is a very important determinant for manganese absorption because of a shared transport mechanism in the gut with manganese. Both are bound to transferrin, and therefore, compete for binding to the protein in the body (Chandra and Tandon, 1973). Iron and manganese share physiological valences of +2 and +3, ionic radius, mitochondria accumulation, and both target and accumulate in the basal ganglia (Garcia, 2006). Both human (Dorner et al., 1989) and animal studies (Kostial et al., 1978; Tehnberg et al., 1985) have found manganese absorption through the gastrointestinal tract to be age dependent; neonates and infants retain a higher proportion of manganese than adults. This phenomenon is not well understood, but may occur because of difference in excretory ability. This may be because the biliary system is not fully developed in infants (Keen et al., 1986). Consequently, children are at greater risk for manganese toxicity because they absorb a greater percent of manganese and excrete less.

Interactions of manganese with cadmium (Cd), nickel (Ni), indium (In), rhodium (Rh), and selenium (Se) have been observed at the level of gastrointestinal absorption (Burch et al., 1975, Doyle and Pfander, 1975; Jacobs et al., 1978). Cadmium has been noted to have an inhibitory effect on manganese uptake (Gruden and Matausic, 1989). Manganese

appears to be capable of increasing the synthesis of the metal-binding protein metallothionine (Waalkes and Klaassen, 1985). Data from a study by Goering and Klaassen suggest that manganese pretreatment increases the amount of Cd^{+2} bound to metallothionine, thereby decreasing hepatotoxicity due to unbound Cd^{+2} (1985).

The addition of manganese to diets deficient in iron leads to depressed hemoglobin levels in blood. Inversely, the effect of manganese is prevented with the addition of iron to the diet (Leach and Lilburn, 1978). In rats, the whole-body retention of orally administered manganese was decreased tenfold upon the increase of iron content in the diet (Kostial et al., 1980).

While the actual mechanism of manganese induced neurotoxicity has not been clearly elucidated, damage seems to occur postsynaptic to the nigrostrial system, predominantly in the globus pallidus (Nordberg, 2007). The globus pallidus is also referred to as the pallidum. The substantia nigra of the midbrain is functionally linked to the basal ganglia (Tortora, 2005). Manganese has a high affinity for neuromelanin and deposition is highest in melanin containing tissues. As a result, manganese toxicity causes depigmentation of the substantia nigra. Neuromelanin levels are highest where dopaminergic pathways are most active (Aschner, 2002). Chronic exposure to high levels of manganese appears to result in neurotoxicity by causing degeneration in neurons within the extrapyramidal system, specifically the globus pallidus and striatum of the basal ganglia (Liu et al., 2006). The collective components of the basal ganglia are responsible for an individual's overall level of responsiveness to stimuli. Damage to this

region of the brain can lead to under- or over-responsiveness to stimuli as well as excessive movement (Castro, 2002). The globus pallidus is involved in the regulation of voluntary movements at a subconscious level. The striatum plays a role in the planning and modulation of movement pathways but is also involved in a variety of other cognitive processes involving executive function. Several cholinergic synaptic mechanisms are disrupted by manganese such as presynaptic choline uptake, quantal release of acetylcholine into the synaptic cleft, postsynaptic binding of acetylcholine to receptors and its synaptic degradation by actylcholinesterase. Cholinergic afferents play an active role in the physiology of locomotion, cognition, emotion and behavioral responses (Finkelstein et al., 2007).

There are many theories as to how and why this damage occurs. It is believed the process is likely a multi factor process including a number of proposed theories. Manganese may lead to an increased production of free radicals, reactive oxygen species, and other cytotxic metabolites by enhancing the auto-oxidation of various intracellular catecholamines such as dopamine. In its divalent oxidative state, manganese may cause a direct toxic effect to dopamine containing cells or create an excitototoxic mechanism in which the activation of glutamate gated cation channels contributes to neuronal degeneration (Aschner, 2002). Excitotoxicity is the process of neuronal damage and degeneration through excessive stimulation of receptors for which glutamate functions physiologically as a transmitter. Glutamate is the major excitatory neurotransmitter used by many pathways of the brain including the corticostriatal pathway (the caudate and

substantia nigra). It is also proposed that GABA regulation and glutamatergic tranmission are disturbed by manganese (Nordberg, 2007).

The most vulnerable organ to manganese toxicity is the brain. An accumulation of manganese in brain tissue results in a progressive disorder of the extrapyramidal system similar to Parkinson's disease (Crossgrove, 2004). In normal brains, manganese is found to be distributed heterogeneously; the highest concentrations are found in the globus pallidus, substantia nigra, and subthalamic nuclei which comprise the basal ganglia (Larsen et al., 1979).

Evidence from animal data suggests that manganese distributes in brain regions as follows: substantia nigra, striatum, hippocampus, frontal cortex. The globus pallidus and the striatum of the basal ganglia are the primary brain regions targeted in manganism (Walter et al., 2003). Each cerebral hemisphere has three masses of gray matter collectively referred to as the basal ganglia. The term "basal ganglia" refers to structures in the brain to which damage causes distinctive kinds of movement disorders. The five major nuclei included in this group are the putamen, caudate nucleus, nucleus accumbens, substantia nigra and the globus pallidus (Nolte, 2009). Collectively, the putamen, caudate nucleus, and nucleus accumbens are referred to as the striatum.
In nonhuman primates, there is evidence that exposure to manganese causes a decreased level of the metabolite N-acetlyasprate (NAA). NAA is a metabolite of N-acetyl-aspartyl glutamate (NAAG) which is the most abundant neuropeptide in the brain. NAAG is important in glutamatergic neurotransmission. Decreased levels of NAA in the brain may

reflect neuronal loss or dysfunction in the brain. The study animals demonstrated subtle deficits in spatial working memory and increased frequency of stereotypic and compulsive like behavior. Mn^{2+} has been shown to inhibit N-methyl D-aspartate (NMDA) receptor function in an activity dependent way. NMDA receptors play an essential role in synaptic plasticity as well as learning and memory function (Guilarte, 2007).

Manganese is distributed to the brain through the capillary endothelial cells of the blood-brain barrier (BBB), by the choroid plexus of the blood-cerebropinal fluid (CSF) barrier, or from the nasal cavity via the olfactory nerve directly to the brain (Crossgrove, 2004). When manganese is at normal or slightly elevated levels (80nM), influx to the brain occurs primarily through the capillary endothelium of the BBB. At much more elevated manganese plasma concentrations, brain influx occurs primarily via the CSF. (Murphy, 1991; Rabin, 1993 cited in Crossgrove et al., 2004). The half-life of manganese in Rhesus monkey brains is thought to exceed 100 days. Manganese brain efflux is not widely understood. It is not thought to occur through a transporter, but more likely to occur slowly by diffusion (Crossgrove et al., 2004).

Toxicological Studies of Manganese Exposure in Animals

Substantial evidence from studies of laboratory animals shows that manganese is neurotoxic (ATSDR, 2000). The manifestations of manganese toxicity include cognitive impairment and behavioral disturbances. Laboratory studies have identified the regions of

the brain that are targeted, and subsequently altered, by an excess of manganese. Consequently an understanding of the underlying mechanisms through which manganese causes behavioral, cognitive and locomotor disturbances has emerged. Manganese readily crosses the blood brain barrier by binding to the iron-transporting protein transferrin. Once across the blood brain barrier, several neurotransmitters are targeted for toxicity including GABA and monoamines such as dopamine, noradrenalin, and serotonin (Casarett et al.,1996). A connection between chronic manganese exposure and behavioral disorders such as Attention Deficit Hyperactivity Disorder (ADHD) is plausible. The dopaminergic and GABAergic systems that play a role in hyperactivity in children are vulnerable to manganese (Li et al, 2006). In rats, manganese has been shown to deplete dopamine levels in the serum and brains, which in turn may cause a decline in neurocognitive function such as memory, attention, and problem solving (Casarett et al., 1996). In humans, behavioral disorders such as attention deficit disorder are associated with reduced dopamine levels in the brain (Rom et al., 1998).

A significant study examining the behavioral consequences of chronic low-level exposure to manganese was conducted by Schneider et al., in 2006. Five cynomolgus macaque monkeys were intravenously injected with manganese sulfate (10-15mg/kg/week) for 272 ±17 days and one macaque received only vehicle injections. Prior to exposure the animals were trained to perform various tasks measuring motor and cognitive function. Videotaped analyses were used to measure overall behavior. Subtle deficits were observed in the exposed animals' spatial working memory, spontaneous activity and manual dexterity upon conclusion of the exposure period. No change or improvement

was observed in the control animal. Behavioral rating scores, based on a Parkinson symptom rating scale for non-human primates, were significantly different from baseline in the exposed animals from week twenty through the end of the study. Certain stereotypic and compulsive-like behaviors increased in manganese intoxicated animals as well. No behavioral changes were noted in the control animal (Schneider, 2006).

In a study conducted to determine toxicological endpoints in the brain, Morello et al. (2008) found that rats exposed to chronic manganese sustained ultra-structural damage in neurons and glial cells. Manganese was found in the mitochondria of astrocytes at levels seven times that of control mice while the manganese levels in the mitochondria of neurons doubled. The increased presence of manganese in the mitochondria of astrocytes and neurons could be because superoxide dismutase, a manganese dependent enzyme, is preferentially located in the mitochondria of neurons in the brain (Morello et al., 2008).

In a study by Liu et al., conducted in 2006 in which manganese toxicity was observed by comparing an exposure group of twelve week old female mice dosed with 100mg/kg $MnCl2•6H2O$ water solution via gastric gavage once daily for eight weeks with a control group which received 0.9% saline. Upon histochemical staining with fluorojade and cresyl fast violet, exposure to manganese was found to cause neuronal injury in the striatum and globus pallidus. The striatum plays a role in the planning and modulation of movement pathways but is also involved in a variety of other cognitive processes involving executive function.

Lethality studies in rats have found varying LD50s depending on the route of exposure (gavage or dietary ingestion) and chemical species of manganese (ATSDR 2000). The LD50 in rats exposed via gavage ranged from 331 mg manganese/kg-day (as manganese chloride) to 1,082 mg manganese/kg-day (as manganese acetate) (Kostial et al., 1989, Smyth et al., 1969). However, no deaths were observed in rats dosed with 1,300 mg manganese/kg-day of manganese sulfate in their feed (NTP, 1993). Rat studies have suggested the youngest and oldest rats demonstrate the greatest oral toxicity to manganese chloride (Kostial et al., 1989). Increased susceptibility of younger rats may reflect high intestinal absorption and body retention of manganese.

Although animal studies are invaluable in the field of toxicology, there are several limitations when attempting to extrapolate non-primate manganese study data to humans. For example, the dietary requirement for manganese in humans is approximately two orders of magnitude lower than for rodents. In addition, manganese has a propensity for accumulation in the melanin pigment. Rodents have a relative lack of neuromelanin which may explain why some psychologic symptoms (irritability, emotional lability, tremor, gait disorders) are present in primates but not in rodents (Lyden et al., 1985).

In summary, toxicologic studies of manganese exposure in laboratory animals, although conducted at higher levels than those encountered by children in the community, show neurotoxicity compatible with disorders in cognition and aberrant behavior. These studies provide the biological plausibility that justifies epidemiologic studies in humans.

Occupational Exposure to Manganese and Human Health

The toxic effects of manganese in humans were first described by Couper in 1837 (Couper, 1837). Symptoms similar to those of Parkinson's disease were observed in five men working in a manganese ore-crushing plant in France. The analogous neurological symptoms appear to be due to similar patterns of pathologic damage in proximal regions of the basal ganglia and central nervous system. In the 1950s, Chilean manganese-ore miners reported symptoms including emotional liability, postural instability, and frequent hallucinations which was termed "manganese madness" (Chu, 1995). Manganese toxicity in workers typically progresses in two phases: asthenia, anorexia, apathy, headaches, hypersomnia, spasms, weariness of the legs, arthralgias, and irritability is followed by expressionless face, speech disturbance, altered gait, and fine tremor. In advanced cases, muscular rigidity, staggering gait and fine tremor may occur (Mergler, 1999). Occupational exposure to moderate levels of manganese (<1 mg/m3) over a long period of time is associated with neuromotor and cognitive deficits and mood changes (Roels et al., 1987; Iregren et al., 1990, Chia et al., 1993, Mergler et al., 1994, Lucchini et al., 1999). Between 4 and 25% of workers who are chronically exposed via inhalation to high levels of ambient manganese exhibit symptoms of chronic manganese poisoning (Kondakis et al., 1989). These symptoms include poor hand steadiness, difficulty performing rapid alternating movements, muscular rigidity, and postural instability, poor memory, slow reaction time, decreased cognitive flexibility, depression, irritability, anxiety, aggressiveness and emotional disorders (Bouchard et al., 2007).

In a recent study, occupational exposure to manganese was found to significantly increase the risk for neurological impairment in welders. Sixty-two welders with known clinical histories of exposure to manganese were compared to forty-six matched regional controls. Welders self reported acute symptoms such as development of tremors, mood changes, neurological problems, sleep disturbance, headaches, and sexual dysfunction. A series of tests were administered to measure a number of neurological markers which included cognitive flexibility, information processing, working memory, visuo-motor tracking speed, visuo-spatial skills, verbal skills, and motor skills. Adjusted odds ratios were significant for information processing (6.1-8.0), visuo-motor tracking speed (3.6-17.8), visuo-spatial skills (4.9), and motor skills (2.6-17.4). These results indicate a higher prevalence of neuropsychological dysfunction in manganese exposed welders than in unexposed controls (Bowler et al., 2006).

Occupational evidence of CNS manganese toxicity (manganism) has been described in welders, miners, ferromanganese-alloy industry workers, manufacturers of dry cell batteries, persons working with fertilizers and fungicides, and others exposed to manganese through inhalation of dusts, mists, and fumes. Environmental exposure to manganese is increasing because of its use in industrial purposes, pesticides and the anti knock agent for engines (MMT, Cl-2). (Zatta et al., 2003). Metal fume fever or manganese pneumonitis is caused by acute exposure to manganese. Recently, occupational exposure to manganese was found to significantly increase the risk for neurological impairment in welders (Bowler et al., 2007). Elevated odds ratios were

reported for information processing, visuo-motor tracking speed, visual-spatial skills and motor skills (Bowler et al., 2007).

Long term, low-level exposure to manganese in ferroalloy workers are associated with neurological symptoms, and changes in motor functions requiring alternating and rapid movements, short term memory, and some tremor parameters. Although the exposed subjects did not exhibit clinical symptoms of manganese intoxication the symptoms that were present indicated the early signs of neurobehavioral effects (Kawamura et al., 1941). Similarly, a follow up study examining the long term effects of chronic occupational manganese exposure suggested that the clinical symptoms of neurological and motor impairment persist and that deterioration continues over time (Bouchard et al., 2005; Bouchard et al., 2007). The aging process is associated with changes in the brain dopamine system and subsequent normal neurobehavioral performance loss. Elimination of manganese from the central nervous system requires a long time which may explain the onset of neurotoxic effects and Parkinsonian symptoms later in life (Zatta et al., 2003). Because it is believed that manganese affects the dopaminergic system as well, the combined effect of age and manganese have been found to be synergistic (Bouchard et 2005). Manganese exposure results in significantly poorer performance with increasing age, which would suggest a significant manganese x Age interaction (Bouchard et al., 2005).

In another follow up study by Bouchard et al., exposure to manganese in manganese ferro-alloy plant workers was associated with ongoing deficits for some neuromotor

functions, cognitive flexibility, and adverse mood states (Bouchard et al., 2007). The average duration of manganese exposure in the sample of 115 workers was 15.3 years. Upon initial examination of the exposed and referent group, testing indicated overall poorer performance in the exposed group ($p < 0.01$) and remained significantly poorer at the time of follow up ($p < 0.05$). Some scores, such as the Confusion-Bewilderment scale and drawing were poorer in the exposed at the time of follow up than at the initial evaluation (Bouchard et al., 2007).

Epidemiological Studies of Lead and Human Health

Understanding the etiology of manganese toxicity in humans may be aided by considering epidemiologic studies of lead toxicity, which have proved invaluable in determining the source and pattern of lead-induced disease in human populations Epidemiological studies have proven invaluable in the field of public health. Over the past centuries, mining and the use of lead has resulted in an increase in lead concentrations in surface soils. Efforts have been made in the past several decades to reduce the amount of lead released into the environment and subsequently, human populations. Exposure to lead occurs primarily in the industrial setting. However, because of industrial activities such as smelting, the release of fumes containing lead fumes, mining, etc. many people not involved directly in industrial processes can be exposed. For example, organic lead compounds can easily become part of the food chain because they are readily taken up by plants (Feldman 1999).

Children are more susceptible to elevated lead levels because gastrointestinal absorption of lead is significantly greater in children than in adults (Feldman 1999), children (especially those under the age of five) frequently mouth objects and consume substances other that food (Raymond 2009). Elevated blood lead levels early in life may damage sensitive brain structures and permanently alter behavior and intelligence (Surkan et al., 2007). For example, infants and young children with increased blood lead manifested neuro-behavioral disruptions such as decreased attention span, reading disabilities, and failure to graduate from high school as adolescents (Needleman et al., 1990).

It is now well established that lead is a major contributor to intellectual impairment and behavioral disorders at relatively low doses originally thought to be below the thresholds for toxicity (Bellinger et al., 1983; 1984; 1986; Needleman et al., 1972; 1979; 1991). Reviewing and reconstructing the research progression that lead to the current understanding of the neruotoxicity caused by lead and mercury is critical for preventing future cognitive impairment. Children are far more susceptible to neruotoxicity from harmful agents because they are still developing. When exposed at a young age, they are at a greater risk for suffering permanent cognitive or behavioral impairment. From epidemiological studies, evidence of cognitive impairment at exposure levels previously thought safe caused the Centers for Disease Control (CDC) to reduced the lowest adverse level of lead four times since the early 1970s (Surkan et al., 2007).

Evidence from studies of laboratory animals suggest that exposure to lead causes behavioral disruptions such as impulsivity and lack of ability to control inappropriate

responding. Primate and rat fixed interval operant schedules studies have shown exposure increases rate of responding with response patterns sometimes atypical of fixed interval responding (Rice, 1988; Rice, 1979). In a fixed ratio waiting-for-reward experiment with rats, Brockel et al., (1998) rats were chronically exposed after weaning to 0, 50, or 150ppm lead acetate in water. After forty days, the rats were trained on a fixed ratio waiting-for-reward paradigm in which fifty presses of a lever produced food. Upon delivery of the fixed ratio pellet, a "free" pellet could be earned by waiting increased time intervals (delivery upon a two seconds, four seconds, six seconds, etc.). Another press of the lever before delivery of the free "wait" pellet reinstated the fixed ratio requirement of 50 lever presses. Exposure to lead increased fixed ratio response rates while decreasing the mean longest waiting time. This experiment demonstrated that lead exposed rats were more likely to demonstrate behaviors such as impulsivity, reinforcement delay and inability to inhibit responding (Brockel, 1998).

Needleman et al., (1979) questioned whether or not blood lead levels lower than those known to cause health effects could have adverse effects on the brain. Like manganese, biological markers for exposure to lead are inadequate. Blood levels measure recent exposure but will eventually return to normal. Therefore, markers for past exposures, even if excessive, cannot be obtained in this way. To measure past exposure to lead, the investigators used dentine lead levels from first and second grade students as and indicator of exposure. Of all sampled, those in the highest 10th percentile (>24 ppm) or lowest 10th percentile (<6ppm) were classified as high and low lead levels respectively. Teachers were asked to complete behavioral assessment surveys about the subjects.

When the results were evaluated, non-adaptive classroom behavior followed a dose dependent curve related to dentine lead level. These findings suggest that previously thought sub-clinical levels of lead exposure may actually have adverse neuropsychologic deficits causing behavioral disturbances.

It is now well established that lead is a major contributor to intellectual impairment and behavioral disorders at relatively low does originally thought to be below the thresholds for toxicity (Needleman et al., 1979, Bellinger et al., 1983). Reconstructing the progression of research that led to the current understanding of the neurotoxicity caused by lead provides an excellent model on which to base studies of potential negative effects of manganese exposures in children. As shown for lead, exposure at younger ages is associated with a higher risk for neurotoxicity and permanent cognitive or behavioral impairment. Currently, the CDC's definition of elevated blood level is 10µg/dL. Increasing evidence from epidemiological studies has shown that lead levels below 10 µg/dL are associated with adverse cognitive effects in children. Surkan et al., (2007) evaluated the cognitive abilities of children with blood lead levels >10 µg/dL and found that those with lead levels between 5-10 µg/dL were found to have significantly lower scores on IQ, achievement, attention, and working memory than children with blood lead levels between 1-2 µg/dL. This study suggests that 10µg/dL is not an appropriate lowest observed adverse effect level (Surkan, 2007). Based on the current literature, epidemiologic research on manganese may find many neurotoxic and behavioral impairment parallels to that of lead.

Exposure to Manganese and Neurobehavioral Effects in Humans

While manganese toxicity via inhalation exposure is well documented in occupationally exposed persons, information addressing toxicity from oral exposure in children is scarce. This may be due to limited gastrointestinal absorption of manganese. However, recent evidence has shown manganese to be more readily absorbed from drinking water than food (Ljung, 2007). Although food is the primary source of manganese intake, water is thought to facilitate manganese absorption through the gastrointestinal tract. Using radio labeled manganese to measure manganese absorption in adults, Johnson et al., (1991) found that absorption ranged from 1.4% to 5.5% from plant food and 7.8% to 10.2% from manganese chloride dissolved in water. Separate ideas

Several reports show that neurotoxicity occurs in children drinking water with high (>1000 µg/L) concentrations of manganese (Kawamura et al., 1941, Woolf et al., 2002). Limited evidence of a potential association between ingestion of elevated levels of manganese and learning problems comes from early studies showing that manganese levels in hair are higher in learning-disabled and hyperactive children than in normal functioning children (Collipp et al., 1983; Pihl et al., 1977).

A case study conducted by Woolf et al., (2002) further supports the relationship between chronic exposure to high levels of manganese and neurotoxic effects in children. The study reports on the results of a ten year old boy who had been consuming well water contaminated with 1210 µgMn/L for five years. Elevated levels of manganese were found

in his whole blood, urine, and hair. Although the child seemed in good health, his ability to coordinate rapid alternating motor movements was weak as well as poor visual and verbal memory. Additionally, his teachers had regularly noted a difficulty with listening skills and following directions.

The state of knowledge regarding manganese exposure in children is similar to that which existed for lead approximately 40 years ago. Several epidemiological studies have established a link between high levels of manganese in drinking water and neurotoxic effects. Kawamura et al., (1941) conducted on of the first studies to examine manganese toxicity from drinking water. Manganism-like symptoms (mask-like face, muscle rigidity and tremors, and mental disturbance) were reported among Japanese families whose drinking water was contaminated with 14000 µgMn/L manganese. Fifteen out of twenty five people examined demonstrated clinical symptoms. Three deaths occurred within the exposed population (including one suicide). The brains were examined upon autopsy and extreme macroscopic and microscopic changes were seen, especially in the globus pallidus (EPA, 2004). The occurrence of such high levels was a result of batteries leaching into the well water. However, it is unknown if these symptoms resulted entirely from manganese exposure as other metals and xenobiotics which could have been present in the water source were not discussed.

Two important epidemiological studies examined the relationship between elevated manganese levels in water and toxic effects in children. The first, conducted in the Chinese province of Shanxi, compared 92 children aged 11 to 13 whose water was

contaminated with elevated levels of manganese (241- 346 µg/L) to children whose drinking water had low levels of manganese (30-40 µg/L) (He et al., 1994). The exposed children performed significantly more poorly ($p<0.01$) in school and on neurobehavioral exams than control students. School performance was measured as mastery of the native language and other subjects; neurobehavioral performance was measured using the WHO core test battery. Manual dexterity and rapidity, short-term memory, and visual identification were significantly lower among children in the exposed group (He et al., 1994). Although the study had several weaknesses, the results suggested that high levels of manganese in drinking water affect neurobehavioral function in children.

The second, conducted in Bangladesh, showed that manganese may affect intellectual function resulting in lower IQ (Wasserman, 2006). The authors conducted a cross-sectional study of 142 ten year old children whose well water supply was contaminated with a mean concentration of 793 µg/L manganese. Intellectual function was measured using the Wechsler Intelligence Scale for Children. Blood samples were taken to measure blood lead, arsenic, manganese, and hemoglobin concentrations. manganese was found to be significantly associated with reduced Full-Scale, Performance and Verbal raw scores in a dose-response fashion (Wasserman et al., 2006).

Most recently, a pilot study conducted in Quebec, Canada examined the relationship between exposure to chronic levels of manganese and hyperactive behavior in children (Bouchard et al., 2007). Forty-six children between the ages of 6 and 15 were grouped into high (610 µg/L) or low (160 µg/L) exposure groups based on the well from which

their primary water source was supplied. Hair manganese concentration, versus blood manganese concentration, was correlated with hyperactive and oppositional behaviors. The high exposure group was found to have significantly higher amounts of manganese hair concentration and was more strongly associated with hyperactive behaviors (Bouchard et al., 2007).

In the Groote Eylandt region of Australia, the Aboriginal population was part of a study examining the relationship between high levels of manganese in the soil (40,000 to 50,000 ppm) and adverse health effects. Because of the elevated manganese levels in the soil, the fruits and vegetables grown there contained elevated concentrations of manganese. In this populations, a high occurrence of Parkinson-like neurobehavioral syndrome, stillbirths, ataxia, oculomotor disturbances, muscle atrophy and weakness were observed. Arrests in this population is the highest in Australia, suggesting (by the authors of the study) that high levels of manganese consumption are related to violent behavior (Stauber et al., 1987; Kilburn, 1987)

CHAPTER 3

Research Design and Methods

Study Design

This ecological case-control study evaluated the relationship between the amount of manganese in drinking water at the city level and cognitive function in school children via standardized test scores at the grade level. This ecological design allowed a greater number of subjects to be included in the study.

Schools within cities with “high” levels of manganese in drinking water (>30µg/L) were treated as cases. Schools within cities with “non-detect” levels of manganese (<2µg/L) were treated as controls. Schools within cities with “low” levels of manganese (2-29µg/L) were used in analysis as cases when comparing all exposure to non-detect, and as controls when combined with non-detect compared to high level exposure schools. For analysis purposes, all manganese values less than 2µg/L were divided by 2 to uniformly decrease the values.

Exposure categories had to be defined differently than originally planned because the manganese values found in the drinking water samples were significantly less than

anticipated. Because there were only two samples greater than the USEPA's SMCL of 50µg/L, the high exposure category cutoff was moved from the originally planned 50µg/L to 30µg/L. This increased the number of high exposure cities from two to seven. 30µg/L is the World Health Organization's recommended drinking water level. ND was set at less than 2µg/L because this was the detection limit of manganese by inductively coupled plasma (ICP) used for the analysis of water samples.

Exposure Assessment

California has a statewide monitoring system for drinking water manganese which was used to identify school districts for water sampling and analysis. The state also provides on-line access to the California Standardized Test scores which were used to measure intellectual function in this cross-sectional study.

The California Department of Public Health Drinking Water Program was used to identify high and low manganese exposure areas. Data provided in the annual drinking water quality reports for cities identified as high exposure areas were used to identify school districts for sampling and analysis of water manganese levels (Figure 1). These reports include range values, average value, and whether the standard concentration has been exceeded (Appendix 2). Historically, about 30 percent of drinking water sources monitoring for manganese have reported detections, reflecting its natural occurrence, and about 20 percent have reported detections greater than the 50 µg/L secondary maximum

contaminant level (SMCL). Based on 2003 statistics from the EPA, 17.2% of public water systems sampled in the state of California had manganese concentration levels above 150 µg/L. School districts with higher ranges of values were targeted, in theory, to assure heterogeneity in exposures. The goal, initially, was to identify fifty cities with suspected manganese concentrations above 50 µg/L and fifty below that value. However, upon collection and analysis, actual manganese concentrations were much lower than expected.

Upon identification of high exposure source water systems, schools within each selected city were selected by zip code corresponding to the water system service area. Districts with overlapping boundaries that did not fall clearly within the distribution system for the water systems were eliminated from consideration. Both exposed and non exposed schools within cities were chosen based on results of inductively coupled plasma optical emission spectrometry (ICP-OES) analysis for manganese content. Exposure for the school district was validated by collection of drinking water samples from schools after contact with principals or school administrators. Two separate water sample collections were completed for the study. Between April 2008 and June 2008, the first set of water samples were obtained by mailing collection containers via Federal Express containing 60 ml acid cleaned polyethylene bottles containing 1 ml 7 N high purity HCL as a preservative to 100 school districts chosen based on drinking water quality reports (Figure 1). In order to ensure the collected sample was representative of the real water composition, it was necessary to include in the collection protocol that the water from the sample source was run for several minutes or collected in mid-day (Gertig, 2008).

Because of the poor response rate (35%) from the school mailing sampling, and consequent low representation of high levels of manganese in drinking water, it was necessary to collect more water samples. For the second set, water samples were collected personally from public drinking water fountains found in parks or elementary schools in California by Eryn Murphy (Figure 2) using a USGS map (Figure 4) as a template to identify high, medium and low manganese concentrations. In order to ensure the collected sample was representative of the real water composition, water from the sample source was run for several minutes or collected in mid-day (Gertig, 2008).

All laboratory analysis for manganese in water was conducted at the Texas A&M Cooperative Extension Soil, Water and Forage Testing Laboratory. Concentration ranges and the nature of the analytes dictate the method of analysis and procedures for sample handling (Hashemi, 2000). ICP was selected as the method for analysis because it has proven to be highly efficient for metals in aqueous samples. Its sensitivity is greater than Flame Atomic Absorption Spectometry (FAAS), it is highly stable, possesses a large dynamic range, good reproducibility and low background. Samples may be analyzed with little or no pretreatment as well (Hashemi, 2000).

Cognitive Assessment

STAR reports the percentage of students who performed “Advanced”, “Proficient”, “Basic”, “Below Basic”, and “Far Below Basic” for each subject test as well as the mean

scale score for each grade. The State Board of Education adopted standards that specify what all California children are expected to know and be able to do in each grade or course. STAR data are publicly available for schools, counties, districts, and the state and contain five separate components including the California Achievement Tests, a national norm-referenced test. For each component, scores are reported for more than four million students in categories including: all students, students with disabilities, economic status, English-language fluency, ethnicity, gender, parent education and special program participation.

Standardized Testing in California

Standardized tests are used as a tool to measure the current level of knowledge or skill in a particular area, or ability (Wortham, 2001). The extent to which a person has acquired certain information or has mastered identified skills is referred to as achievement. Achievement tests, such as the California Standards Test (CST), are tools used to evaluate achievement related to specific prior instruction in areas such as math, reading recognition, reading comprehension, spelling, and general information. The results of group achievement tests administered by a school district may be used to measure student progress and assess the need for future instruction for individuals. Collectively, the test results can be used to measure the progress of students both between and within schools to evaluate the effectiveness of school programs.

The CSTs are primarily multiple choice and cover four subject areas: English language arts (grades 2-11); mathematics (grades 2-11); history/social science (grades 8, 10, and 11); and science (for grades 5, 8, 10, and high school students who are taking specific subjects like biology, chemistry, or integrated science). CSTs are criterion-referenced tests, and students are scored as "far below basic, below basic, basic, proficient, and advanced." The state goal is for every student to score at "proficient" or above. Only California students take these standards-based tests so their results cannot be compared to test scores of students in other states or nations (Wortham, 2001).

Reading, language, and mathematics tests were administered to students in grades two through eleven. The Stanford 9 was implemented to compare the achievement of general skills of individual students in the United States to the achievement of a national sample of students tested in the same grade at the same time of the school year (California Department of Education, 2007).

In California, both norm-referenced and criterion-referenced tests are used to measure students' ability and performance. Norm-referenced tests compare the scores of individuals against the scores of a representative group of individuals, or norm group. The norm-referenced tests used in the STAR program is the California Achievement Tests Sixth Edition survey (CAT/6) which generally measures a student's mastery of basic skills in comparison to a national sample of students and are typically reported as national percentiles. At the state or national level, test results can be used to evaluate and compare instructional effectiveness (Wortham, 2001).

Norm-referenced tests, such as the California Achievement Tests Sixth Edition survey (CAT/6), generally measure a student's mastery of basic skills in comparison to a national sample of students and are typically reported as national percentiles. At the state or national level, test results can be used to evaluate and compare instructional effectiveness. Specific types of improvements can be implemented for schools in districts falling below the state or national set standard for achievement (Wortham, 2001).

The criterion test used in the STAR program is the California Standards Tests (CST). These tests in English-language arts, mathematics, science, and history-social science are based on the state's academic content standards developed by the State Board of Education of what teachers are expected to be teaching and what students are expected to be learning. Achievement related to specific prior instruction in areas such as math, reading recognition, reading comprehension, spelling, and general information is evaluated. The results of group achievement tests administered by a school district may be used to measure student progress and assess the need for future instruction for individuals. Collectively, the test results can be used to measure the progress of students both between and within schools to evaluate the effectiveness of school programs (Wortham, 2001). Because these tests are not norm-referenced, the results cannot be compared with the rest of the nation (Wortham, 2001). The State Board of Education adopted these standards that specify what all California children are expected to know and be able to do. The CSTs are used to determine students' achievement of the California Content Standards for each grade or course. Students' scores are determined to

be advanced, proficient, basic, below basic, or far below basic based on a comparison to preset criteria (California Department of Education, 2007).

California Standardized Testing and Reporting Program (STAR) was authorized in 1997. Initially, the program designated the Stanford Achievement Test Series, Ninth Edition (Stanford 9) as the national norm-referenced achievement test. All students in grades two through eleven were to take the tests except for those students who "were receiving special education services with individualized education programs (IEPs) that specified that the students were to have and alternate assessment", and "students whose parents/guardians submitted written requests to exempt the students from testing." Reading, language, and mathematics tests were administered to students in grades two through eleven (California Department of Education, 2007).

The Stanford 9 was implemented to compare the achievement of general skills of individual students in the United States to the achievement of a national sample of students tested in the same grade at the same time of the school year. In 2002, the State Board of Education selected the California Achievement Tests for the STAR program. The Stanford 9 was replaced by the Sixth Edition Survey (CAT/6 Survey) as the national norm-referenced test for the Program. Currently, the CAT/6 survey is administered to students in grades three and seven. Students in grades two through eleven who do not require alternative testing take the multiple choice California Standards Tests (CSTs). Fourth and seventh grade students complete an English-language arts written assessment in addition to the multiple choice CST.

The California Standards Tests in English-language arts, mathematics, science, and history-social science are comprised of items that were developed specifically to assess students' performance on California's Academic Content Standards. The State Board of Education adopted these standards that specify what all California children are expected to know and be able to do. The CSTs are used to determine students' achievement of the California Content Standards for each grade or course. Student's scores are determined to be advanced, proficient, basic, below basic, or far below basic based on a comparison to preset criteria.

Subjects Tested in California

English-Language Arts

Students in grades two through eleven are required to take the California Standards Tests English-Language Arts. The English-Language Arts CSTs for grades two and three consist of 65 multiple-choice questions with an additional 6 field-test questions. For grades four through eleven, the tests consist of 75 multiple-choice questions with an additional 6 field-test questions. At grades four and seven, the English-Language Arts CSTs also include a writing component, the California Writing Standards Test, which addresses a writing applications standard selected for testing each year.

Mathematics

Grade-level mathematics tests are administered to students in grades two through seven. Students in grades eight and nine who are not taking a standards-based math course take the General Mathematics CST. Students in grades eight through eleven take the CSTs as an end of course test in Algebra I, Geometry, Algebra II, and Integrated Mathematics 1, 2, and 3. For students in grades nine through eleven who have completed Algebra II, Integrated Mathematics 3, or a higher math course, the Summative High School Mathematics CST is administered. The CSTs in mathematics consist of 65 multiple-choice questions with an additional 6 field-test questions.

Science

Students in grades five, eight, and ten take a science test. CSTs in Biology, Chemistry, Earth Science, Physics, and Integrated/Coordinated Science 1, 2, 3, and 4 are end-of-course tests taken by students in grades nine through eleven. The CSTs in science consist of 60 multiple-choice questions with an additional 6 field-test questions.

Statistical Analyses

Cognitive assessment was measured by comparing the California Standardized Testing and Reporting (STAR) system scores from 2009 for students in grades three through five in high (>30µg/L), low (≥2µg/L to 29µg/L), and non-dectect (ND) (<2µg /L) manganese exposure groups. Because some cities were sampled more than once, the

average manganese value from the samples was used to represent the cities' exposure group. The mean scale score for each grade was compared between exposure groups as well as the percentage of proficiency on a gradient scale. Exposure-response was also analyzed as continuous data.

Tests scores, demographic, and grade level information were downloaded as text files from the California STAR program website and imported to Microsoft Access. Drinking water manganese levels, test scores, demographic information, and grade level comprised a large Microsoft Access data base which was manipulated to extract desired data about each grade level, test, city, etc. These data were then imported into SAS and analyzed.

Univariate Analysis

Statistical analyses were performed using the general linear model procedure of the statistical package SAS (SAS Institute Inc., Cary, NC). Crude analysis was done as a one-, two- or three-way analysis of variance (ANOVA). Factors named "Mean Scale Score", "Manganese Group", "Grade", and "Test Name" were used in the model.

Confounder Selection

Confounders available in this data set included levels of other potentially neurotoxic metals in the drinking water, the student to teaher ratio in the school, the students' sex, ancestry, English fluency, parent education, and economic status. For confounder

selection, Spearman's Rank Correlation Test was used to measure each variable's correlation against Mean Scale Score. Manganese Group and the three variables with the highest correlation coefficient were entered into the adjusted models: Percent Not Economically Disadvantaged, Percent Economically Disadvantaged, and Percent Hispanic. Correlation was not determined between potential confounding variables and manganese levels because manganese group was the independent variable.

Multivariate Analysis

Multivariate analyses of the relationship between test scores and water manganese concentration were conducted using PROC GLM and PROC MIXED for analysis of covariance in the exposure modeled as a categorical variable with three levels of exposure: "high", "low" and "non-detect". Because of the unbalanced design of the model, PROC ANOVA was not an appropriate command. The Mixed Effects Model in SAS is similar to the General Linear Model (GLM), but allows more flexibility and was specifically designed to fit both fixed and random effects in the model. The mixed effect model is ideal for analyzing data nested within naturally occurring hierarchies such as students within classes (Singer, 1998). In this study, "city" was the random effect. Data for GLM analysis were averaged by city because only one water sample was taken for each city. Each city's test scores, demographic information percents, and drinking water contaminate values were averaged so that each city had one value for each of these categories. These averaged values were then used in the model (Table 31). In the previous models, each city had multiple test scores, demographic information percents,

and drinking water contaminate values because each city's schools were represented in the model individually (Table 8).

Separate analyses were conducted for English, Math, and Science tests with combined grades and for each test in each grade. Associations were tested using a two-tailed test based on the type III sums of squares. Differences in the adjusted means between the highest and lowest exposure categories were compared using the least significant differences statistic. The availability of data for ethnicity, parent education and economic status was important for adjusting for potentially important covariates in each model.

CHAPTER 4

Results

Exposure Assessment

One water sample per city was taken and analyzed for manganese and used to represent exposure for each school in the city. Water sample collections were conducted at two different times. In May-July 2009 water sample vials were mailed to selected schools and mailed back to the author for analysis. Because of the low response rate, a second set of samples were collected in August 2009 personally by the author in California from public water fountains.

The total number of schools and students in the Non-Detect (ND)(<2µg/L), Low (2-29µg/L), and High (>30µg/L) manganese exposure groups are displayed in Table 1. A map of sampled cities' manganese levels can be found in figure 3. Tables 2, 3, and 4 present the number of samples in the ND, Low and High manganese exposure groups from the first, second, and combined rounds of sampling (respectively). The results of this merger (and final sample size) are presented in Table 5. More than 50% of the schools fell in the non-detect manganese group.

The manganese levels ascertained from drinking water quality reports did not correspond with the manganese levels reported from our laboratory analysis (Appendix A). Two manganese levels were reported on most water quality reports: “Average Amount Detected” and “High Level Detected”. Reports displayed one or both of these values. For this reason, sensitivity and specificity were evaluated for both categories using manganese values from drinking water quality reports as the “Gold Standard” (Tables 6 and 7). The water quality report manganese values were used at the Gold Standard because they represent an average amount of manganese levels in drinking water from repeated sampling. Manganese values were categorized into Non-Detect (ND) (<2µg/L), Low (≥2µg /L to 29µg /L) and High (>30µg /L) groups. The sensitivity and specificity of the average amount and high manganese values reported from analysis were calculated using the water quality reports values as the standard of comparison (Gold Standard). The sensitivity for the average and high manganese value was 29.5% and 1%, respectively, and the specificity 8% and 27.5%, respectively.

Unadjusted Analyses

Summarized PROC MEANS statistics for potential confounding variables for all grades and tests are presented in Table 8. The mean manganese level of all samples taken was 6.21µg/L. Because individual level data are not available, the data are presented at the grade level. These variables were correlated with Mean Scale Score to produce a Spearman Correlation Coefficient (Tables 9 - 12).

Analysis of variance (ANOVA) was performed to evaluate differences between Mean Scale Score across three levels of manganese exposure: ND, low, and high. Differences in the unadjusted means were compared using the least squared mean (LSM) and contrast statistic. LSM were compared four ways: "High vs. Low and ND", "High and Low vs ND", "High vs. Low", and "High vs. ND". Significant differences were observed between manganese exposure groups in English test scores ($p = 0.04$), and math test scores were found to have borderline significance ($p = 0.08$) when 3rd, 4th, and 5th grade students were combined in the unadjusted analyses. Every contrast for English test scores was found to be significant. The contrast in math tests between "High vs Low and ND" had borderline significance ($p = 0.06$), and "High and Low vs ND" was statistically significant ($p =0.03$) (Table 13, Figure 5). When the tests were separated by grade, the difference between manganese exposure groups' Mean Scale Score was not significant in 3rd and 4th grade math and English test scores (Tables 14 – 15). The difference between manganese exposure group was significant for 5th grade science test scores ($p = 0.003$) (Table 16). There were no statistically significant least square means contrasts (Tables 14-16). The LSM for Mean Scale Score did not follow the expected trend across manganese groups. LSM were greater for each test when grades were combined and separated in the high manganese exposure group (Tables 13 -16). While the LSM mean scale score was still highest in the "high" manganese exposure group, tests separated by grade yielded no statistically significant contrasts (Tables 14 -16), with the exception of borderline significance in 4th grade math (Table 15). These results, contradictory to this

study's hypothesis, appear to suggest that Mean Scale Score does not have an inverse relationship with higher manganese levels.

Confounder Selection

The Spearman Correlation Coefficient for potential confounders vs. Mean Scale Score in all students and all tests combined resulted in Percent Not Economically Disadvantaged, Percent Economically Disadvantaged, and Percent Hispanic as the three most highly correlated variables (respectively) (Table 9). Percent Hispanic and Percent Economically Disadvantaged were negatively correlated with Mean Scale Score. Manganese had the only statistically insignificant correlation with Mean Scale Score (p =0.17) and the lowest correlation coefficient (0.01784). The Spearman Correlation Coefficient for potential confounders vs. Mean Scale Score for all grades combined resulted in Percent Not Economically Disadvantaged, Percent Economically Disadvantaged, and Percent Hispanic as the three most highly correlated variables (respectively) for English-Language Arts (Table 10). Percent Hispanic and Percent Economically Disadvantaged were negatively correlated with Mean Scale Score. Manganese (p =0.59), Copper (p =0.06), and Student Teacher Ratio (p =0.15) had insignificant correlations with Mean Scale Score. Manganese had the lowest correlation coefficient (0.01). The Spearman Correlation Coefficient for potential confounders vs. Mean Scale Score for all grades combined resulted in Percent Not Economically Disadvantaged, Percent Economically Disadvantaged, and Percent Hispanic as the three most highly correlated variables (respectively) for Mathematics (Table 11). Percent Hispanic and Percent Economically

Disadvantaged were negatively correlated with Mean Scale Score. Manganese (p =0.29) and Percent White (p =0.14) had insignificant correlations with Mean Scale Score. Manganese had the lowest correlation coefficient (0.02). The Spearman Correlation Coefficient for potential confounders vs. Mean Scale Score for 5th grades resulted in Percent Not Economically Disadvantaged, Percent Economically Disadvantaged, and Percent Hispanic as the three most highly correlated variables (respectively) for English-Language Arts (Table 12). Percent Hispanic and Percent Economically Disadvantaged were negatively correlated with Mean Scale Score. Manganese (p =0.34), Cadmium (p =0.07), Copper (p =0.21), and Student Teacher Ratio (p =0.77) had insignificant correlations with Mean Scale Score. Student Teacher Ratio had the lowest correlation coefficient (0.01).

Adjusted Analyses

Analysis of covariance (ANACOVA) was performed to evaluate differences between Mean Scale Score across three levels of manganese exposure for the adjusted analysis. When adjusted for Percent Not Economically Disadvantaged, Percent Economically Disadvantaged, and Percent Hispanic, the ANACOVA model yielded statistically significant results across tests when 3rd, 4th, and 5th grades were combined and analyzed separately (p= <.0001) (Tables 17 – 18) The overall F test is significant, indicating that the Mean Scale Score was not equal in all three manganese groups when grades are combined and separated (Tables 17 – 18). Manganese Group was not found to be statistically significant in any of the grades or tests (Table 17). Percent Hispanic had a

statistically significant effect on mean scale score at 0.05 for all grades and tests. Percent Not Economically Disadvantaged had a significant effect on mean scale score in 3rd and 4th grade English-Language Arts and Mathematics. Percent Economically Disadvantaged was only found to be statistically significant in 5th grade English-Language Arts (Table 17). The R^2 indicates that the model accounts for 49%, 35%, and 55% of the variation in Mean Scale Score for English-Language Arts, Math, and Science, respectively (Table 18, Figure 7).

When adjusted, mean scale scores contrasts no longer held any statistically significant differences in any grade or test (Tables 19 – 21). The LSM mean scale scores still did not follow expected trends across manganese groups. In 3rd and 4th grade math and English the LSM scores were greatest in the High manganese exposure group (Tables 19 – 20), but were not consistently highest in the "high" exposure group in 5th grade tests (Table 21).

Mixed Effects Model

Unadjusted Mixed Effects Analysis

Analysis of variance (ANOVA) was performed to evaluate differences between Mean Scale Score across three levels of manganese exposure: ND, low, and high. No significant associations were observed in all three tests when 3rd, 4th, and 5th grade students were combined in the mixed effects unadjusted analyses (Table 22). Differences

in the unadjusted means were compared using the least squared mean (LSM) and contrast statistic. LSM were compared four ways: “High vs. Low and ND”, “High and Low vs ND”, “High vs. Low”, and “High vs. ND”. There were, however, no statistically significant least squares mean contrasts for any grade or test (Tables 23 – 25). The LSM for Mean Scale Score did not follow the expected trend across manganese groups, but was no longer consistently greatest in the High manganese exposure group. While the LSM mean scale score had less variability when adjusted for random effects than the unadjusted model, there were still no statistically significant differences found between manganese exposure groups.

Adjusted Mixed Effects Analysis

Analysis of covariance (ANACOVA) was performed to evaluate differences between Mean Scale Score across three levels of manganese exposure for the mixed effects adjusted analysis. When adjusted for Percent Not Economically Disadvantaged, Percent Economically Disadvantaged, and Percent Hispanic, the ANACOVA model yielded no statistically significant results for difference in Mean Scale score among manganese groups when 3rd, 4th, and 5th grades were analyzed together and separately (Tables 26 - 30). The LSM mean scale scores did not follow any pattern across manganese exposure groups, and no contrasts were statistically significant when all grades were combined (Table 26).

Manganese group was not found to be statistically significant in any of the grades or tests (Tables 27). Percent Hispanic had a statistically significant effect on mean scale score at 0.05 for all grades and tests except 4th grade math and 5th grade math. Percent Not Economically Disadvantaged had a significant effect on mean scale score in 3rd and 4th grade English-Language Arts and Mathematics. Percent Economically Disadvantaged was only found to be statistically significant in 5th grade English-Language Arts (Table 27).

Mean scale scores contrasts remained statistically insignificant in all grades and tests (Tables 28 – 30). The LSM mean scale scores still did not follow expected trends across manganese groups. LSM mean scale scores were very similar across manganese groups, but remained highest in the high exposure group for 3rd and 4th grade English and math tests (Table 28 – 29).

Generalized Linear Model

Summarized PROC MEANS statistics for potential confounding variables for all grades and tests are presented in Table 31. The mean manganese level of samples taken and used in the GLM was 0.0078µg/L. Because individual level data are not available, the data are presented at the average city level. N for this table is 562 because each school's grades' tests scores were averaged by city. For 3rd and 4th grade there are two tests associated with the manganese values and three tests in the 5th grade. Ideally, for each of the 82 samples taken there are seven tests associated with the manganese values. Some entities

are not present because not all schools had all three grades. These variables were correlated with Mean Scale Score to produce a Spearman Correlation Coefficient (Table 32). As found previously, Percent Hispanic, Percent Economically Disadvantaged, and Percent Not Economically Disadvantaged were the three variables most highly correlated with Mean Scale Score. Unlike the previous correlation analysis, manganese was significantly correlated with Mean Scale Score ($p = 0.04$), but the correlation was not negative as predicted (Table 32).

Unadjusted Generalized Linear Model

When tests scores were averaged within cities, there was no statistically significant association between mean scale scores and manganese as a continuous variable. In each grade and test, mean scale scores in the low manganese group were highest. There were no spastically significant ANOVA models (Table 33).

Adjusted Generalized Linear Model

The GLM model was adjusted for Percent Not Economically Disadvantaged, Percent Economically Disadvantaged, and Percent Hispanic. For all grades and tests Percent Not Economically Disadvantaged was statistically significantly associated with Mean Scale Score. Percent Hispanic was significantly associated with Mean Scale Score in 3rd grade English and 5th grade science scores (Table 34).

When manganese was placed into the GLM as a categorical variable, the results were similar to the analysis with manganese as a continuous variable. Percent Not Economically Disadvantaged was statistically significantly associated with Mean Scale Score for all tests and grades. Percent Hispanic was statistically significantly associated with Mean Scale Score in 5th grade science and held border line significance in 3rd grade English scores (Table 35).

CHAPTER 5

DISCUSSION

The purpose of this study was to test the hypothesis that low level exposure to manganese in children through drinking water is associated with impaired cognitive performance on standardized tests of intellectual function. The results of this study did not show an inverse relationship between manganese levels in drinking water and cognitive performance on standardized tests of intellectual function as hypothesized. This may be due to the relatively small number of water samples classified in the high manganese exposure group, failure to detect subtle cognitive impairments across exposure groups at the population level, or other unknown factors.

Identifying and characterizing any effects of low-dose exposure to environmental constituents is complicated by many factors. In our study, the effects of chronic exposure to manganese may be subclinical. They could be subtle in magnitude and within the range of normal variation. Low-dose exposure studies are nearly all observational by design. Because cognition is multi-determined, it is comprised of many influences including biological, psychological, and social.

This study may not have been successful elucidating a relationship between elevated levels of manganese in drinking water and cognitive performance on standardized tests because of the low number of high manganese values. Previous studies with statistically significant findings found manganese in drinking water upwards of 600µg/L (Bouchard, 2007; Wasserman, 2005). Because of the much smaller than anticipated high manganese levels, the standard error of estimated association for that exposure group was relatively large. Consequently, the LSM test scores were not significantly different from those in the low and ND manganese exposure groups. A comparison of only LSM test scores in the low and ND categories was not conducted because the effect of such low estimated exposure to manganese would not likely be detectable.

The analysis with the most statistically significant effects was the unadjusted and grade combined contrast of Mean Scale Score for each test (Table 13, Figure 5). A majority of the contrasts between LSM Mean Scale Score for different manganese groups were significant. When the same model was adjusted for city as a random effect, and when each city's test scores and demographic data were averaged, none of the significance remained. This may be explained by the fact that the unadjusted presented significance because of chance. With multiple entries associated with each city and manganese value, the model had more power than when adjusted. The same is true in the unadjusted analysis when the combined grade data is separated by grade and test. By separating the grades, power, and consequently significance, was lost.

Throughout adjustment and model design, Percent Not Economically Disadvantaged and Percent Hispanic remained consistently significantly associated with Mean Scale Score throughout the study. The association for Percent Not Economically Disadvantaged suggests a positive correlation with test score, and Percent Hispanic a negative correlation. This seems plausible within the context of this study as it is generally understood that more affluent populations have better access to many factors, such as nutrition and health care which may improve scores on cognitive tests. Hispanic populations may not speak English as well as their native English speaking classmates and therefore not perform as well on standardized tests.

When comparing the basic statistics (mean, standard deviation, and median) of the raw data and the data which was averaged by city the differences are subtle but clearly impacted the results of the analysis. The mean Mean Scale Score for the raw data (366.19) only differed from the averaged by city's mean Mean Scale Score (362.13) by 4.06 points, but the standard deviation was 9.73 points greater in the raw data. The majority of standard deviations for other categories were less in the averaged data.

California was chosen for this study because of the large amount of drinking water quality data and standardized test score data available online. In addition, based on 2003 statistics from the EPA, 17.2% of public water systems sampled in the state of California had manganese concentration levels above 150 µg /L.

In the majority of studies examining the neurotoxic and behavioral effects of manganese on humans, inhalation was the route of exposure and many were occupational exposures. While inhalation is an important route of exposure, it has been studied extensively in humans and is not typically a common exposure route for children. In order to better understand and prevent manganese toxicity in children it is important to examine the effects of ingested manganese on neuro-function and behavior, and what amount can be safely ingested.

Past studies have used individual level data to study the impact of ingested manganese on cognitive and behavioral function. Biological markers such as hair, blood, and urine manganese levels were measured and examined against individual cognitive and behavioral tests (Bouchard, 2005, 2007; Collipp, 1983; Mergler, 1999; Wasserman, 2006; Woolf, 2002). Because of financial and temporal restrictions, individual level data were not able to be used in this study.

The disparity between drinking water reports and the values found from the analysis of samples could be a result of newly implemented drinking water standards improvements. Although manganese is not considered a regulated contaminant, California is very progressive in their health standards. Many drinking water quality reports with very high levels of manganese were accompanied by a letter expressing the possible health effects of manganese. Some of these cities implemented filtration systems specifically aimed at reducing the amount of manganese in the drinking water.

Limitations

To date, no ecologic studies have examined the effects of elevated levels of manganese in drinking water and its effects on cognitive ability in children. Ecologic studies serve mainly to generate hypotheses. However, because population data are used to draw conclusions, ecological fallacy can occur and incorrect assumptions may be made.
In this study, it would appear that elevated levels of manganese in manganese may actually cause an increase in children's standardized test scores. This said, no matter what the study design, a causal association between exposure and disease cannot be proved. Epidemiologic studies may provide an estimated measure of association with the aide of statistics.

A significant limitation of this study was the lack of individual data. When measuring a sensitive, multi-factoral characteristic such as cognitive performance, it is important to control for as many contributing factors by gathering as much individual level data as possible.

Bias

Several possible biases may have affected the results of this study. Confounding may have biased results because many important confounders such as mobility and nutritional status were unavailable. Confounding factors cannot typically be taken into account in most ecologic studies because of the unavailability of such data. Although some

confounders were controlled at the population level, cognitive performance and its measure is very idiosyncratic. At the population level, unfortunately, it is very difficult to measure subtle reductions in cognitive performance because of exposure to manganese.

Information bias may have occurred in the exposure or outcome assessment. Due to the lack of elevated manganese levels initially predicted for the study, new classification criteria for “high”, “low”, and “ND” were created. This may have weakened or muted the effects of manganese level on mean scale score. Because of the ecologic nature of the study, many individuals may have been misclassified in the exposure category. The drinking water system from which they drink may have been different from the one sampled (although an attempt to control for this was made), some may drink from private wells, drink bottled water, have private filtration systems, or may not have lived in the sampled region long enough for symptoms to manifest.

Selection bias may have been introduced when collecting water samples. Because of the reliance on drinking water quality reports (Figure 1) and the USGS map of manganese in ground water (Figure 4), the selection of collection sites depended on the accuracy, and currency of the data provided which may have been inaccurate or misleading. Cities with more money for reporting these data may have also had the money to properly filter manganese from the water. Cities with more money typically have better educational institutions as well. Unfortunately, the drinking water quality reports were not as accurate or current as expected based on the sensitivity and specificity analysis conducted in this study (Tables 6 and 7).

In addition to the aforementioned problems, there were also challenges with carrying out this study. The initial sampling plan for the study was to have water samples mailed to us from targeted schools. Of 100 schools, only thirty five returned water samples. This sample size was far too small and we did not have enough high exposure samples. After analysis of the samples, it was revealed that the actual manganese levels were much smaller than reported in the drinking water quality reports used to target exposure areas.

There is also a possibility that manganese levels were higher in the past (as suggested from drinking water quality reports). Because of the concern for aesthetics and taste quality of the water, more and more cities and private well owners have implemented the use of inexpensive and effective water filtration systems such as greensand manganese and irons filters.

Past studies on manganese exposure in manganese and neuro-behavioral impairment have yielded statistically significant results (Bouchard 2007, He 1994, Kawamura 1941, Wasserman 2006). In these studies, drinking water manganese levels were >500µg/L, individual level demographic, biological, psychological, and IQ data was available for study subject. In this study, many subjects were used in analysis, but none at the individual level. Cognitive and behavioral impairment characteristics are very difficult to identify at a grade level.

Strengths

With an ecological study such as ours these factors are difficult to identify, measure, and control. The effects of these factors may seriously drown out the effect of manganese on cognition. Although our study focused on a single toxicant, manganese, most individuals are exposed to a variety and mixture of chemicals. An effect maybe erroneously attributed to a particular chemical when it actually reflects the influence of a correlated exposure (Bellinger and Adams, 2001). In most human studies exposure assessments relies on biomarkers to estimate toxicity on the brain. However, because manganese has such a short half life in the body, there are no reliable biomarkers to measure long term exposure or body burden. Because brain measurement is not possible (for living subjects) exposure misclassification becomes a problem. Because of the lack of reliable biomarkers, indirect exposure assessment measures were used.

Conclusions

Although no definitive evidence concerning the effect of manganese in drinking water on standardized test scores was found in this study, further investigation should not be ruled out. The biological plausibility of an association is strong and has been supported in previous studies (Wasserman, 2006; Bouchard, 2005).

Although there are currently no reliable biomarkers for manganese exposure and body burden, the use of biological markers in concurrence with individual level data on manganese intake from food, water, and dietary supplements would certainly provide a

more accurate exposure assessment. Individual cognitive and behavioral assessments may present a more accurate inference about the relationship between manganese exposure and cognitive impairment in children.

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TABLES

Table 1: Total Number of Schools and Students in Each Manganese Exposure Group

Manganese Group	Schools	Students
Non-Detect (<2)	528	285602
Low (2-29)	264	153332
High (>30)	52	25676
Total	844	464612

Table 2: First Set of California Drinking Water Samples Stratified by Level of Manganese Detected

Manganese Detected in Water Sample (µg/L)	Number of Samples (n , %)
Non-Detect (< 2)	14 (40.00)
Low (2-29)	19 (54.29)
High (>30)	2 (5.71)
Total	35

Table 3: Second Set of California Drinking Water Samples Stratified by Level of Manganese Detected

Manganese Detected in Water Sample (µg/L)	Number of Samples (n , %)
Non-Detect (< 2)	37 (58.73)
Low (2-29)	21 (33.33)
High (>30)	5 (7.94)
Total	63

Table 4: All California Drinking Water Samples Stratified by Level of Manganese Detected

Manganese Detected in Water Sample (µg/L)	Number of Samples (n , %)
Non-Detect (< 2)	51 (52.04)
Low (2-29)	40 (40.82)
High (>30)	7 (7.14)
Total	98

Table 5: Cities in California Sampled Stratified by Level of Manganese Detected

Manganese Detected in Water Sample (µg/L)	Number of Samples (n , %)
Non-Detect (< 2)	42 (51.85)
Low (2-29)	33 (40.74)
High (>30)	6 (7.41)
Total	81

Table 6: Specificity and Sensitivity of the Reported Manganese Average from Water Quality Reports

	Water Quality Report Reported Manganese Average		
ICP Results	High	Low	Total
High	3	2	5
Low	13	57	70
Total	16	59	75

Specificity = 8%
Sensitivity = 29.5%

Table 7: Specificity and Sensitivity of the Reported High Levels of Manganese from Water Quality Reports

	Water Quality Report Reported High Manganese		
ICP Results	High	Low	Total
High	2	0	2
Low	53	21	74
Total	55	21	76

Specificity = 27.5%
Sensitivity = 1%

Table 8 : N, Mean, Standard Deviation and Median Statistics of Potential Confounders to Standardized Test Scores

Variable	N	Mean	Std Dev	Median
Mean Scale Score	5782	366.19	37.361	362.00
Manganese	5782	6.21	12.67	0.50
Percent White	343	34.73	20.49	31.00
Percent Asian	1657	22.12	10.93	20.00
Percent Male	5715	48.12	4.50	48.00
Percent Hispanic	4527	54.10	24.70	56.00
Percent Black	672	14.56	48.73	13.00
Percent Fluent	5744	60.20	21.16	59.00
Percent Not Fluent	4255	32.06	15.79	30.00
Percent Parent High School Graduate	4284	24.98	9.02	24.00
Percent Parent Some College and Beyond	4703	24.29	8.45	23.00
Percent Parent No Diploma	2968	28.81	13.56	26.00
Percent Female	5682	47.42	4.24	47.00
Percent Not Economically Disadvantaged	4720	52.23	26.25	53.00
Percent Economically Disadvantaged	5050	57.41	25.91	61.00
Pb	5782	0.01120	0.01032	0.01000
Cd	5782	0.0007450	0.00130	0.00050
Cu	5782	0.10680	0.14520	0.05100
Fe	5782	0.10026	0.55401	0.00500
Student Teacher Ratio	5782	19.43835	2.41965	19.000

Table 9: Spearman Correlation Coefficients between potential confounders and Mean Scale Score

Variable	Spearman Correlation Coefficient	*p* Value	Rank
Manganese	0.01784	0.17	19
Percent White	0.20190	0.0002	11
Percent Asian	0.35714	<0.0001	8
Percent Male	0.05826	<0.0001	15
Percent Hispanic	-0.53335	<0.0001	3*
Percent Black	-0.38993	<0.0001	7
Percent Fluent	0.44102	<0.0001	6
Percent Not Fluent	-0.49544	<0.0001	5
Percent Parent High School Graduate	-0.24415	<0.0001	10
Percent Female	0.06971	<0.0001	14
Percent Not Economically Disadvantaged	0.64897	<0.0001	1*
Percent Parent with Some College and Beyond	0.51354	<0.0001	4
Percent Parent with No Diploma	-0.26305	<0.0001	9
Percent Economically Disadvantaged	-0.60877	<0.0001	2*
Lead	0.08334	<0.0001	13
Cadmium	-0.05621	<0.0001	16
Copper	0.03987	0.002	17
Iron	-0.12667	<0.0001	12
Student Teacher Ratio	0.03141	0.02	18

* Variables were chosen for the model

Table 10: Spearman Correlation Coefficients between potential confounders and Mean Scale Score in English-Language Arts

Variable	Spearman Correlation Coefficient	*p* Value	Rank
Manganese	0.01067	0.59	19
Percent White	0.34261	<.0001	10
Percent Asian	0.34647	<.0001	9
Percent Male	0.05705	0.004	16
Percent Hispanic	-0.65077	<.0001	3*
Percent Black	-0.40315	<.0001	7
Percent Fluent	0.54546	<.0001	6
Percent Not Fluent	-0.59386	<.0001	5
Percent Parent High School Graduate	-0.28568	<.0001	11
Percent Female	0.08594	<.0001	14
Percent Not Economically Disadvantaged	0.75364	<.0001	1*
Percent Parent with Some College and Beyond	0.60278	<.0001	4
Percent Parent with No Diploma	-0.34979	<.0001	8
Percent Economically Disadvantaged	-0.72115	<.0001	2*
Lead	0.10030	<.0001	13
Cadmium	-0.05885	0.003	15
Copper	0.03740	0.062	17
Iron	-0.15088	<.0001	12
Student Teacher Ratio	0.02853	0.155	18

*Variable was chosen to be in model

Table 11: Spearman Correlation Coefficients between potential confounders and Mean Scale Score in Mathematics

Variable	Spearman Correlation Coefficient	*p* Value	Rank
Manganese	0.02121	0.29	19
Percent White	0.12039	0.14	12
Percent Asian	0.41383	<.0001	7
Percent Male	0.06201	0.002	15
Percent Hispanic	-0.50756	<.0001	3*
Percent Black	-0.43261	<.0001	6
Percent Fluent	0.38374	<.0001	8
Percent Not Fluent	-0.47254	<.0001	5
Percent Parent High School Graduate	-0.25560	<.0001	9
Percent Female	0.06095	0.002	16
Percent Not Economically Disadvantaged	0.63219	<.0001	1*
Percent Parent with Some College and Beyond	0.48506	<.0001	4
Percent Parent with No Diploma	-0.22086	<.0001	10
Percent Economically Disadvantaged	-0.58774	<.0001	2*
Lead	0.07894	<.0001	13
Cadmium	-0.06659	0.0009	14
Copper	0.05421	0.007	17
Iron	-0.12385	<.0001	11
Student Teacher Ratio	0.04787	0.01	18

*Variable was chosen to be in model

Table 12: Spearman Correlation Coefficients Between Potential Confounders and Mean Scale Score In Science

Variable	Spearman Correlation Coefficient	*p* Value	Rank
Manganese	0.03293	0.34	18
Percent White	0.46343	0.01	8
Percent Asian	0.44010	<.0001	9
Percent Male	0.08550	0.01	14
Percent Hispanic	-0.68174	<.0001	3*
Percent Black	-0.49323	<.0001	7
Percent Fluent	0.57214	<.0001	6
Percent Not Fluent	-0.59202	<.0001	5
Percent Parent High School Graduate	-0.28507	<.0001	11
Percent Female	0.06754	0.05	15
Percent Not Economically Disadvantaged	0.76058	<.0001	1*
Percent Parent with Some College and Beyond	0.64508	<.0001	4
Percent Parent with No Diploma	-0.38378	<.0001	10
Percent Economically Disadvantaged	-0.75192	<.0001	2*
Lead	0.09209	0.008	13
Cadmium	-0.06234	0.07	16
Copper	0.04321	0.21	17
Iron	-0.135939	<.0001	12
Student Teacher Ratio	0.00990	0.77	19

*Variable was chosen to be in model

Table 13: Unadjusted Associations Between Manganese Exposure and Test Scores for 3rd, 4th, and 5th Grade Students in California, 2009

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	361.16	355.20	354.67	$p = 0.02$	$p = 0.03$	$p = 0.03$	$p = 0.01$
Math[2]	387.32	381.62	380.02	$p = 0.06$	$p = 0.03$	$p = 0.11$	$p = 0.03$
Science[3]	360.65	356.15	353.43	$p = 0.26$	$p = 0.11$	$p = 0.41$	$p = 0.17$

[1] $p = 0.05$, $R^2 = 0.002441$
[2] $p = 0.09$, $R^2 = 0.001962$
[3] $p = 0.28$, $R^2 = 0.003095$

Table 14: Unadjusted Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 3rd Grade Students in California, 2009

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	346.51	340.78	340.60	$p = 0.17$	$p = 0.24$	$p = 0.20$	$p = 0.17$
Math[2]	393.90	386.93	386.85	$p = 0.23$	$p = 0.31$	$p = 0.26$	$p = 0.23$

[1] $p = 0.39$, $R^2 = 0.002259$
[2] $p = 0.49$, $R^2 = 0.001732$

Table 15: Unadjusted Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 4th Grade Students in California, 2009

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	374.17	367.23	366.55	$p = 0.09$	$p = 0.11$	$p = 0.13$	$p = 0.08$
Math[2]	390.44	382.55	380.33	$p = 0.08$	$p = 0.05$	$p = 0.15$	$p = 0.05$

[1] p=0.23, R^2=0.003561
[2] p=0.14, R^2=0.004721

Table 16: Unadjusted Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 5th Grade Students in California, 2009

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	363.10	357.67	356.79	$p = 0.13$	$p = 0.13$	$p = 0.19$	$p = 0.11$
Math[2]	377.63	375.34	372.93	$p = 0.57$	$p = 0.34$	$p = 0.72$	$p = 0.45$
Science[3]	360.65	356.15	353.43	$p = 0.25$	$p = 0.11$	$p = 0.41$	$p = 0.16$

[1] p=0.28, R^2=0.003038
[2] p=0.61, R^2=0.001162
[3] p=0.003, R^2=0.00309

Table 17: Adjusted Associations Between Manganese Exposure Groups and Test Scores for 3rd, 4th and 5th Grade Students in California, 2009*

	ANACOVA Model	R^2	Manganese Group	Percent Not Economically Disadvantaged	Percent Economically Disadvantaged	Percent Hispanic
3rd Grade						
English	*p*= <0.0001	0.592300	*p*= 0.41	*p*= 0.002	*p*= 0.89	*p*= <0.0001
Math	*p*= <0.0001	0.408667	*p*= 0.49	*p*= 0.006	*p*= 0.51	*p*= <0.0001
4th Grade						
English	*p*= <0.0001	0.614513	*p*= 0.39	*p*= 0.004	*p*= 0.18	*p*= <0.0001
Math	*p*= <0.0001	0.387380	*p*= 0.28	*p*= 0.002	*p*= 0.72	*p*= 0.02
5th Grade						
English	*p*= <0.0001	0.626571	*p*= 0.33	*p*= 0.76	*p*= 0.001	*p*= <0.0001
Math	*p*= <0.0001	0.324019	*p*= 0.91	*p*= 0.42	*p*= 0.15	*p*= 0.03
Science	*p*= <0.0001	0.554113	*p*= 0.81	*p*= 0.21	*p*= 0.11	*p*= <0.0001

*Adjusted for Percent not Economically Disadvantaged, Percent Economically Disadvantaged, Percent Hispanic

Table 18: Adjusted Associations Between Manganese Exposure and Test Scores for Combined 3rd, 4th, and 5th Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	352.84	350.90	352.54	p = 0.52	p = 0.54	p = 0.29	p = 0.86
Math[2]	379.47	376.99	377.09	p = 0.35	p = 0.49	p = 0.37	p = 0.37
Science[3]	347.92	349.85	349.97	p = 0.53	p = 0. 58	p = 0.56	p = 0.52

[1] p= <.0001, R^2 =0.494362
[2] p= <.0001, R^2 =0.34997
[3] p= <.0001, R^2 =0.554113
*Adjusted for Percent not Economically Disadvantaged, Percent Economically Disadvantaged, Percent Hispanic

Table 19: Adjusted Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 3rd Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English	339.68	337.21	338.87	$p = 0.52$	$p = 0.78$	$p = 0.36$	$p = 0.76$
Math	387.82	382.91	384.83	$p = 0.36$	$p = 0.84$	$p = 0.28$	$p = 0.49$

*Adjusted for Percent not Economically Disadvantaged, Percent Economically Disadvantaged, Percent Hispanic

Table 20: Adjusted Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 4th Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English	365.38	362.62	364.22	$p = 0.44$	$p = 0.89$	$p = 0.30$	$p = 0.65$
Math	383.52	378.24	377.04	$p = 0.14$	$p = 0.12$	$p = 0.21$	$p = 0.11$

*Adjusted for Percent not Economically Disadvantaged, Percent Economically Disadvantaged, Percent Hispanic

Table 21: Adjusted Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 5th Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English	354.19	352.76	354.54	$p = 0.80$	$p = 0.43$	$p = 0.52$	$p = 0.87$
Math	367.49	369.50	369.50	$p = 0.68$	$p = 0.74$	$p = 0.69$	$p = 0.68$
Science	347.92	349.85	349.97	$p = 0.53$	$p = 0.58$	$p = 0.56$	$p = 0.52$

*Adjusted for Percent not Economically Disadvantaged, Percent Economically Disadvantaged, Percent Hispanic

Table 22: Unadjusted Mixed Effect Model Associations Between Manganese Exposure and Test Scores for Combined 3rd, 4th, and 5th Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	354.31	355.26	351.78	$p = 0.92$	$p = 0.58$	$p = 0.97$	$p = 0.77$
Math[2]	375.83	379.62	372.88	$p = 0.97$	$p = 0.48$	$p = 0.74$	$p = 0.79$
Science[3]	354.06	356.77	351.65	$p = 0.98$	$p = 0.60$	$p = 0.82$	$p = 0.83$

[1] $p= 0.76$
[2] $p= 0.52$
[3] $p= 0.71$
*Adjusted for random effects

Table 23: Unadjusted Mixed Effect Model Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 3rd Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	343.19	341.96	337.53	$p = 0.71$	$p = 0.39$	$p = 0.90$	$p = 0.55$
Math[2]	385.73	386.51	379.76	$p = 0.83$	$p = 0.40$	$p = 0.95$	$p = 0.63$

[1] $p= 0.62$
[2] $p= 0.56$
*Adjusted for random effects

Table 24 Unadjusted Mixed Effect Model Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 4th Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	368.69	366.73	364.84	$p = 0.76$	$p = 0.63$	$p = 0.84$	$p = 0.69$
Math[2]	384.84	381.43	376.11	$p = 0.55$	$p = 0.28$	$p = 0.75$	$p = 0.41$

[1] $p= 0.88$
[2] $p= 0.51$
*Adjusted for random effects

Table 25: Unadjusted Mixed Effect Model Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 5th Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	357.26	358.05	355.69	p = 0.9647	p = 0.72	p = 0.93	p = 0.86
Math[2]	369.17	373.13	367.17	p = 0.9388	p = 0.62	p = 0.76	p = 0.87
Science[3]	354.06	356.77	351.65	p = 0.9896	p = 0.60	p = 0.82	p = 0.83

[1] p= 0.88
[2] p= 0.68
[3] p= 0.71
*Adjusted for random effects

Table 26: Adjusted Mixed Effect Model Associations Between Manganese Exposure and Test Scores for Combined 3rd, 4th, and 5th Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English[1]	351.81	351.25	350.98	$p = 0.86$	$p = 0.83$	$p = 0.89$	$p = 0.84$
Math[2]	373.99	377.05	373.02	$p = 0.88$	$p = 0.57$	$p = 0.66$	$p = 0.89$
Science[3]	347.20	351.62	347.31	$p = 0.73$	$p = 0.62$	$p = 0.52$	$p = 0.98$

[1] $p = 0.98$
[2] $p = 0.53$
[3] $p = 0.48$
*Adjusted for Percent not Economically Disadvantaged, Percent Economically Disadvantaged, Percent Hispanic

Table 27: Adjusted Mixed Effect Model Associations Between Manganese Exposure Groups and Test Scores for 3rd, 4th, and 5thGrade Students in California, 2009*

	Manganese Group	Percent Not Economically Disadvantaged	Percent Economically Disadvantaged	Percent Hispanic
3rd Grade				
English	$p = 0.89$	$p = 0.003$	$p = 0.71$	$p =< 0.0001$
Math	$p = 0.83$	$p = 0.03$	$p = 0.85$	$p =< 0.0001$
4th Grade				
English	$p = 0.09$	$p = 0.0004$	$p = 0.47$	$p = 0.0005$
Math	$p = 0.35$	$p = 0.001$	$p = 0.72$	$p = 0.10$
5th Grade				
English	$p = 0.98$	$p = 0.21$	$p = 0.009$	$p = <0.0001$
Math	$p = 0.59$	$p = 0.30$	$p = 0.15$	$p = 0.18$
Science	$p = 0.48$	$p = 0.12$	$p = 0.10$	$p =< 0.0001$

*Adjusted for Random Effects

Table 28: Adjusted Mixed Effect Model Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 3rd Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English	339.25	337.90	337.12	*p*= 0.71	*p*= 0.63	*p*= 0.78	*p*= 0.66
Math	384.84	383.26	381.32	*p*= 0.72	*p*= 0.55	*p*= 0.83	*p*= 0.63

*Adjusted for random effects, Percent not Economically Disadvantaged, Percent Economically Disadvantaged, Percent Hispanic

Table 29: Adjusted Mixed Effect Model Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 4th Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English	364.12	362.59	363.10	*p*= 0.78	*p*= 0.93	*p*= 0.75	*p*= 0.83
Math	381.94	378.62	374.48	*p*= 0.39	*p*= 0.16	*p*= 0.61	*p*= 0.25

*Adjusted for random effects, Percent not Economically Disadvantaged, Percent Economically Disadvantaged, Percent Hispanic

Table 30: Adjusted Mixed Effect Model Least Squares Means and Contrasts Between Manganese Exposure Groups and Test Scores for 5th Grade Students in California, 2009*

	Least Squares Mean			Contrasts			
	High	Low	ND	High vs Low and ND	High and Low vs ND	High vs Low	High vs. ND
English	353.03	353.60	353.30	*p*= 0.92	*p*= 0.99	*p*= 0.90	*p*= 0.95
Math	364.07	368.88	364.23	*p*= 0.76	*p*= 0.67	*p*= 0.58	*p*= 0.98
Science	347.20	351.62	347.31	*p*= 0.73	*p*= 0.61	*p*= 0.52	*p*= 0.98

*Adjusted for random effects, Percent not Economically Disadvantaged, Percent Economically Disadvantaged, Percent Hispanic

Table 31: N, Mean, Standard Deviation and Median Statistics of Potential Confounders to Standardized Test Scores[1]

Variable	N	Mean	Std Dev	Median
Mean Scale Score	562	362.13	27.63	360.42
Manganese	562	7.8	14.0	1.0
Percent White	196	39.5	22.3	32.00
Percent Asian	247	15.65	6.5	13.25
Percent Male	562	48.65	3.8	48.50
Percent Hispanic	506	48.31	22.0	49.25
Percent Black	119	13.01	3.8	12.76
Percent Fluent	562	65.52	17.8	66.00
Percent Not Fluent	464	31.61	12.5	30.00
Percent Parent High School Graduate	506	25.98	8.9	24.92
Percent Parent Some College and Beyond	511	24.83	6.7	24.26
Percent Parent No Diploma	408	26.72	10.1	24.00
Percent Female	562	47.93	2.8	47.75
Percent Not Economically Disadvantaged	527	48.43	19.3	45.70
Percent Economically Disadvantaged	562	54.71	20.1	56.32
Pb	562	0.0112	0.00999	0.01
Cd	562	0.00067	0.00109	0.0005
Cu	562	0.096	0.14977	0.03
Fe	562	0.10528	0.53850	0.01
Student Teacher Ratio	562	19.21	2.03122	19.25

[1]Means are averaged by city

Table 32: Spearman Correlation Coefficients between potential confounders and Average Mean Scale Score for All Students and Tests in Each California City Sampled

Variable	Spearman Correlation Coefficient	*p* Value	Rank
Manganese	0.08640	0.04	15
Percent White	0.36593	<.0001	8
Percent Asian	0.29750	<.0001	10
Percent Male	0.05346	0.21	16
Percent Hispanic	-0.53447	<.0001	3
Percent Black	-0.37200	<.0001	7
Percent Fluent	0.39820	<.0001	6
Percent Not Fluent	-0.43282	<.0001	5
Percent Parent High School Graduate	-0.32677	<.0001	9
Percent Female	0.09073	0.03	14
Percent Not Economically Disadvantaged	0.61768	<.0001	1
Percent Parent with Some College and Beyond	0.47183	<.0001	4
Percent Parent with No Diploma	-0.29264	<.0001	11
Percent Economically Disadvantaged	-0.60997	<.0001	2
Lead	0.02266	0.59	18
Cadmium	0.20092	0.2	12
Copper	0.01257	0.77	19
Iron	-0.05299	0.21	17
Student Teacher Ratio	-0.14265	0.0007	13

[1]Means are averaged by city

Table 33: Unadjusted Least Squares Means Test Scores for 3rd, 4th, and 5th Grade Students in California[1]

	ANOVA	Least Squares Mean		
	p	High	Low	ND
3rd Grade				
English	0.53	339.59	341.31	335.91
Math	0.55	377.81	384.31	377.31
4th Grade				
English	0.89	364.66	365.53	363.01
Math	0.47	377.97	379.55	372.70
5th Grade				
English	0.75	353.36	357.74	354.60
Math	0.42	359.94	372.50	364.75
Science	0.59	349.32	356.49	349.32

[1]Means are averaged by city

Table 34: Adjusted Associations Between Manganese Exposure and Test Scores for 3rd, 4th, and 5thGrade Students in California, 2009[1]

	Manganese	Percent Not Economically Disadvantaged	Percent Economically Disadvantaged	Percent Hispanic
3rd Grade				
English	$p = 0.99$	$p = 0.002$	$p = 0.84$	$p = 0.02$
Math	$p = 0.57$	$p = 0.04$	$p = 0.89$	$p = 0.18$
4th Grade				
English	$p = 0.87$	$p = <0.0001$	$p = 0.59$	$p = 0.31$
Math	$p = 0.42$	$p = 0.0003$	$p = 0.55$	$p = 0.37$
5th Grade				
English	$p = 0.96$	$p = 0.0002$	$p = 0.43$	$p = 0.28$
Math	$p = 0.79$	$p = 0.05$	$p = 0.37$	$p = 0.87$
Science	$p = 0.78$	$p = 0.0003$	$p = 0.83$	$p = 0.02$

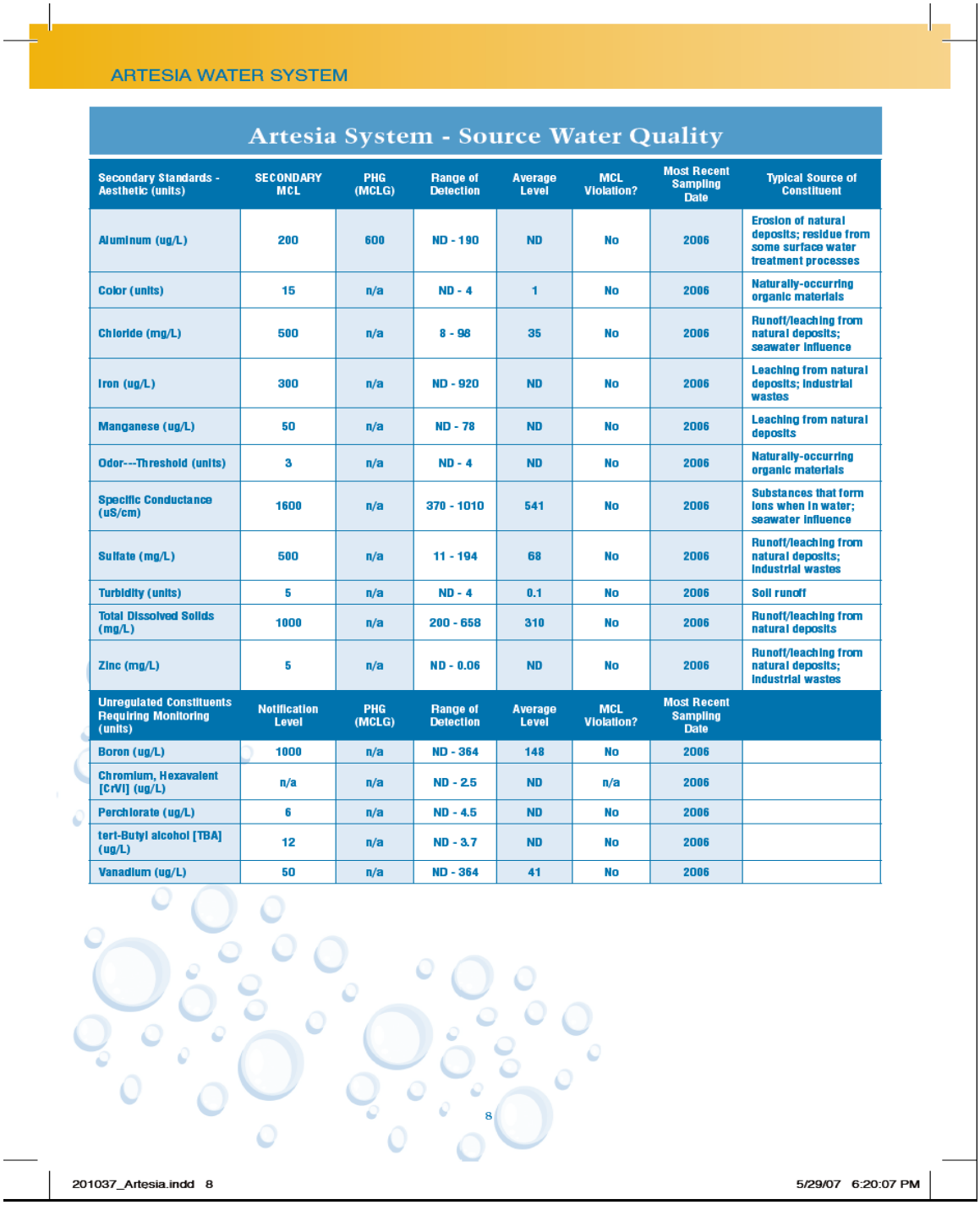
[1]Means are averaged by city

Table 35: Adjusted Associations Between Manganese Exposure Groups and Test Scores for 3rd, 4th, and 5thGrade Students in California, 2009[1]

	Manganese Group	Percent Not Economically Disadvantaged	Percent Economically Disadvantaged	Percent Hispanic
3rd Grade				
English	$p = 0.77$	$p = 0.002$	$p = 0.80$	$p = 0.08$
Math	$p = 0.75$	$p = 0.04$	$p = 0.87$	$p = 0.22$
4th Grade				
English	$p = 0.99$	$p = <0.0001$	$p = 0.60$	$p = 0.33$
Math	$p = 0.58$	$p = 0.0004$	$p = 0.60$	$p = 0.43$
5th Grade				
English	$p = 0.84$	$p = 0.0003$	$p = 0.42$	$p = 0.31$
Math	$p = 0.54$	$p = 0.05$	$p = 0.34$	$p = 0.82$
Science	$p = 0.63$	$p = 0.0004$	$p = 0.88$	$p = 0.02$

[1]Means are averaged by city

FIGURES



ARTESIA WATER SYSTEM

Artesia System - Source Water Quality

Secondary Standards - Aesthetic (units)	SECONDARY MCL	PHG (MCLG)	Range of Detection	Average Level	MCL Violation?	Most Recent Sampling Date	Typical Source of Constituent
Aluminum (ug/L)	200	600	ND - 190	ND	No	2006	Erosion of natural deposits; residue from some surface water treatment processes
Color (units)	15	n/a	ND - 4	1	No	2006	Naturally-occurring organic materials
Chloride (mg/L)	500	n/a	8 - 98	35	No	2006	Runoff/leaching from natural deposits; seawater influence
Iron (ug/L)	300	n/a	ND - 920	ND	No	2006	Leaching from natural deposits; industrial wastes
Manganese (ug/L)	50	n/a	ND - 78	ND	No	2006	Leaching from natural deposits
Odor---Threshold (units)	3	n/a	ND - 4	ND	No	2006	Naturally-occurring organic materials
Specific Conductance (uS/cm)	1600	n/a	370 - 1010	541	No	2006	Substances that form ions when in water; seawater influence
Sulfate (mg/L)	500	n/a	11 - 194	68	No	2006	Runoff/leaching from natural deposits; industrial wastes
Turbidity (units)	5	n/a	ND - 4	0.1	No	2006	Soil runoff
Total Dissolved Solids (mg/L)	1000	n/a	200 - 658	310	No	2006	Runoff/leaching from natural deposits
Zinc (mg/L)	5	n/a	ND - 0.06	ND	No	2006	Runoff/leaching from natural deposits; industrial wastes
Unregulated Constituents Requiring Monitoring (units)	**Notification Level**	**PHG (MCLG)**	**Range of Detection**	**Average Level**	**MCL Violation?**	**Most Recent Sampling Date**	
Boron (ug/L)	1000	n/a	ND - 364	148	No	2006	
Chromium, Hexavalent [CrVI] (ug/L)	n/a	n/a	ND - 2.5	ND	n/a	2006	
Perchlorate (ug/L)	6	n/a	ND - 4.5	ND	No	2006	
tert-Butyl alcohol [TBA] (ug/L)	12	n/a	ND - 3.7	ND	No	2006	
Vanadium (ug/L)	50	n/a	ND - 364	41	No	2006	

8

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Figure 1: Sample Drinking Water Quality Report from Artesia, California 2007

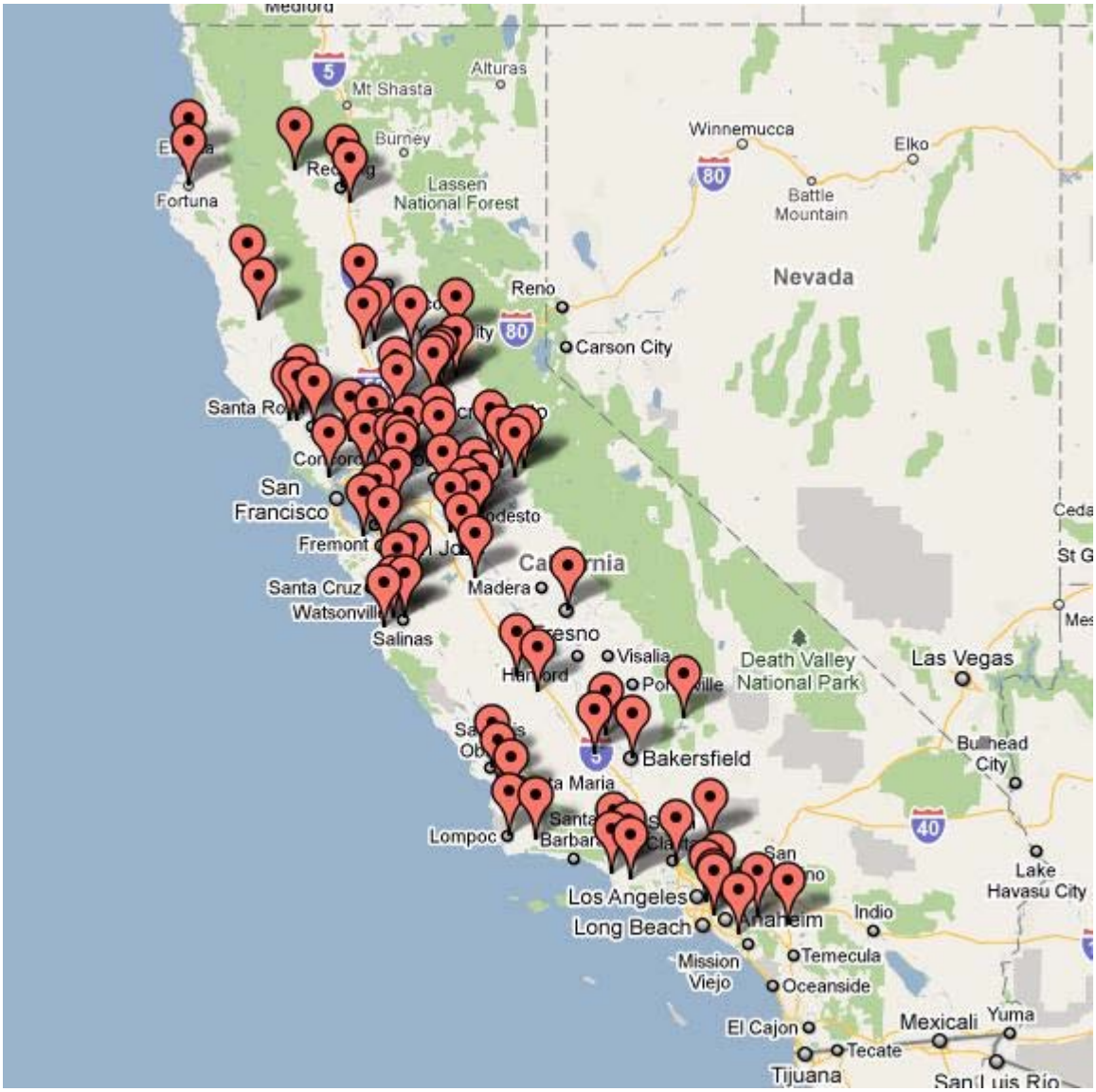

Figure 2: All California Cities Sampled

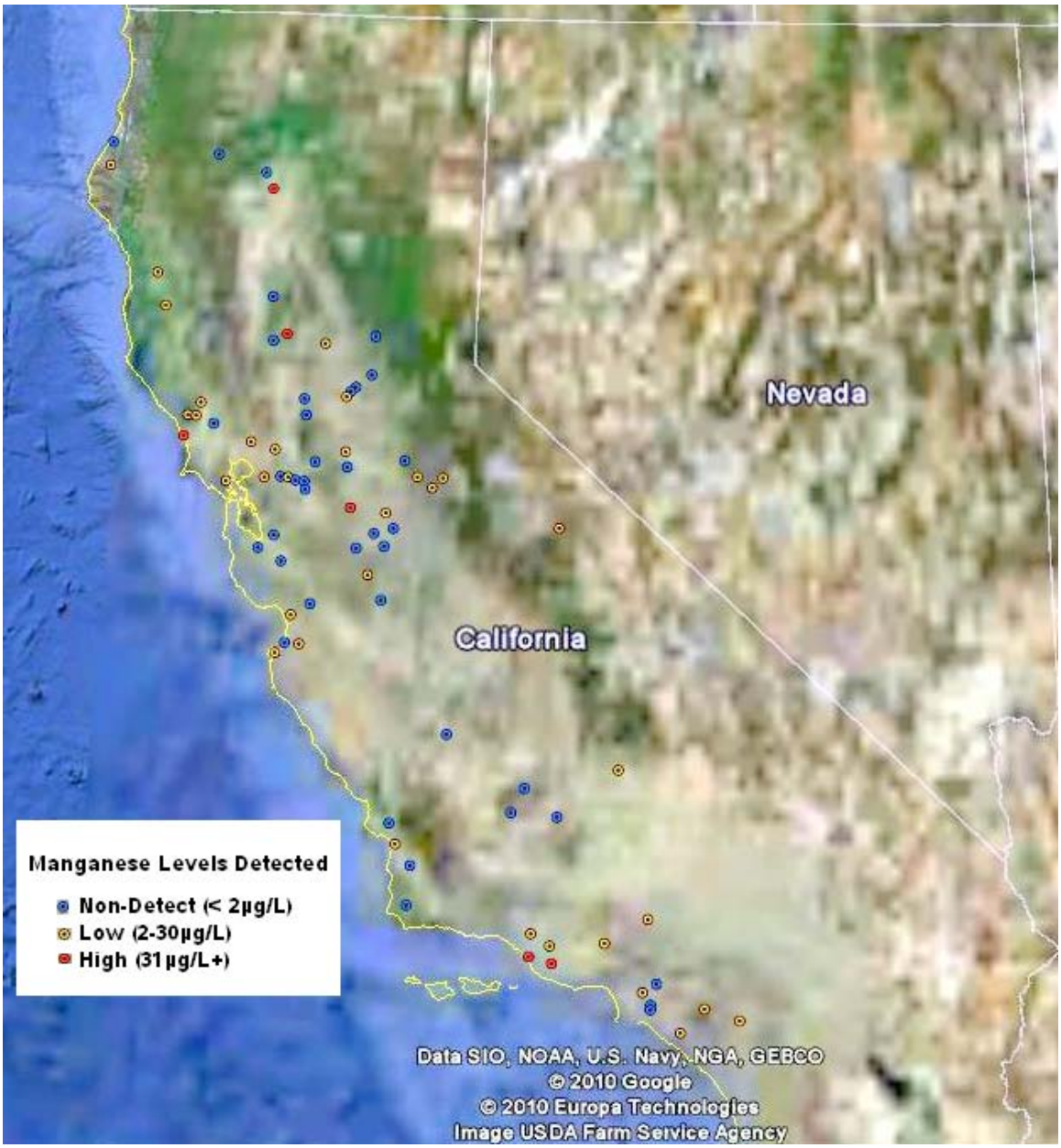

Figure 3: Locations Sampled in California for Manganese in Drinking Water

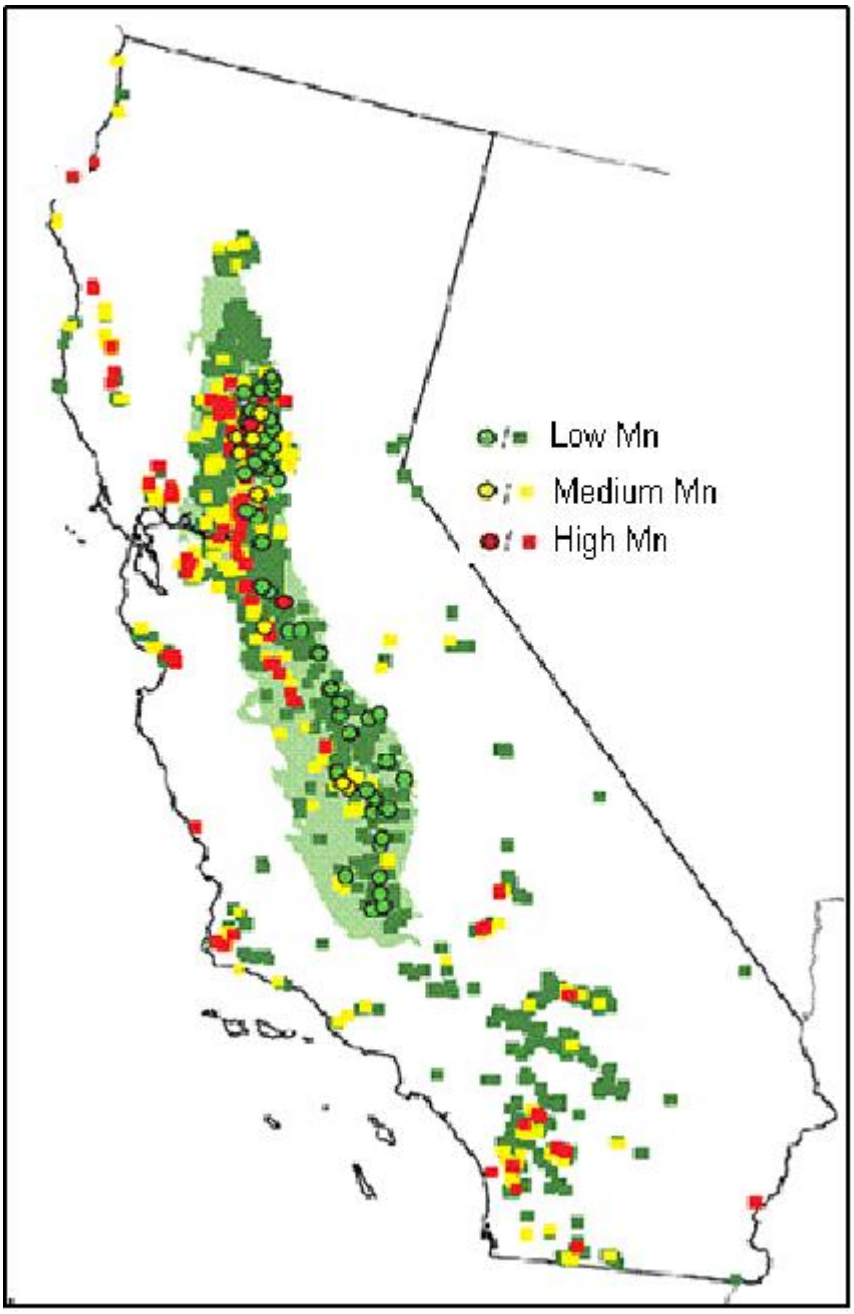

Figure 4: USGS Map of Manganese in Ground Water in California

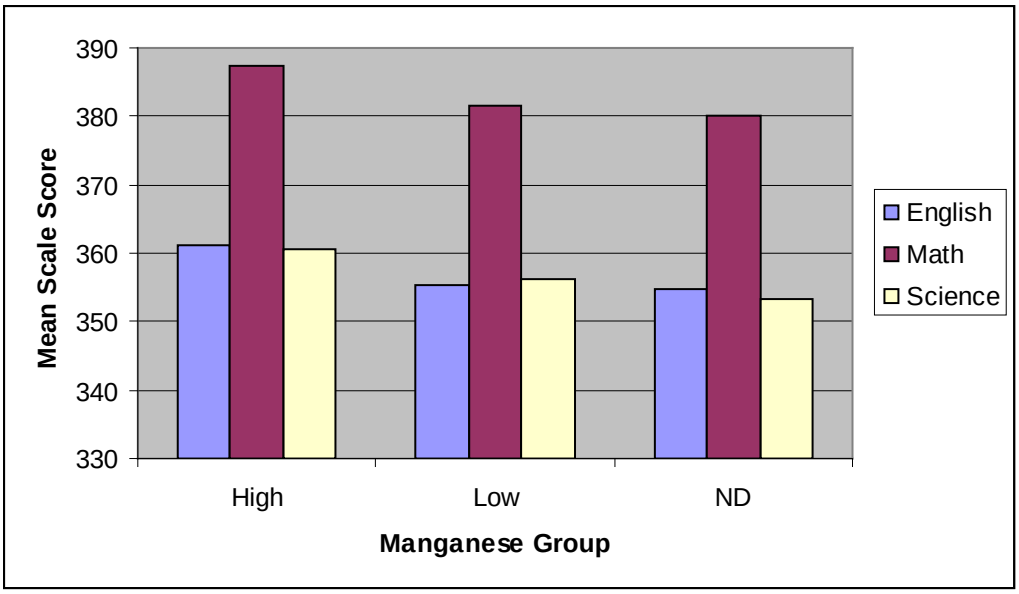

Figure 5: Unadjusted Associations Between Manganese Exposure and Test Scores for 3rd, 4th, and 5th Grade Students in California, 2009

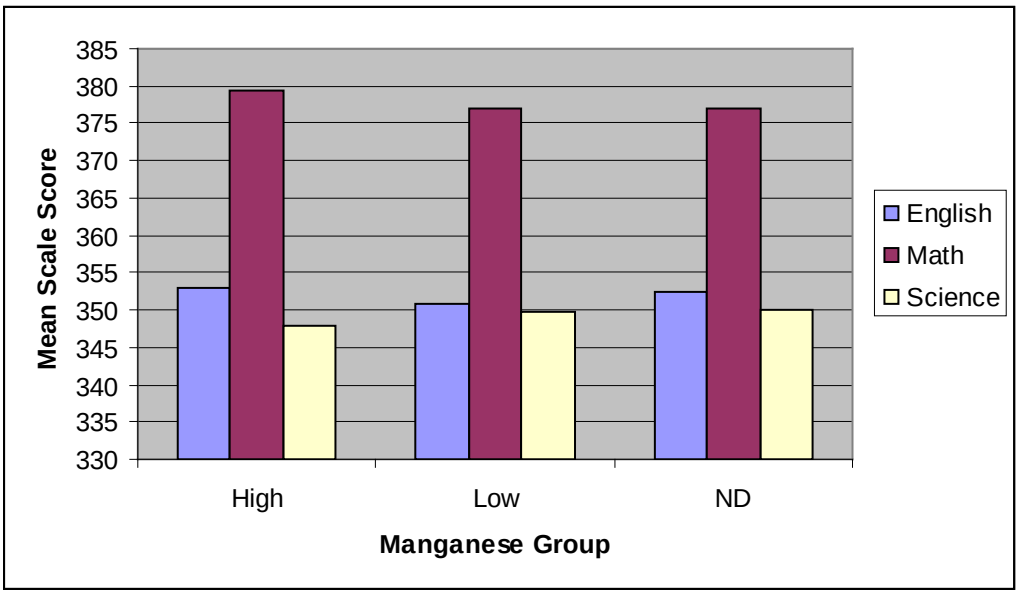

Figure 6: Adjusted Associations Between Manganese Exposure and Test Scores for 3rd, 4th, and 5th Grade Students in California, 2009*

APPENDIX A

Comparison of Manganese levels from drinking water quality reports versus Manganese levels from sample analysis*

	Value from Water Quality Report		Value from Analysis
City	High	Average Detected	Actual Amount
Anderson	110		37
Angels camp	65	ND	18
Antelope	91	47	2
Antioch	7.9	4	ND
Arroyo Grande	33	ND	9
Artesia	78	ND	ND
Auburn	462	17	ND
Avenal	26	12	ND
Bakersfield	142	10	ND
Bay Point	46	3	ND
Brentwood	220	ND	ND
Buttonwillow	20	20	ND
Camarillo	67	ND	49
Cedar Ridge	150		3
Ceres	31.6	1	ND
City of Commerce	170	104	7
Coalinga	10	8	ND
Colusa	140	80	58
Corona	140	80	9
Davis	22	22	ND
El Monte	20	20	ND
Eureka		43.3 - 89.3	ND
Fairfield	91.1	33	2
Fortuna	72	ND	2
Fremont	77	8	ND
Fresno	69.6	8	ND
Galt	98	25	5
Gilroy	280		ND
Grass Valley	30	1	ND
Guerneville	405	49	12
Gustine	40	16	25
Healdsburg	24	1	7
Irvine	79	16	2
Isleton	100	ND	ND
Kernville	30	6	10
Laytonville	30	0	13
Livermore	20	6	23
Lodi	57.5	20	ND
Lompoc	3.7	1	ND
Los Banos	468	408	ND
Manteca	30	10	51

Marina	310	48	ND
Martinez	48	22	5
Marysville	26	20	13
Monterey	48	22	3
Napa	90	37	3
Newhall	100	50	8
Norwalk	43	ND	ND
Oakdale	170	33	21
Oakley	46	14	ND
Ojai	86	4	10
Palmdale	19	10	17
Palo Alto	26	20	ND
Patterson	170	ND	ND
Perris	75	ND	6
Pittsburg	220	30	9
Redding	56	8	ND
Rio Dell	92	79	2
Rocklin	120	ND	ND
Roseville		ND	ND
Salinas		ND	28
San Andreas	70	ND	ND
San Jose	80	28	ND
San Luis Obispo	7	7	ND
San Rafael	24	24	4
Santa Maria	91	47	ND
Santa Paula	27	1	4
Santa Rosa	186	47	ND
Solvang	100	ND	ND
Sonora	20		4
Turlock	140	20	ND
Ventura	41	3	56
Wasco	77	8	ND
Waterford	85	20	ND
Watsonville	44	22	5
Weaverville	90	22	ND
Willows	9	9	ND
Windsor	940	269	ND
Woodland	78	9	ND

*Not all sampled cities are represented this table