

5-2011

Lead concentrations in extracted primary teeth among Clark County pediatric patients

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LEAD CONCENTRATIONS IN EXTRACTED PRIMARY TEETH AMONG
CLARK COUNTY PEDIATRIC PATIENTS

by

Jennifer Anne Berger

Bachelor of Science
University of Nevada Las Vegas
2007

A thesis submitted in partial fulfillment
of the requirements for the

Master of Public Health
Department of Environmental and Occupational Health
School of Community Health Sciences
Division of Health Sciences

Graduate College
University of Nevada, Las Vegas
May 2011

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THE GRADUATE COLLEGE

We recommend the thesis prepared under our supervision by

Jennifer Anne Berger

entitled

Lead Concentrations in Extracted Primary Teeth Among Clark County Pediatric Patients

be accepted in partial fulfillment of the requirements for the degree of

Master of Public Health

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May 2011

ABSTRACT

Lead Concentrations in Extracted Primary Teeth Among Clark County, Pediatric Patients

by

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Childhood lead poisoning is a completely preventable condition, yet only a small portion of children in Nevada are screened for elevated blood lead levels. In 2009 only 6.11% of children in Nevada were screened for lead indicating that Nevada would benefit from an alternative method of screening for childhood lead exposure. Deciduous teeth are not currently recognized by the Centers for Disease Control and Prevention as diagnostic samples for the measurement for lead exposure. However, this unique and innovative detection method utilizes opportunistic samples that will contribute to the childhood lead poisoning prevention efforts in Nevada.

The objectives of this study were to measure the lead concentrations of extracted deciduous teeth from children, identify demographic and environmental factors associated with increased tooth lead concentrations, and evaluate the effectiveness of measuring lead in teeth as a biomonitoring tool for at risk populations in Clark County, Nevada. Over the course of the study, 93 parents and legal guardians were approached at the UNLV School of Dental Medicine pediatric dental clinic to participate in the study. Seventy children (2 to 13 years old) were included in the study. In total, 147 whole teeth

were collected from extractions performed by pediatric residents. Samples were analyzed by Inductively Coupled Plasma-Mass Spectrometry for lead (parts per million).

Tooth lead concentrations ranged from 0.1 parts per million (ppm) to 1.99 ppm lead, with an average mean (\pm standard deviation) lead concentration of 0.46 ± 0.41 ppm.

Hispanic children, children living in 1978 or pre-1978 housing, and children living in low income zip codes had higher tooth lead concentrations than other Clark County children.

Results were consistent with identified at risk groups for childhood lead poisoning by the Southern Nevada Health District.

Several distinct advantages to using lead concentrations of deciduous teeth for screening include access to high risk groups at pediatric clinics, high participation percentage, on-site sample collection, and simple sample processing. Future research should focus on the standardization of methodology and address the lack of direct correlation between tooth lead concentrations and blood lead level, which is acceptable diagnostic test for childhood lead poisoning. Although the results of this study did not impact the number of children screened for childhood lead poisoning in Clark County Nevada, it did indirectly serve as a reminder to parents to have their child's blood lead level tested.

ACKNOWLEDGEMENTS

To my thesis committee, Dr. Shawn Gerstenberger, Dr. Chad Cross, Dr. Timothy Bungum, and Dr. Deborah Keil, thank you for your support, guidance, and patience as I attended your courses and worked on my thesis project. Dr. Gerstenberger, it has been an honor to be a graduate assistant in your laboratory and work on the Healthy Homes Initiative. I can only hope that I met all of your expectations. You gave me guidance and direction when I needed it most, yet left me to learn principles on my own. I am a better student, writer, researcher, and person for having worked with you. Dr. Tim Bungum, everything I know about epidemiology I learned from you. Dr. Chad Cross, thank you for making biostatistics less intimidating, assisting me with my statistics for this project, and taking the time to always answer my questions. Dr. Deborah Keil, as a professor you challenged to me to gain knowledge and as a mentor you introduced me to the field of toxicology and encouraged me to obtain my Master of Public Health. The opportunities and experiences you provided have shaped the researcher and snowboarder I am today.

This project started as a merely a hallway discussion of a great idea, but through the combined efforts of Dr. Karl Kingsley and Dr. Shawn Gerstenberger, measuring lead in children's teeth became a reality. I will always be grateful to Dr. Kingsley for writing and submitting the IRB for this project. His quality of research, dedication and positive attitude are truly inspirational.

To the pediatric residents and dental assistants at the pediatric clinic, thank you for taking the time to assist with recruitment and sample collection. The pediatric clinic was a very positive and enjoyable environment to work in, even with the occasional child crying in the background. Amol Amin, Yvonne Giraud, Sabrina La Monica, and Jason

Nita were essential team members in the recruitment of participants at the UNLV School of Dental Medicine. Thank you for all the hours you spent at the pediatric clinics to make this project possible.

No graduate college experience would be complete without the friendship and support of fellow students and graduate assistants: Erika Torres, Maraya Morse, Jonathon LaValley, Mackenzie Burns, and Sabrina La Monica, Ashley Watters, Sean Comeau, and Scott Rainville. I'm thankful that I was able to share this experience with you. Erika, thank you for your support, fun trips the gym, and guidance.

This work is dedicated to my husband, Benjamin Ritchie, and my parents, Kenneth and Joan Berger, for their unconditional love and support throughout my educational career. Thank you for always having faith that I will succeed and achieve my goals.

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CHAPTER 1

INTRODUCTION

The vulnerability of young children to lead has been well documented. Even in light of this, childhood lead poisoning continues to be a prominent childhood condition in the United States. Childhood lead poisoning is currently defined by the U.S. Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) as a BLL exceeding 10 ug/dL (Barbosa et al., 2005). To address this public health issue, children are regularly screened in the United States for childhood lead poisoning. In 1978, the Centers for Disease Control and Prevention (CDC) recommended universal screening for children that are 9 months to 6 years of age (Wengrovitz & Brown, 2009). A 1985 statement indicated that priority should be given to the screening of children 1 to 3 years of age who either live in dilapidated housing, live near industrial areas, or have parents that are occupationally exposed to lead (CDC, 1985). Childhood Lead Poisoning Prevention Programs (CLLPPs) were authorized and initiated by the Lead Contamination Control Act of 1988 to develop state programs and policies, screen children, and provide education to the public (CDC, 2009a). In 1989, federal Medicaid laws mandated that Medicaid eligible (1 to 6 years) were required to receive lead screening through Medicaid's Early and Periodic Screening, Diagnostic, and Treatment (EPSDT) service. Since this time, the CDC has modified its recommendation from universal screening to targeted screening for children at the greatest risk for lead exposure. The U.S. Department of Health and Human Services set the precedence for eliminating childhood lead poisoning by making it an objective (8-11) for *Healthy People*

2010 (Objective 8-11) and a proposed objective (EH HP2020–13) for *Healthy People 2020* (U.S. Department of Health and Human Services).

Despite decades of lead poisoning prevention efforts, data from 2003- 2004 National Health and Nutrition Examination Survey (NHANES) indicated that 2.3% of young children (1 to 5 years) have blood lead levels (BLLs) that exceed the CDC action level of 10 ug/dL (Miranda et al., 2007). Nationally, only 7.9% of children less than 3 years were tested for lead poisoning in 2007. Although the number of children less than 3 years of age living in the United States increased 6.1%, the number of children screened decreased 6.6% (CDC, 2010). This reflects a need for additional methods to identify and screen children in the United States in order to eliminated childhood lead poisoning, a completely preventable disease.

The actual status of childhood lead poisoning in Nevada is currently unknown due to a lack of data. In the NHANES report summary for childhood lead poisoning, the CDC categorizes Nevada with 16 other states under “state data not available for” (CDC, 2009b). This gives the illusion that children living in Nevada are not at risk for lead exposure (Rothweiler, Cabb, & Gerstenberger, 2007). Screening in Nevada is performed, but the number of children screened in Clark County, Nevada is neither representative nor generalizable to the Nevada population as a whole. Between 2004 and 2005, only 2,791 children in Clark County were tested for blood lead levels (BLLs) (Rothweiler et al., 2007). Of these children, 17 were discovered to have elevated blood lead levels (EBLLs) of greater than 10ug/dL, with 15 children belonging to the Hispanic race (Rothweiler et al., 2007). In 2006, the Southern Nevada Health District (SNHD) and the University of Nevada, Las Vegas (UNLV) were awarded a five year grant from the CDC

to establish the Clark County Childhood Lead Poisoning Prevention Program (CCCLPPP) in Nevada. This program strives to significantly reduce and eliminate childhood lead poisoning in Clark County. This program has increased the amount of yearly screenings, which is evident by the 10,595 children (Age 0 to 72 months) that were screened between July 2008 and June 2009 in Clark County (SNHD, 2011). Yet, screening and reporting deficiencies have resulted in a lack of cumulative Nevada childhood lead poisoning data (Rothweiler et al., 2007).

Currently, Nevada is unable to meet the CDC objectives for the CLPPPs at the state level, which are 1) to estimate the extent of elevated BLLs among children, 2) assess the follow-up of children with elevated blood-lead levels, 3) to examine potential sources of lead exposure, and 4) to help allocate resources for lead poisoning prevention activities (CDC, 2009c). In 2008, annual blood lead screening rates for Clark County reached 5.72%, which was only 0.23% less than the target percentage. The 2009 screening rates increased to 6.11 % (based on 2008 population estimate). Although screening rates are improving annually, not enough children in Nevada are being screened for childhood lead poisoning. As a result, Nevada does not have extensive childhood lead poisoning data (CDC, 2009b). Due to the low number of children screened annually in Nevada, it is unlikely that all communities at risk are receiving adequate lead poisoning screening and prevention education. Today, Nevada is challenged by the ability of the local health departments to locate at risk communities in need of outreach, education, screening, treatment, and improved access to lead testing clinics. This indicates a need for an alternative method of biomonitoring childhood lead exposure, in order to identify children at risk in Clark County and Nevada.

Study Purpose and Significance

This study addresses the need for alternative lead sampling and analytical methodologies for the identification of children at risk for childhood lead poisoning and distinguishes risk factors of lead exposure specific to children residing in Clark County, Nevada. The overarching goal was to explore the use deciduous teeth extracted from pediatric dental patients as an epidemiological biomonitoring tool for childhood lead exposure in Clark County, Nevada. Although tooth lead concentrations are not recognized by the CDC as an acceptable diagnostic indicator of lead exposure, the utilization of opportunistic samples will assist in the identification of communities at risk in Clark County. This alternative detection method will contribute to the childhood lead poisoning monitoring and prevention efforts in Nevada.

CHAPTER 2

REVIEW OF RELATED LITERATURE

Characteristics of Lead

Lead is a ubiquitous bluish-gray, heavy metal found in the earth's crust that has been mobilized in the environment by recent anthropological activities (Williams, James & Roberts, 2000). Lead has an atomic weight of 207.2, with 5 naturally occurring isotopes (204, 206, 207, 208, and 210). With the exception of tin, lead has the lowest melting point (327°C) compared to other common metals (Landsdown & Yule, 1986). Lead is malleable, corrosion-resistant, ductile, and is present primarily in its divalent form (Pb^{2+}) (Levin et al., 2008; Klaassen, 2008). Compounds of lead are divided into two categories, inorganic lead and organic lead. Inorganic lead compounds are used as pigments in ceramic glazes, paints and dyes (Klaassen, 2008). Organic lead compounds contain carbon and hydrogen, with "organolead" compounds referring to compounds with at least one lead-carbon bond. Tetra-ethyl lead and tetramethyl lead are organolead compounds historically were used in gasoline to increase octane ratings (Landsdown & Yale, 1986; ATSDR, 2007).

Malleability, lack of mechanical strength, and softness are characteristics unique to lead. Contact with air results in the creation of a lead carbonate film, which gives lead a dull grey appearance and makes it corrosion resistant. Lead is combined with other metals to create alloys, which retain the desirable properties of lead while increasing its hardness and mechanical strength, while retaining its corrosion-resistance properties (Landsdown & Yule, 1986). Lead's properties allow for easy smelting and the creation

of lead alloys for the production of storage batteries, ammunition, cable covers, and radiation shields (Williams, James & Roberts, 2000; Klaassen, 2008; ATSDR, 2007).

Lead in the Environment

Historically, airborne lead from the combustion of gasoline containing tetraethyl lead and the use of lead in residential paint were two major sources of environmental lead contamination. A significant increase in U.S. lead consumption occurred in 1921 with the introduction of tetraethyl lead to gasoline as an anti-knocking agent, which was vital to the high-power, high-compression engines used in World War II (Lewis, 1985). The greatest increase in environmental lead occurred between 1950 and 2000, which is a reflection of U.S. and international use of leaded gasoline. The burning of coal, oil and waste have also significantly contributed to airborne lead concentrations (ATSDR, 2007). In 1970, the Clean Air Act mandated the introduction of unleaded gasoline and required 1975 automakers to equip new cars with catalytic converters, which used unleaded gasoline. After a 25 year phase-out, leaded gasoline and fuel were banned January 1, 1996 (Wigle, 2003; Lewis, 1985). The 1970s legislation decreased the amount of environmental lead by removing tetraethyl lead from gasoline and reducing smokestack emissions from smelters (American Academy of Pediatrics Committee on Environmental Health, 2005). U.S. lead emissions declined from 221,000 tons in 1970 to less than 4,000 tons in 1997 (Wigle, 2003).

The environmental impact and direct hazard of lead-based paint in pre-1978 housing has extended into the 21st century. Lead was added to paint as a pigment, an anti-corroding agent, an anti-microbial additive, and as a drying agent. Lead carbonate was

extensively used in pre-1970s white paints. In 1978, under the , the Consumer Product Safety Commission (CPSC) banned the sale of residential paint containing greater than 0.06 % lead by weight (CPSC, 2008). However, Jacobs et al. (2002) estimates that 1.2 million homes contain significant lead based hazards and are occupied by low-income families with children under 6 years of age. Seven million dollars in federal appropriations was given to the U.S. Department of Housing and Urban Development (HUD) between 1992 and 2002 to control lead hazards in low-income housing and other federal agencies have invested in housing rehabilitation. The number of homes containing lead-based paint decreased from 64 million homes in 1990 to 38 million homes in 2000 (CDC, 2005). Lead from gasoline, house paint, and other sources will remain a potential source of lead exposure for future generations due to the strong adherence of lead to soil particles, where it remains near the surface (ATSDR, 2007).

Reductions in environmental lead have significantly impacted the BLLs of children throughout the United States. Between 1976 and 1980, approximately 14.2 million U.S. children had BLLs greater than 10ug/dL, with a median BLL of 15ug/dL. In comparison, only 0.9 million children had elevated BLLs between 1991 and 1994, with the median BLL decreased to 1.9 ug/dL in 1999 (American Academy of Pediatrics Committee on Environmental Health, 2005; Wigle, 2003). Even though BLLs have decreased in the U.S., lead is still persistent in the environment and will continue to be significant source of lead exposure to children through contaminated soil, dust and deteriorated lead-based paint (American Academy of Pediatrics Committee on Environmental Health, 2005)

Toxicokinetics of Lead

Absorption

Lead is absorbed through two main routes: gastrointestinal or pulmonary absorption, with ingestion as the most significant route of exposure (Landsdown & Yule, 1986). The bioavailability of the lead is dependent on the form of lead (i.e. inorganic, organic, or metallic), the quantity ingested, the age of the individual, and the current dietary status. A diet high in calcium inhibits the binding of lead to intestinal binding sites; thereby reducing absorption. In a state calcium deficiency, vitamin D and calbindin-D, a calcium-binding protein in the intestines, are activated to enhance the absorption of calcium. However, if calcium is not available in a sufficient quantity, lead and other trace metals will be absorbed in the place of calcium. Iron deficiency in children also facilitates gastrointestinal lead absorption (Wigle, 2003). Adults absorb approximately 15% of ingested lead, while children and pregnant women absorb nearly 50% of ingested lead (Williams, James & Roberts, 2000; Wigle, 2003). Pulmonary lead exposure is considered insignificant and is mainly a concern for occupational exposure (Klaassen, 2008; Williams, James & Roberts, 2000). The health effects of lead are the same regardless of the route of exposure (Williams, James & Roberts, 2000).

Circulation and Storage

After absorption, 99% of the lead is bound to the hemoglobin portion of erythrocytes and is circulated via the vascular system to soft tissues (liver and kidney), bone, and hair. Lead has a half-life of approximately 30 days in the blood (Klaassen, 2008). BLLs only indicate recent lead exposure, but the potential of earlier lead poisoning cannot be ruled out (American Academy of Pediatrics Committee on Environmental Health, 2005).

During systemic circulation, lead interrupts the heme biosynthesis pathway. Cytoplasmic delta-aminolevulinic acid dehydratase (ALAD) and ferrochelatase are particularly sensitive to elevated lead concentrations (Klaassen, 2008; Barbosa et al., 2005).

Specifically, ALAD is progressively inhibited in the blood by lead concentrations of greater than 5 ug/dL lead (Wigle, 2003). These enzymes, and the resulting compounds, can be used as biomarkers to determine the extent of lead exposure (Barbosa et al., 2005).

BLLs may also reflect the recirculation of lead from bone storage, which has been reported to contribute greater than 90% of lead in blood (Bergdahl & Skerfving, 2008; Barbosa et al., 2005). Lead has an average half-life of 32 years in bone (McPherson & Pincus, 2007). In children, 70% of lead body burden is stored in the bones and increases to 95% in adulthood (Klaassen, 2008). Lead in the blood has a second half-life of 4 years, due to the recirculation of lead released from the bone storage compartment (ADSTR, 2007). Lead mobilization from bone is dependent on the rate of biological activity. Trabecular bones are a significant source of endogenous lead, due to greater level of biological activity, surface area, and volume of blood flow in comparison to cortical bones (Barbosa et al., 2005). A particular demographic of concern is pregnant women due to the mobilization of lead during pregnancy as bone is catabolized to assist in the creation of the fetal skeleton (ATSDR, 2007). Circulation and storage are important factors to consider in the evaluation of childhood lead poisoning by blood lead test, Rapid skeletal growth may conceal lead exposure by rapidly decreasing the concentration of circulating lead (Barbosa et al., 2005).

Lead concentrations in bone and teeth reflect cumulative exposure overtime (Barbosa et al., 2005), therefore past-exposures. Due to the unique composition and prenatal

formation of tooth components, lead concentrations can reflect both *in utero* exposure and lead exposure occurring prior to the age of 6 years (Fergusson & Purchase, 1987). Evidence supports that teeth and bone share similar qualities, such as a high affinity for metals as similar accumulation rates (Arruda-Neto et al., 2009). However, the loss and recirculation of lead occurs at a much slower rate from teeth than bone (Fergusson & Purchase, 1987). The appearance of a Burtonian blue line (at gum line) reflects an elevated lead concentration in teeth and the overall lead accumulation within the body (Moore, 1986). Lead concentrations in teeth increase with age and are dependent on the level of lead exposure (Landsdown & Yule, 1986).

Lead is also distributed to soft tissues throughout the body, with the liver serving as the main soft tissue compartment (ATSDR, 2007). The greatest percentage of lead uptake by the organs occurs in the liver, kidney, heart, and brain (Landsdown & Yule, 1986). During pregnancy, lead crosses the placental barrier and accumulates in fetal tissue, particularly the brain. Additionally, lead targets the proximal tubules of the kidneys and is capable of inducing nephrotoxicity in the form of proximal tubular nephropathy, glomerular sclerosis, and interstitial fibrosis (Klaassen, 2008)). A decreased glomerular filtration rate has been detected in adults with BLLs less than 20ug/dL (ATSDR, 2007). Even though only 2% of absorbed lead is distributed to the brain(ATSDR, 2007), neurological damage in children ranges from cognitive and academic deficiencies at BLLs less than 5ug/ to lead encephalopathy or death after an acute exposure (Klaassen, 2008).

Excretion

Urine and feces are the main routes of excretion for most heavy metals (Williams, James & Roberts, 2000). The excretion rates of lead may be influenced by dietary components, such as calcium and vitamin D, and therapeutic compounds used to treat lead poisoning (Landsdown & Yule, 1986). Minor routes of lead excretion include sweat, saliva, hair, nails, and breast milk (ATSDR, 2007). Saliva, feces, urine, hair and nails have been investigated as alternative biomarkers for lead poisoning. However, clearance levels, inconsistencies in lead excretion, and variation due to age do not support routes of excretion as reliable biomarkers for lead poisoning (Barbosa et al., 2005).

Childhood Lead Poisoning

Prior to 1970, individuals with BLLs of ≤ 60 ug/dL were considered safe from the permanent neurological effects of lead exposure. Overtime, the CDC has decreased the limit of concern, with the last decrease to 10 ug/dL in 1991 (Richardson, 2005). The progression of tolerable BLLs by the CDC is summarized in Table 1 (Richardson, 2005). However, current studies indicate that children with blood lead levels less than 10ug/dL can still suffer from permanent IQ and hearing deficits (Wigle, 2003), suggesting that 10 ug/dL may not be a safe level of exposure. Currently, there is no known threshold (safe level of exposure) for the permanent health effects of lead (Lanphear et al., 2005). It is time for the CDC and WHO to reevaluate the 10 ug/dL action limit and consider lowering this value as a result of low limits of detection by improved instrumentation and evidence that negative health effects occur at BLLs < 10 ug/dL .

Table 1. Changes in CDC EBLL Benchmarks for Children by Year (Richardson, 2005)

Year	CDC EBLL Benchmark (ug/dL)
Pre-1970*	60
1971	40
1975	30
1985	25
1991	10

*Adults and Children

Childhood Exposure

Young children, less than six years old, are disproportionately exposed to environmental contaminants and generally exhibit more severe health effects than adults (Landrigan et al., 1999). Most cases of childhood lead exposure can be attributed to the ingestion of deteriorating lead-based paint, soil or dust (Raymond & Anderson, 2009). As lead-based paint deteriorates, it contaminates interior surfaces and soils at the exterior perimeter of then home (American Academy of Pediatrics Committee on Environmental Health, 2005). Leaded dust from soil, paint and industrial emission is of particular concern due to its ability to adhere to exposed surfaces. Ingestion of dust is the most common route of lead exposure (Levin et al., 2008). Young children exhibit increased hand-to-mouth behavior and are in close proximity to the ground, which increases exposure to leaded dust. Additionally, this age group may display pica-like behavior and may directly consume paint chips (Gorospe & Gerstenberger, 2008; American Academy of Pediatric Committee on Environmental Health, 2005). Drinking water is also a potential source of lead exposure, but is less likely (Levin et al., 2008).

The emergence of global free trade and increased international travel have resulted in atypical sources of lead exposure, such as folk remedies, imported condiments, imported

candies, glazed ceramics and toys. In 2003, it was estimated that greater than 40% of Americans use a form of folk remedy. Between 1966 and 2006, there were 47 cases of EBLs in children due to the ingestion of a folk remedy (Gorospe & Gerstenberger, 2008). Household items such as ceramic pottery, dinnerware, vinyl lunchboxes, and vinyl mini-blinds are suspected sources of childhood lead exposure (Levin et al., 2008). In greater than 30% of childhood cases of EBLs there is no lead-based paint hazards present (Levin et al., 2008). Identifying atypical items as sources of lead exposure is often complicated due to the abundant usage of lead and an increase of unregulated imported items, nutritional supplements, food items and ethnic accessories (Gorospe & Gerstenberger, 2008).

Health Effects

The diagnosis of lead poisoning in children presents a unique challenge due to the nonspecific symptoms exhibited at low concentrations. Neurological changes that occur as a result of lead exposure range from cognitive deficiencies, behavioral changes (i.e. inattentiveness and hyperactivity), and increased aggression to delinquency (American Academy of Pediatrics Committee on Environmental Health, 2005). Lamphear et al. (2005) estimates that an increase in BLL from 1 to 10ug/dL results in a 7.4 point drop in IQ scores. Although symptoms are not generally identified at low level exposures, there is no evidence that the neurological effects of lead exposure are reversible, even with treatment (American Academy of Pediatrics Committee on Environmental Health, 2005).

In contrast to the subclinical symptoms of chronic or low level lead exposure, characteristic symptoms, abdominal or neurological, only manifest after acute lead exposure (Gorospe & Gerstenberger, 2008). Growth deficits are seen in children with

BLLs greater than 10ug/dL and anemia may occur at BLLs \geq 20 ug/dL (Wigle, 2003). At BLLs \geq 60 ug/dL, children may complain of headaches, abdominal pain, and become agitated or have a decreased level of activity (American Academy of Pediatrics Committee on Environmental Health, 2005). Encephalopathy may appear at BLLs $>$ 80 ug/dL, followed by acute encephalopathy and death at BLLs $>$ 125 ug/dL (Wigle, 2003).

High Risk Populations

Although there has been a substantial decrease in BLLs among all ages and ethnic groups in the United States, health disparities in childhood lead poisoning among subpopulations have not been eliminated. High risk groups are identified as children 0 to 6 years of age, children living in inner-city urban areas, children of low-income households, and minority groups (Rothweiler et al., 2007). Low-income and residing in an inner-city neighborhood are well established risk factors for childhood lead poisoning. Needleman et al. (1972) reported that inner-city children of Philadelphia (referred to as the “lead belt”) had mean tooth lead concentrations of 51.1 ± 109.0 ppm, compared to 11.1 ± 14.8 ppm lead in teeth collected from children living in the suburbs (Needleman et al., 1972). Data from NHANES has been used for several decades to track changes in childhood lead poisoning in the U.S. Additionally, a CDC analysis of the NHANES data for 1991 to 1994 estimated that 93% of children with BLLs \geq 20 ug/dL were Medicaid eligible. This is consistent with previous studies (1980-1990) that identified Medicaid eligibility and poverty as risks for increased lead exposure. Between 1991 to 1994, the highest rates of elevated blood lead levels (EBLLs) occurred among children living in pre-1946 housing, children of low-income families, and children that were either of non-

Hispanic black or Mexican-American decent (Wengrovitz & Brown, 2009). According to 1999- 2000 NHANES data, non-Hispanic blacks and Mexican Americans have a higher percentage of elevated BLLs than non-Hispanic whites, with non-Hispanic black children (age 1 to 5 years) and elderly (≥ 60 years) having the highest prevalence of elevated BLLs (CDC, 2005).

Although there continues to be a difference in BLLs between white children and minority children in the United States, the gap has been reduced. Between 1991 and 1994, 11.2% of black children had EBLLs in comparison to 2.3% of white children. In 2009, this gap was reduced as BLLs in black and white children significantly decreased to 3.4% and 1.2%, respectively (Wengrovitz & Brown, 2009). The change in EBLLs by subgroups of children is also represented by the change in geometric mean BLL from NHANES. This data is summarized in Table 2.

Table 2. Geometric Mean BLL (ug/dL) by race (Wengrovitz & Brown, 2009)

Race/Ethnicity	NHANES 1991-1994	NHANES 1999-2004
Black Children (non-Hispanic)	4.3	2.8
White Children	2.3	1.7

High Risk Populations in Nevada

Nevada contains communities at risks for lead exposure. There has been a recent influx of immigrants and 39% of the Clark County population belongs to a minority group. In 2006, Hispanics made up 26% of the Clark County population (Rothweiler et al., 2007). African American and Hispanic children are more likely than Caucasian

children to have elevated blood lead levels (CDC, 2005). In 2003 approximately 11% of Nevadans lived below the poverty level and 17% of children in Nevada were uninsured. In 2004, the Great Basin Primary Care Association reported that 56,000 children in Clark County were uninsured. Hispanic children are more likely to be both uninsured and live in poverty. Furthermore, 35,775 homes in North Las Vegas, Las Vegas, Mesquite and Boulder City potentially contain lead-based paint hazards, with 10,441 (29%) of these homes are occupied by low and very low income families (Rothweiler et al., 2007). Generally, Nevada has a younger housing stock, but children living these homes are at risk for lead exposure. These figures contradict the assumption that Nevada's children are not a risk for lead exposure or childhood lead poisoning.

Biomonitoring of Lead Exposure

Biomarkers for lead exposure extend beyond the use of whole blood, serum and plasma to include hair, bone, teeth, urine, and cerebrospinal fluid (Bergdahl & Skerfving, 2008; ATSDR, 2007). However, selecting the appropriate biomarker should entail an evaluation of practical usage, the portion of lead body burden represented by the biomarker, and analytical accuracy and precision. Since there is a poor correlation between biomarkers reflecting short-term exposure (e.g. blood) and those reflecting long-term exposure and storage (i.e. bone and teeth), it is important to select the most useful biomarker or combinations of biomarkers (Bergdahl & Skerfving, 2008). The use of biomonitoring alternatives could potentially expand the number of children tested for BLLs and improve the number of communities that receive educational outreach. The

following sections will evaluate blood, bone and teeth as valuable biomarkers for monitoring lead exposure.

Blood

Testing for BLLs is routinely performed and is a cost-effective way to assess recent lead exposure in adults and children. BLL testing is the most common method for childhood lead poisoning screening (ATSDR, 2007). As lead exposure and uptake increases, lead binding sites on red blood cells become saturated and BLLs increase (Bergdahl & Skerfving, 2008). Lead in blood can be measured by several different analytical methods, including flame atomic absorption (AAS), graphite furnace atomic absorption spectrometry (GFAAS), anode stripping voltametry (ASV), inductively coupled plasma mass spectrometry (ICPMS), and isotope dilution mass spectrometry (IDMS). IDMS is considered the most reliable method for low concentrations, but is expensive and requires a high level of technical skill. ICPMS is being increasingly used for the detection of trace metals due to its reliability and lower detection limits (ATSDR, 2007). Lead exposure can also be determined by the activity or quantity of components of heme synthesis in the blood. Decreased delta-aminolevulinic acid dehydratase (ALAD) activity, increased free erythrocyte protoporphyrin, and elevated zinc protoporphyrin in whole blood are indicative of inorganic lead exposure (McPherson & Pincus, 2007).

Sampling by venipuncture is invasive and is considered a disadvantage and barrier to blood lead testing (Bergdahl & Skerfving, 2008). Other barriers to blood lead testing for children include parental refusal, lack of education about the health effects of lead exposure, lack of transportation to testing location, and caregivers not aware of locations

that perform BLL testing (Polivka & Gottesman, 2005). A current alternative to venipuncture is capillary sampling by finger stick that is tested by a U.S. Food and Drug Administration (FDA) approved portable device on-site in clinical offices for instant results (Wengrovitz & Brown, 2009). The SNHD uses the Lead Care II® blood lead analyzer, to increase screening rates through field testing in high risk communities. The CDC recommends capillary sampling to encourage screening rates in deficient areas (Wengrovitz & Brown, 2009). There is a higher risk of falsely elevated BLLs due to contamination with the capillary sampling method compared to BLL testing using a venipuncture collection (Bergdahl & Skerfving, 2008) Confirmation by venous sampling is recommended for all elevated capillary BLLs (Wengrovitz & Brown, 2009). Blood is the most routinely collected sample for measuring lead exposure and is the accepted method of detection of EBLLs in children (Bergdahl & Skerfving, 2008; Wengrovitz & Brown, 2009).

Bone

Lead stored in bone reflects total body burden of lead exposure, but is not a practical or effective method of measuring lead exposure in children. Bone lead is an ideal biomarker for epidemiological studies assessing the long-term effects of lead exposure. However, the mobilization of lead differs between trabecular and cortical bones, therefore study comparisons should be conducted with caution. The measurement of lead in bone can be performed *in vivo* with x-ray fluorescence (XRF) technology, which is a noninvasive procedure with a detection limit of approximately 10ug/g lead (Bergdahl & Skerfving, 2008). A study by Needleman et al. (2002) utilized XRF technology to correlate *in vivo* tibial bone lead concentrations and delinquency status of youth in

Pennsylvania. As anticipated, the mean(\pm standard deviation) bone lead levels of the delinquent youth (11.0 ± 32.7 ppm lead) were significantly higher than the bone lead levels of the control group (1.5 ± 32.1 ppm lead) (Needleman et al., 2002). Disadvantages of bone lead as a biomarker include the transportation of equipment and unnecessary exposure of study participants to radiation from the ^{109}Cd source found in some XRF instruments (Bergdahl & Skerfving, 2008; Needleman et al., 2002). Although bone lead is an indicator of stored lead concentration, the sensitivity of the XRF technology is in the parts per million (ppm) compared to the low detection limits of other methodology and variation in bone thickness affects precision (ATSDR, 2007).

Teeth

In addition to the analysis of blood and bone, teeth (deciduous and permanent) have been explored as a biological sample for measuring past and accumulative lead exposure. Similar to bone, teeth contain a substantial concentration of lead and provide a historical record of lead exposure from the pre-natal period, during the formation of the teeth, to the natural shedding or extraction of the tooth. (Bergdahl & Skerfving, 2008). Children's primary (deciduous) teeth for analysis are easily obtained from schools or dental clinics and are stable in storage for long periods of time (Ferguson & Purchase, 1987). Since lead concentrations in blood are transitory, measuring lead in teeth offers a method to analyze store lead in calcified tissues without performing bone biopsies (Needleman et al., 1972). Teeth are more susceptible to contamination, so an extensive cleaning process is required prior to analysis to remove organic materials, such as blood, tissue or grease, and any surface contamination (Fergusson & Purchase, 1987). XRF technology has been utilized to determine tooth lead concentration *in situ* (Shapiro et al., 1978). Although the

analysis of tooth lead concentrations is not routinely performed, teeth contain a sufficient concentration of lead to meet the detection limits of most instrumentation (Bergdahl & Skerfving, 2008). Challenges with the analysis of trace elements in teeth include the heterogeneous composition of teeth, potential variation in concentrations between types teeth, and differences in pretreatment protocols, sample type (whole or dissected teeth), and analytical methodology (Fergusson & Purchase, 1987).

Lead in Deciduous Teeth as a Biomarker for Childhood Lead Exposure

Pioneers of childhood lead poisoning in the United States studied children's deciduous teeth as an indicator of the total body burden of lead, reaffirmed the harmful effects of lead exposure, and assessed the relationship between tooth lead levels and childhood lead poisoning symptoms. An early study by Altshuler et al. (1962) evaluated the use of lead levels in deciduous teeth from lead poisoned children as an index for the total body burden of lead. There was no significant difference between mean lead concentration in the teeth from the deceased from lead encephalopathy (164.4 ppm) and the survivors of acute lead poisoning (116.6 ppm). The authors concluded that teeth are a more reliable indicator of past exposure, with tooth lead concentrations increasing from time of tooth eruption. Needleman et al. (1972; 1974) further confirmed the relationship between childhood lead poisoning and tooth lead concentrations and introduced data supporting income, race, and ethnicity as health disparities in regard to childhood lead poisoning. A controversial study by Needleman et al. (1979) compared dentine lead concentrations of first and second grade aged children to scores on a neuropsychologic battery and teacher's behavioral rating (i.e. distraction, organization, and following directions). Children with high dentine lead levels were rated poorly on 9 of 11 criteria

by teachers and were less competent in the verbal procession and auditory processing of the neuropsychologic battery (Needleman et al., 1979). The charges of misconduct against H.L. Needleman regarding the 1979 study were cleared by a panel at the University of Pittsburgh (J.P., 1992).

Composition of Teeth

Teeth are heterogeneous in nature with two main zones of teeth, the enamel and dentine, which greatly differ in formation, composition and lead concentrations (see Figure 1) (Fergusson & Purchase, 1987). There is also a layer of cementum that covers the root dentine (Bath-Balogh & Fehrenbach, 1997). Dentine makes up the bulk of the tooth, but enamel covers the crown of the tooth. Enamel is highly mineralized and is considered to be one of the hardest biological tissues. The principle mineral in enamel is calcium hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$], with only 1-2% organic matrix and 2% water by weight of enamel. Although enamel can withstand shearing, impact, and abrasion forces, it cannot be repaired or replaced once damaged. Surface enamel is harder, less porous, and in contact with the oral cavity environment (Berkovitz, Holland, & Moxham, 2009). Dentine is a more elastic and sensitive tissue and is composed of small, tubules in a mineralized collagen matrix. In contrast to enamel, dentine is formed throughout life, is permeable, and is similar to bone in composition (Berkovitz, Holland & Moxham, 2009). In contrast to bone, dentine does not actively participate in calcium homeostasis, does not undergo remodeling, and the physiological resorption of dentine only occurs prior to the natural shedding of deciduous teeth (Linde & Goldberg, 1993).

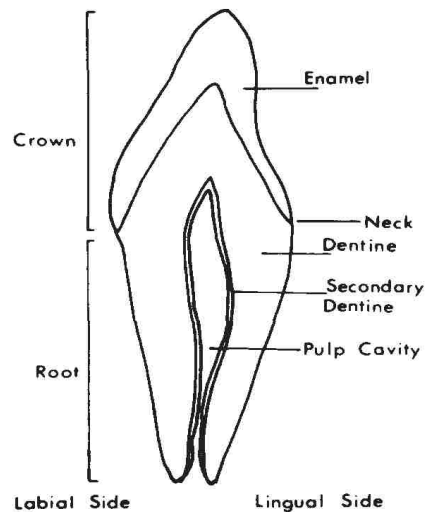


Figure 1. Composition of teeth (Fergusson & Purchase, 1987)

Deciduous Teeth

Children have a total of 20 deciduous teeth, with each tooth developing and erupting at a different time (Table 3). Deciduous teeth are labeled A through T starting with the right side of the top jaw to the left and then down to the bottom jaw to the right. Children have 8 incisors, 4 canine teeth, and 8 molars. By the age of 3 years, all of the deciduous teeth have erupted with permanent teeth appearing at approximately 6 years (Berkovitz, Holland & Moxham, 2009). However, the development of deciduous teeth begins *in utero* between the 6th and 7th week of embryonic development. Due to differences in formation, eruption, and therefore the amount lead accumulation, it is optimal to compare lead concentrations from teeth of the same type (Berkovitz & Laufer, 1990). Table 3 summarizes the onset of formation and eruption of deciduous teeth. A portion of the tooth is lost prior to shedding in the form of bone resorption by cells called odontoclasts (Bath-Balogh & Fegrenbach, 1997). Even teeth that are extracted may have evidence of

resorption. In this case, the degree of resorption may cause variation in the concentration of trace metals, such as lead (Fergusson and Purchase, 1987).

Table 3. The Formation and Eruption of Deciduous Teeth (Fergusson & Purchase, 1987)

Tooth	Onset of Formation		Eruption	
	Upper	Lower	Upper	Lower
Deciduous Teeth (Age in months)				
Central Incisor	4 <i>in utero</i>	4-5 <i>in utero</i>	7-5	6
Lateral Incisor	5 <i>in utero</i>	4-5 <i>in utero</i>	9	7
Canine	4-5 <i>in utero</i>	5 <i>in utero</i>	18	16
First Molar	5 <i>in utero</i>	5 <i>in utero</i>	14	12
Second Molar	6 <i>in utero</i>	6 <i>in utero</i>	24	20

Analytical Methods

The preparation and analysis of deciduous teeth for lead concentrations in both historical and recent studies are inconsistent, which makes study results incomparable. A review paper by Fergusson & Purchase (1987) reported that cleaning the teeth prior to analysis to avoid external contamination is essential. However, methods have varied from simple soaking in distilled water or hydrogen peroxide to using proteolytic enzymes (i.e. papain), acetone, detergents and sonication. Instrumental methods of analysis are similar in variation. Analytical instrumentation for the measurement of tooth lead have included, but are not limited to, X-ray fluorescence, flame atomic absorption (AAS), anode stripping voltammetry (ASV), Graphite Furnace AAS (GFAAS), inductively coupled plasma (Fergusson & Purchase, 1987), and inductively coupled plasma mass spectrometry (ICPMS) (Arora et al., 2006). Refer to Table 4 for a summary of

pretreatment, sample preparation and analytical methods from previous studies. With no standard methodology for the measurement of lead concentrations in teeth, all future studies will be stand-alone, therefore limiting the practical application.

Table 4. Methodologies for the Lead Analysis of Deciduous Teeth

Study	Pretreatment	Sample	Reference	Instrumentation
Altshuller et al. (1962)	Cariou Removal	Whole teeth	-----	Spectrographically
Needleman et al. (1979)	Ultrasonic cleaning	Dentine	-----	ASV
Shapiro et al. (1973)	“Cleansed Mechanically”	Dentine and Circumpulpal Dentine	-----	ASV
Bercovitz & Laufer (1990)	-----	Dentine	-----	GFAAS
Grobler, Theunissen, Kotze (2000)	Distilled water	Dentine, Enamel, Circumpulpal dentine	-----	GFAAS
Arora et al. (2006)	Distilled water	Pre-natal and Post-natal Zones	NIST (SRM-610 & 1486), Calcium	ICPMS
Arruda-Neto et al. (2009)	30% Nitric Acid	Whole teeth	H-5 Animal bone*	ICPMS

* Certified Reference Material from the International Atomic Energy Agency (IAEA)

Limitations

The composition, formation, and natural resorption of deciduous teeth may contribute to variable results and limit the application of lead concentrations in deciduous teeth for identification of children at risk for childhood lead poisoning. Whole tooth analysis is not generally performed due to root resorption, which occurs as a child ages prior to the natural shedding of teeth (Fergusson & Purchase, 1987). The presence of amalgam fillings may also change the concentrations of trace metals. Arruda-Neto et al. (2009) found that carious teeth had significantly higher tooth lead concentrations than non carious teeth, which indicated a 33% increase in lead absorption rates.

Additionally, there is no international quality control or standard reference material available for the analysis of teeth (Fergusson & Purchase, 1987). Studies have used a variety of materials used to maintain analytical accuracy, such as animal bone certified reference material by Arrunda-Neto et al. (2009) and lead- enriched calcium-chloride standard by Bellinger et al. (1991). An internal standard, not containing lead, can also be utilized to verify methodology. Arora et al. (2006) measured lead in deciduous teeth by ICPMS using calcium as an internal standard and Bone Meal SRM-1486 (National Institute of Standards and Technology, USA) to test for analytical accuracy and precision. Another alternative, is the creation of lab-internal animal tissue reference materials using bovine teeth. This process is described by Lüker et al. (1992).

Currently, tooth lead concentrations lack diagnostic value due to the poor correlation between BLLs and tooth lead concentrations (Bergdahl & Skerfving, 2008). There are several studies that assess the relationship between BLLs and tooth lead concentrations. An early study by de la Burd  and Shapiro (1975) found a relationship between EBLLs and increased tooth concentration in an evaluation of children with known lead exposure from overt pica behavior compared to a control group, with no pica behavior. Grobler, Theunissne and Kotze (2000) suggest that the lead concentrations of dentine are 16% higher than whole teeth with a ratio of 80:1 for dentine to whole blood. Using this data, the authors calculated estimated blood lead levels for others studies reporting the mean lead concentrations for whole teeth and dentine. Costa de Almeida et al. (2011) found no statistically significant correlation between enamel lead concentrations of both deciduous and permanent teeth from a cohort of children 6 to 8 years of age and BLL. Despite the findings of several current studies, more research is

needed in this area before BLLs can be calculated from dentine or whole tooth lead concentrations

CHAPTER 3

RESEARCH QUESTIONS, OBJECTIVES & HYPOTHESES

Research Questions

1. What demographic and environmental factors contribute to elevated concentrations of lead in children's deciduous teeth in Clark County, Nevada? (See APPENDIX 4 for demographic and environmental information requested.)
2. What zip codes within Clark County, Nevada will have the greatest concentrations of lead in children's extracted deciduous teeth?
3. Can the measurement of lead concentrations in deciduous teeth be used to direct a childhood lead poisoning primary prevention effort?

Objectives

Objective 1: To determine lead concentrations in deciduous teeth extracted from children that have visited the pediatric clinic at the University of Nevada, Las Vegas (UNLV) School of Dental Medicine.

Objective 2: To identify demographic and environmental factors that are associated with elevated concentrations of lead in deciduous teeth extracted from children at the pediatric dental clinic, UNLV School of Dental Medicine.

Objective 3: Evaluate the effectiveness of teeth as a biomonitoring tool in Clark County, Nevada for the identification of populations at risk for childhood lead poisoning.

Hypotheses

H_{A1}: Hispanic children (and multi-racial, including Hispanic race) will have increased lead concentrations in deciduous teeth compared to non-Hispanic children.

A racial health disparity exists for childhood lead poisoning. There is evidence that African American and Hispanic children are more likely to have elevated blood lead levels than Caucasian children (MMWR, 2005).

H_{A2}: The lead concentrations in deciduous teeth extracted from children 7 years of age or older will be greater compared to younger children, 0 to 6 years of age.

Since the accumulation of lead in the dentine portion of teeth is continuous until root absorption and tooth loss of the deciduous teeth, younger children should have a lower lead concentration than older children.

H_{A3}: Children living in target zip codes (89030, 89101, 89102, 89106, 89108, 89109, 89110, 89115, and 89119) will have greater concentrations of lead in their deciduous teeth than children living in other Clark County zip codes.

Target zip codes were defined as zip codes within Clark County, NV that had a median family income less than \$50,849, the 1999 Nevada median family income, and contained greater than 1,000 families below the poverty level (http://factfinder.census.gov/home/saff/main.html?_lang=en). Poverty level for

1999 was calculated by U.S. Census Bureau as \$16,895 for a family of 4 individuals, including 2 children (U.S. Census Bureau, 2003). See Table 5 for target zip code characteristics. Census 2000 demographic profile highlights were available for 56 Clark County zip codes. Census data for 2005-2009 has not yet been tabulated for Clark County zip codes.

Table 5. Economic Characteristics of Identified Target Zip Code

Target Zip Codes	City	Median Family Income	Families below Poverty Level
89030	North Las Vegas	\$31,632	2,424
89101	Las Vegas	\$28,106	2,245
89102	Las Vegas	\$38,463	1,120
89106	Las Vegas	\$32,894	1,187
89108	Las Vegas	\$46,664	1,554
89109	Las Vegas	\$33,860	1,216
89110	Las Vegas	\$45,456	1,715
89115	Las Vegas	\$32,764	2,230
89119	Las Vegas	\$36,193	1,365

H_{A4}: Deciduous teeth extracted from children living in 1978 or pre-1978 housing will have increased lead concentrations in comparison to teeth extracted from children living in homes built after 1978.

Leaded paint was banned for residential use in 1978. Homes constructed prior to 1978 may have lead hazards due to leaded paint that is chipping, peeling, or creating dust. Children living in homes built after 1978 are less likely to be exposed to lead hazards from deteriorating lead paint. Year of construction for the home or apartment at the provided address and zip code was determined using

the Clark County Assessor Records Search (<http://www.clarkcountynv.gov/depts/assessor/pages/recordsearch.aspx>).

CHAPTER 4

METHODOLOGY

Collection of Data and Samples

UNLV Institutional Review Boards and School of Dental Medicine

Deciduous teeth were collected from November 2010 to March 2011 at the UNLV School of Dental Medicine according to the research protocol approved by the UNLV Biomedical Institutional Review Boards (IRB) March 23, 2010 (APPENDIX 1). The UNLV School of Dental Medicine clinic serves the community by treating and educating patients that are low-income, uninsured and Medicaid dependent. Through 2004, the clinic had screened and educated over 10,000 children in Las Vegas and treated over 3,000 low-income, uninsured, and elderly patients (UNLV, 2010). In total, the pediatric dental clinic of the UNLV School of Dental Medicine has seen 552 children under the age of 5 and 1,217 children between the ages of 5 to 10 years (K. Kingsley, personal communication, July 20, 2010). As reported by Dr. Karl Kingsley in 2010, 38.92% of patients are without dental insurance and 99.96% do not have medical insurance. Calculated from the new patient numbers for July 2009 to June 2010, the pediatric dental clinic has an average of 106 new patients per month (K. Kingsley, personal communication, July 20, 2010).

Recruitment

The parents or legal guardians of potential participants were approached in the waiting room and treatment areas of the pediatric dental clinic at the UNLV School of Dental Medicine. This occurred after a pediatric dental resident determined the course of treatment to include an extraction of a deciduous tooth. Selection of participants was not

random, with all children having a tooth extraction considered potential study participants. The UNLV interns were not involved in the decision to extract teeth or the actual extraction process. Participation in the study did not affect the course of treatment or the decision to perform a routine primary tooth extraction. All parents and legal guardians of children 0 to 14 years of age were approached for participation in the study when student interns were present to acquire parent permission and participant assent.

UNLV interns described the study in detail, summarized the information contained in the parent permission form (APPENDIX 2), and answered any questions about the study. Parents were required to read the parent permission form, initial each page, and sign with printed name below. A translator was provided by the pediatric dental clinic to translate the parent permission form into Spanish as needed to obtain parent permission. Children 7 years of age or older, with the ability to read the assent to participate in research form, were required to read and sign the form (APPENDIX 3). After completion of both the consent and assent process, parents provided demographic information on the intake form regarding the child participating in the study.

Sample Collection

A randomly assigned, unique coded identification number was assigned to each participant. Labels with the unique identifier were placed on the parent permission form, assent form, intake form, and tubes for sample collection.

After the extraction, participants were given a certificate for the “tooth fairy” and the samples were placed in polypropylene conical tubes containing 10 to 20 milliliters of phosphate buffered saline (PBS). The tubes were labeled with the unique identification number and were identified (A through T) by the pediatric dental resident performing the

extraction. Race, gender, age, address and zip code were recorded on the designated intake form (APPENDIX 4) after obtaining parent permission and participant assent. All samples were transported to the Environmental and Occupational Health Laboratory at UNLV for cleaning and preparation for analysis.

Treatment of the Data and Samples

All investigators and student interns completed the UNLV Office for the Protection of Research Subjects Collaborative Institutional Training Initiative (CITI) program to ensure the proper treatment of participants and to maintain participant confidentiality. All consent, assent, and intake forms were stored at the UNLV School of Dental Medicine in a secure laboratory in a locked cabinet. Access to the research files was restricted to study investigators and interns. Upon collection, data was entered into Microsoft[®] Excel and SPSS[®] Statistical Software (PASW 17.0) on password-protected computers at the UNLV School of Dental Medicine and in the Environmental and Occupational Health Laboratory.

Samples were transported to UNLV laboratory by study interns and Dr. Karl Kingsley to the UNLV Environmental and Occupational Health Laboratory. Upon arrival, the unique number identifier, tooth position number, physical condition of each tooth (i.e. degree of decay, condition of roots) and identification of tooth type (e.g. incisors, molars, cuspids) were recorded.

Sample Pretreatment

The pretreatment protocol was created from a consensus of methods described by Fergusson & Purchase (1987) and recent studies using deciduous teeth for lead analysis. Each tooth was placed in a labeled metals-free polypropylene container. Residual organic material, gum tissue and blood, were lysed with 10ml of 3% hydrogen peroxide for approximately for 30 to 45 minutes, or until violent bubbling subsided. A sonicating water bath of 0.5% Citrinox® detergent solution was prepared to remove oils and other contaminants. Teeth were sonicated in detergent solution for 5 minutes. Brief rinsing of teeth with 0.5% nitric acid followed by Millipore® distilled water was used to remove any trace metals present on the external surfaces. Teeth were dried in a 65°C oven for 12 hours in individually labeled VWR 28mm aluminum boats to remove all moisture content. Dry weights were taken and recorded for each sample. Confirmation of balance calibration was performed daily, prior to use with Troemner calibration weights (Thorofare, NJ, USA) at 100g, 10g, 5g, 1g, 10mg and 1mg. The incubation period for dry weights to remove all moisture was determined experimentally. Each tooth was photographed and graded visually for degree of root resorption and for percentage of crown visibly decayed. Teeth were visually identified as teeth having a fully intact root, teeth that have partially undergone root resorption, and teeth with no root remaining. To determine the percentage of crown decayed, the superior surface of crown was visually divided into 4 quadrants and categorized by range of percent decay (0%, ≤10%, 11- 25%, 26- 50%, 50- 75%, and >75%). Storage containers were labeled with the unique identification number, tooth dry weight, and date of preparation. Samples were stored at room temperature until shipment to an analytical chemistry laboratory for acid digestion

and ICPMS analysis for lead. Teeth with metal caps, metal fillings or no crown were excluded from the study and were not sent for analysis. A participant was excluded from the study if only one tooth containing metal fillings or a cap was extracted. Adult teeth were not accepted. See Table 6 for a list of inclusion criteria. Not all teeth satisfying inclusion criteria were sent for analysis due to financial restraints. At least one tooth (meeting inclusion criteria) per participant was sent for ICPMS analysis. If no samples for an individual participant satisfied inclusion criteria, the participant was excluded from the study.

Table 6. Inclusion Criteria for Participants and Samples

Inclusion Criteria
Participants age 0 to 14 years of age
Parent Permission Form Completed
Assent Form Completed (As Required)
Deciduous Teeth (A to T)
Assent Form Completed (As Required)
Teeth Free of Caps or Metal Fillings

Acid Digestion and Lead Analysis by ICPMS

Acid digestion and analysis of deciduous teeth were performed by Exova (Santa Fe Springs, California). Each tooth was digested in 1.0 mL of nitric acid and heated to 110°C on a HotBlock™ for one hour. During the digestion, 0.5 mL of 30% hydrogen peroxide was added to the solution. Prior to analysis the sample solution was diluted to 10g with nanopure water. The acid digestion methodology was determined by Samina Hussain, Senior Chemist and Metals Group Leader at Exova (Santa Fe Springs, California, US).

ICPMS has recently gained popularity in the measurement of lead in biological and environmental samples due to its high sensitivity, greater reliability and less intensive sample preparation (ATSDR, 2007). The analysis of samples by ICPMS was performed by Exova (Santa Fe Springs, California, US) using Standard operating procedure (SOP) No. 7040 (Revision 10). All Exova SOPs are proprietary and could not be obtained. See APPENDIX 1 for the ICPMS instrument parameters used by Exova for the lead analysis for this study. Internal standard, laboratory fortified blank (LFB) and 1 repeat measurement per set of samples to obtain percent recovery ($\geq 95\%$) in a calcium-rich matrix for quality control and quality assurance. All LFB samples fell within 80 to 120% of the expected values. A concentration of 0.1 ppm Terbium (mass 159) was used as an internal control with an acceptable recovery range of 50 to 125%. The detection limit varied by sample due to low volume dilutions, with a range of 0.004 to 0.06 ug/g lead.

CHAPTER 5

FINDINGS OF THE STUDY

Statistical Analysis of Research Hypotheses

All statistical analyses were performed using PASW (SPSS) version 17.0. The sample with the highest lead concentration was selected, regardless of tooth type or visual characteristics, for each participant that had more than one extraction. In other words, statistical analysis was performed using 70 samples with corresponding participant demographic characteristics. A Shapiro-Wilk test was conducted to test data for normality ($W=0.7$, $p=.000$). Data were found to be non normal. A log transformation was applied, but data could not be adequately transformed. Therefore nonparametric Mann-Whitney tests were utilized to test dichotomous data for all hypotheses ($p \leq .05$).

Analysis of Data

Participant Demographics

During the recruitment period of the study, the parents or legal guardians of 93 children having extractions were approached for participation in the study between November 2010 and March 2011. A 96% ($n=89$) participation percentage was achieved. One parent declined because he wanted to keep the child's tooth as a keepsake and 3 participants wanted to save the teeth for the "tooth fairy." Two parents were approached at a second visit and declined consent due to prior participation in the study. Additionally, two participants were consented twice and donated two sets of samples. Of the 89 participants, 12 (13%) were recruited during a free pediatric dental care event held Saturday, February 5, 2011 at the UNLV School of Dental Medicine in celebration of the American Dental Association's "Give Kids a Smile Day." Gender, race, age, zip code,

and street address were collected for each participating child and recorded on the designated intake form by the parent or legal guardian. Parents or legal guardians that declined to complete the parent permission form did not complete the intake form. Following the recruitment period, fourteen (14) participants were excluded from the study based upon the defined inclusion criteria for tooth sample characteristics (see CHAPTER 4 METHODOLOGY). Missing identification numbers on the consent and intake forms resulted in the exclusion of an additional 3 participants. Identification number and samples were combined for the 2 participants that were enrolled in duplication. All results are representative of the 70 participants that completed all forms and successfully provided at least one sample meeting the inclusion criteria.

Sample Characteristics and Condition

In total, 147 teeth were collected at the pediatric dental clinic during the recruitment period. However, only 107 samples (66 molars, 5 cuspids, 19 incisors, and 17 not identified) from 70 participants were found to meet the inclusion criteria. Each participant had 1 to 7 teeth extracted in a single appointment. The mean (\pm standard deviation) dry weight of the teeth was 0.51 ± 0.34 grams ($n=70$). Not all samples were sent to Exova (Santa Fe Springs, CA, USA) for ICPMS analysis for lead due to funding restraints. However at least one tooth per participant ($n=70$) was analyzed for lead. The 70 teeth that were selected for highest tooth lead concentration for each individual are representative of the 70 participants included in the study.

Prior to the statistical analysis of the research hypotheses, Kruskal-Wallis and Mann-Whitney tests were applied to investigate the effect of tooth type on tooth lead concentrations (unidentified teeth were excluded from this statistical analysis). Tooth

types were defined as primary molars (n=23), secondary molars (n=20), cuspids (n=5), incisors (n=12) and unidentified (n=10). There was no significant difference in lead concentrations between tooth types ($X^2 = 2.8, p=.422$).

Other noted physical characteristics included percentage of crown destroyed by caries and degree of root resorption. Only 37% of teeth were found to have crowns free of caries (0% decay). See Table 7 for the frequency of samples by tooth condition in regard to percentage of crown decay. Statistical analysis revealed that the percentage of crown decay does not significantly affect tooth lead concentrations ($X^2=10.5, p=.061$).

Table 7. Frequency of Teeth by Percentage of Crown Decay

Percentage of Crown Decay	Frequency (n=70)
0%	26
≤10%	11
11-25%	9
26-50%	10
51-75%	8
>75%	6

In regard to root resorption, 46% (n=32) teeth had intact roots, 34% (n=24) showed evidence of resorption, and 20% (n= 14) of those teeth contained no root. The average age for teeth with intact roots, partial root resorption, and no root were approximately 7 years, 9 years, and 10 years, respectively. Figure 2 depicts frequency of teeth sorted by the degree of root resorption by age.

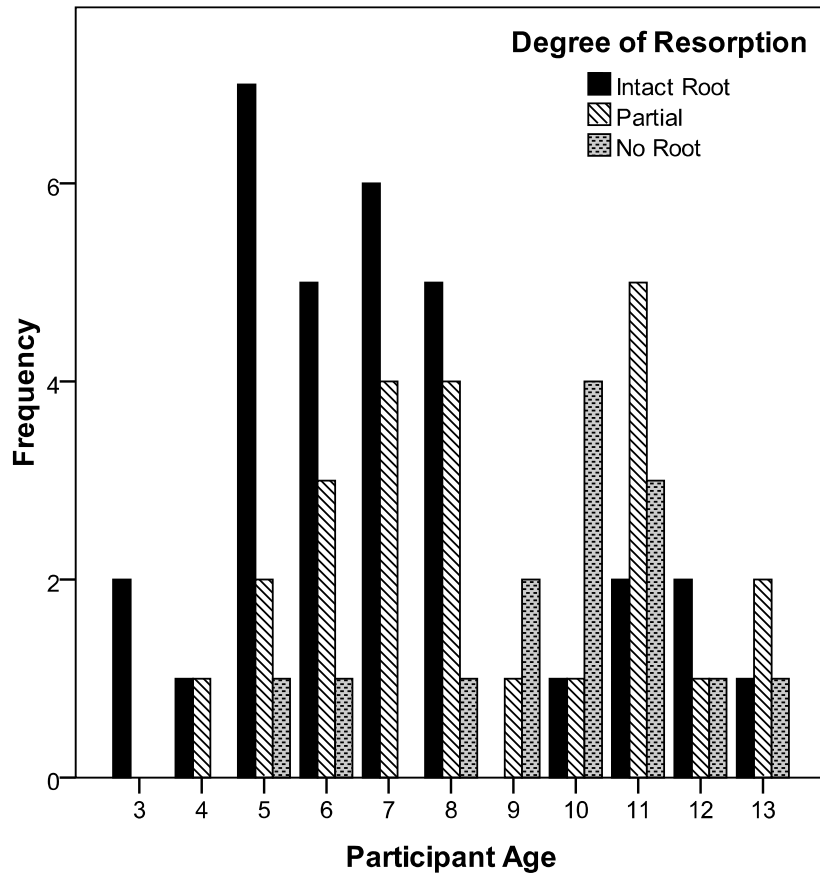


Figure 2. Frequency of Teeth by Age for the Degrees of Root Resorption

Tooth lead concentration was found to be significantly affected by the degree root resorption by Kruskal-Wallis test ($X^2=8.3$, $p=.015$). Subsequent post hoc Bonferonni-adjusted Mann Whitney tests revealed that the significant difference was between the lead concentrations of teeth with no root significantly differed from teeth with partial root resorption ($U=79$, $p=.018$). Interestingly, there was no statistical difference in lead concentration between teeth with intact roots and teeth with partial root resorption ($U=304$, $p=.546$) or no root ($U=136$, $p=.102$). The mean (\pm SD) tooth lead concentration of teeth with intact roots, partial root resorption, and no root were $0.41\pm.31$ (mdn=0.27),

0.60±.52 (mdn= 0.32), and 0.30±.32 (mdn= 0.28) ppm, respectively. See Figure 3 for a visual comparison of mean lead concentrations by degree of root resorption.

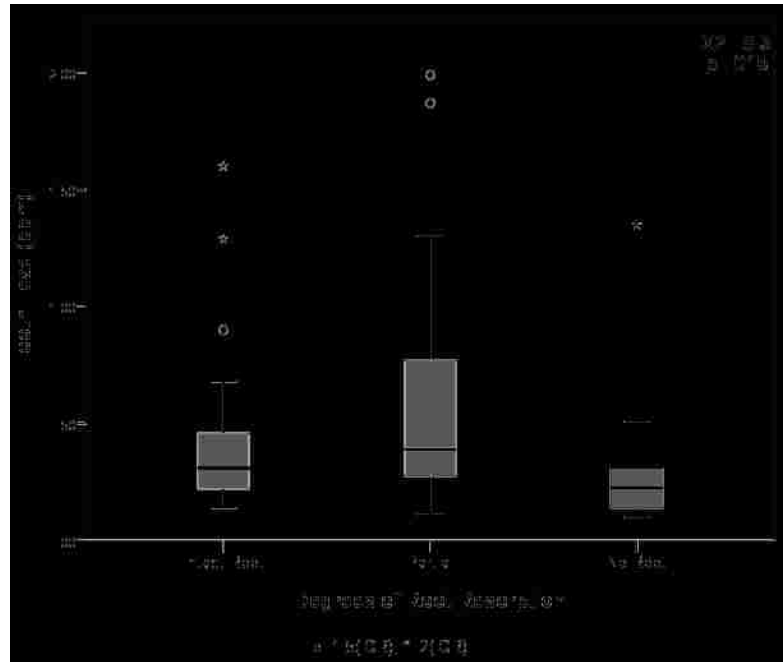


Figure 3. Tooth Lead (ppm) for Teeth with Varying Degrees of Resorption

Participant Race and Tooth Lead Concentrations

The intake form included 5 selections for race: White, Black, Hispanic, American Indian, or other. A majority of participants were identified as Hispanic or multi-racial including Hispanic (67%, n=47), with 63% (n= 44) identified as Hispanic only. All participants recruited on “Give Kids a Smile Day” were of the Hispanic race. The remaining races were significantly less represented in this study. See Figure 4 for the ungrouped, distribution of participant races.

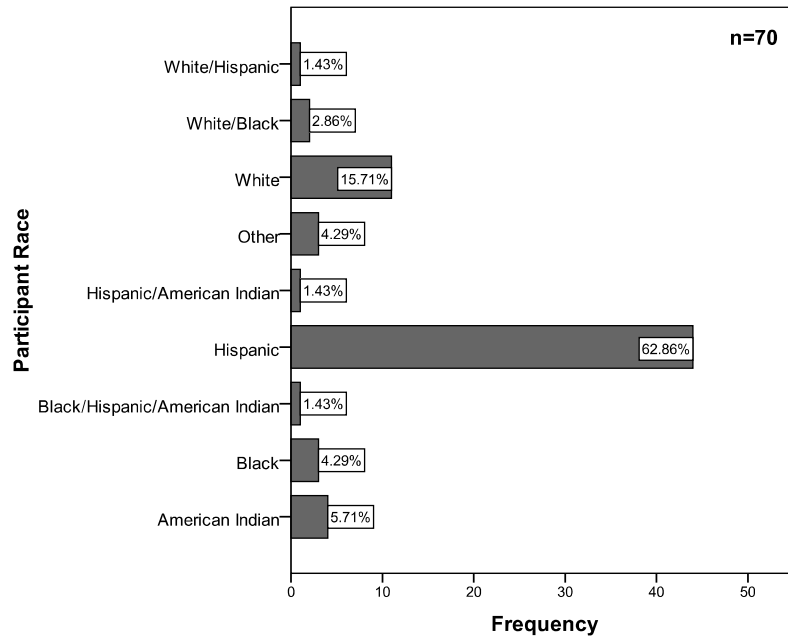


Figure 4. Race Distribution of Participants for Data Analysis

A Mann-Whitney test was used to compare the tooth lead concentrations of Hispanic children (Hispanic and multi-racial, including Hispanic race) and children of other races (Caucasian, Black, American Indian and non-Hispanic multi-racial). There is a significant difference in tooth lead concentration between Hispanic children and non-Hispanic children ($U=325$, $pp=.007$). The mean ($\pm SD$) tooth lead concentration for Hispanic children ($n=47$) was $0.54 \pm .47$ ($mdn=0.35$) ppm lead and non-Hispanic children ($n=23$) had a mean ($\pm SD$) tooth lead concentration of $0.28 \pm .13$ ($mdn=0.27$) ppm lead. Hispanic children had a greater mean tooth concentration than children of other races. See Figure 5.

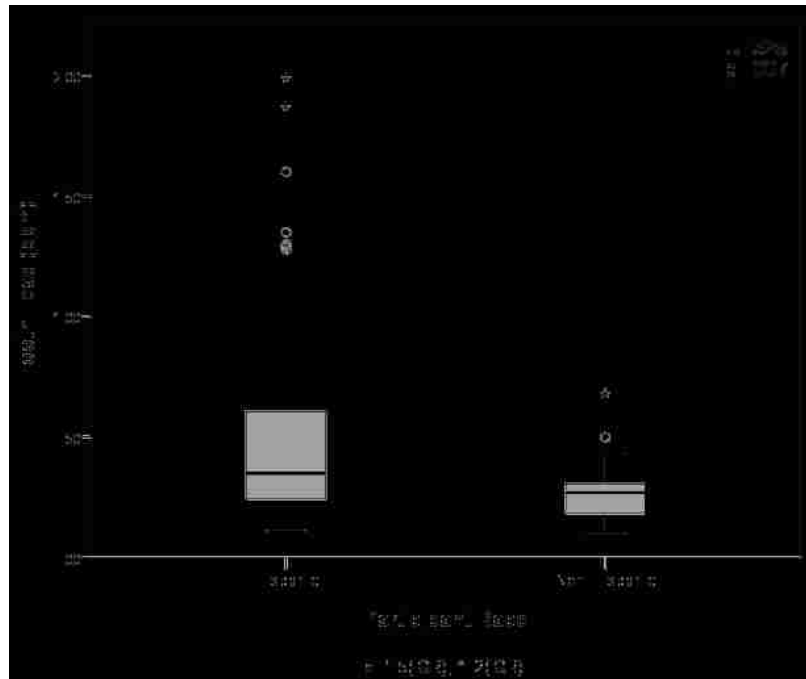


Figure 5. Tooth Lead (ppm) for Hispanic and Non-Hispanic Participants

Participant Age and Tooth Lead Concentrations

The age of participants enrolled in the study ranged from 2 to 13 years of age, with 33% of participants age 6 years or younger (23 of 70 participants). A nearly equal number of males (56%, n= 39) and females (44%, n= 31) participated in the study. See Figure 6 for age and gender distribution of participants.

The second hypothesis was tested using a Mann-Whitney test to determine if a significant difference between the tooth lead concentrations of young children (0 to 6 years of age) and children 7 years of age or older was present. Statistical analysis revealed no significant difference in tooth lead concentrations between the two age groups ($U=472$, $p=.395$). However, younger children ($n= 23$) had a lesser mean ($\pm SD$) tooth lead concentration of 0.28 ± 0.14 (mdn= 0.29) ppm lead compared to 0.50 ± 0.46 (mdn= 0.32) ppm lead for children 7 years of age or older ($n=47$). See Figure 7.

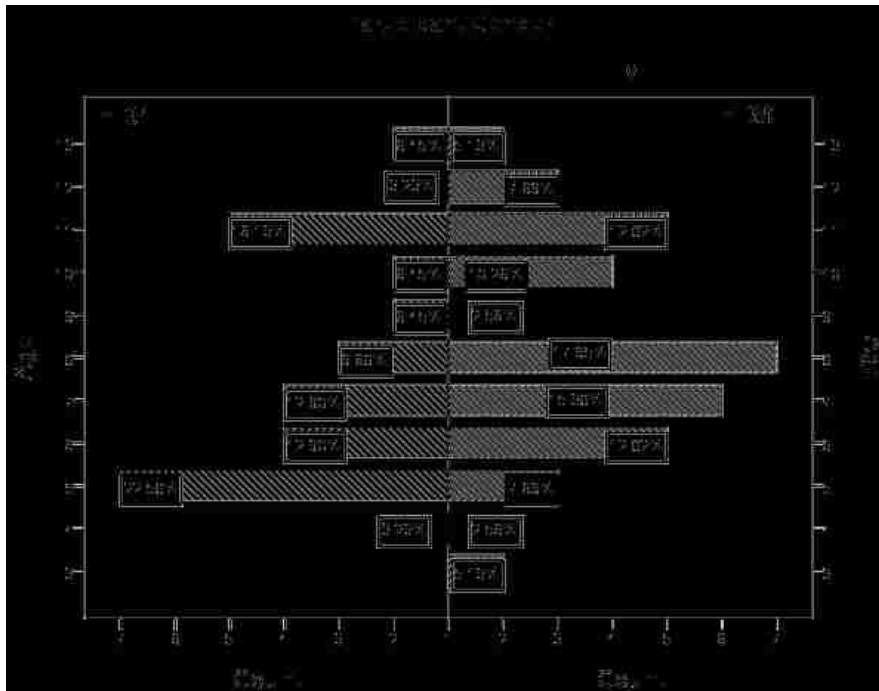


Figure 6. Age and Gender Distribution of Study Participants for Data Analysis

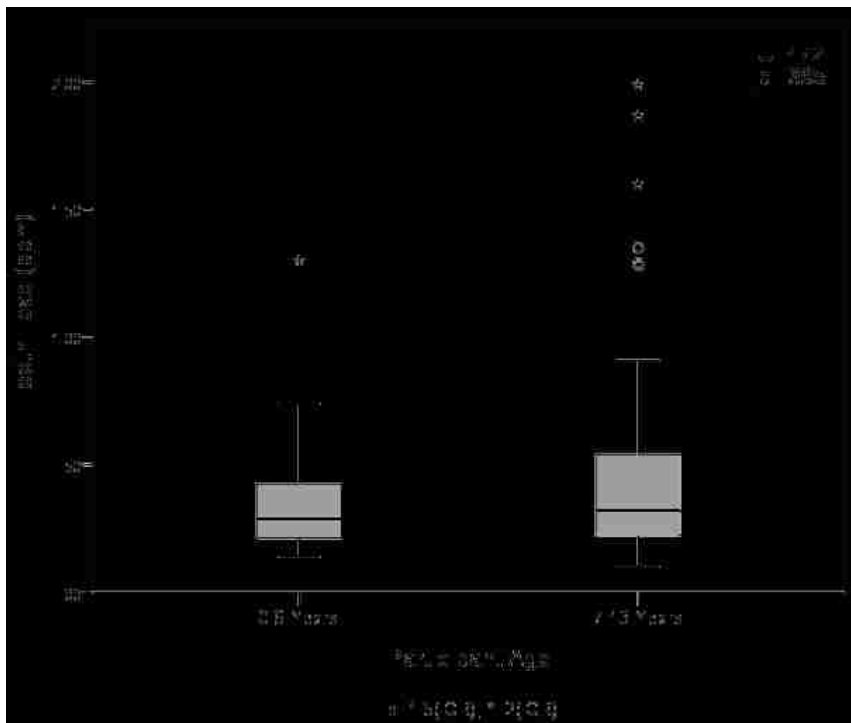


Figure 7. Tooth Lead (ppm) for Young and Older Children

Participant Zip Codes and Tooth Lead Concentrations

Zip codes were provided for 64 participants and twenty-eight (28) Clark County zip codes were represented in this study. A majority of participants resided in Las Vegas (70%, n= 45). See Table 8 for represented Las Vegas zip codes. Other represented areas include Henderson (89114, 89115; n=2), Moapa (89025; n=2), and North Las Vegas (89030-89032, 89085; n=15). Zip code 89030 was the most well represented in this study (North Las Vegas, n=12). There was at least one participant from each of the target zip codes (89030, 89101, 89102, 89106, 89108-89110, 89115, and 89119).

Table 8. List of Las Vegas Zip Codes of Study Participants

Las Vegas Zip Codes						
89101	89104	89108	89115	89119	89144	89147
89102	89106	89110	89117	89120	89145	89156
89103	89107	89113	89118	89122	89146	89183

A Mann-Whitney test revealed a significant difference in tooth lead concentrations between children living in the identified target zip codes and other Clark County zip codes ($U=181$, $p=.000$). Target zip codes were lumped together for analysis. Children living in the target zip codes ($n=37$) had a higher mean (\pm SD) tooth lead concentration of $0.64\pm.49$ ($mdn=0.44$) ppm lead compared to children living in other Clark County zip codes ($n=27$) with a mean (\pm SD) of $0.25\pm.11$ ($mdn=0.23$) ppm lead. See Figure 8. The relationship between frequency of detectable blood lead levels in Clark County, NV by zip code and target zip codes is shown in Figure 9.

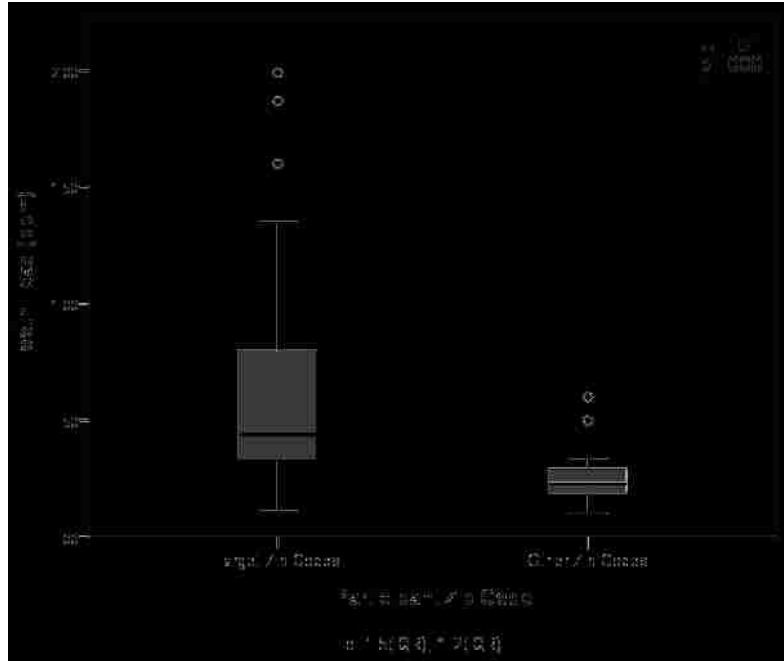


Figure 8. Tooth Lead (ppm) for Children living in Target and Non-Target

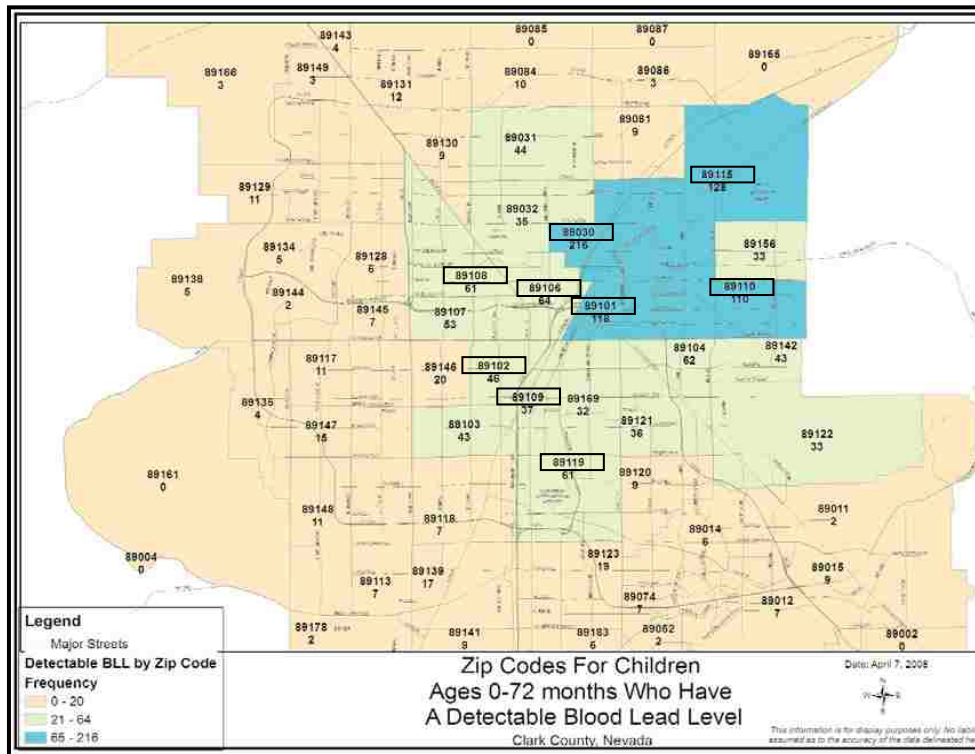


Figure 9. Clark County Map Depicting Frequency of Detectable BLL by Zip Code (Target zip codes are marked; 89025 and 89005 not shown; UNLV & SNHD, 2011)

Housing Construction Year and Tooth Lead Concentrations

A street address was provided for 62 participants, with 2 participants providing P.O. Box addresses instead of a street address. The year of construction for current place of residence was determined for 52 participants using the Clark County Assessor Records Search. Determining the year of construction was limited by revisions made to the IRB protocol and the handwriting of the consenting parent on the intake form (APPENDIX 4), the validity of the provided address, and records available through the Clark County Assessor. Forty-eight percent (48%, n=25) of these participants are currently living in 1978 or pre-1978 housing.

A Mann-Whitney test was applied to determine if tooth lead concentration is related to housing construction year. The statistical analysis of the year of construction of current residence increases revealed year of construction for current place of residence as a significant factor affecting tooth lead concentrations ($U=226$, $p=.042$). The mean (\pm SD) tooth lead concentration for children living in housing built in 1978 or prior (n=25) was $0.50 \pm .38$ (mdn= 0.39) ppm lead. Children residing in housing constructed after 1978 (n=27) had a mean (\pm SD) tooth lead concentration of $0.42 \pm .46$ (mdn= 0.26) ppm lead. Children living in housing constructed in 1978 or prior to 1978, had a greater mean tooth concentration than children living in housing built after 1978. See Figure 10.

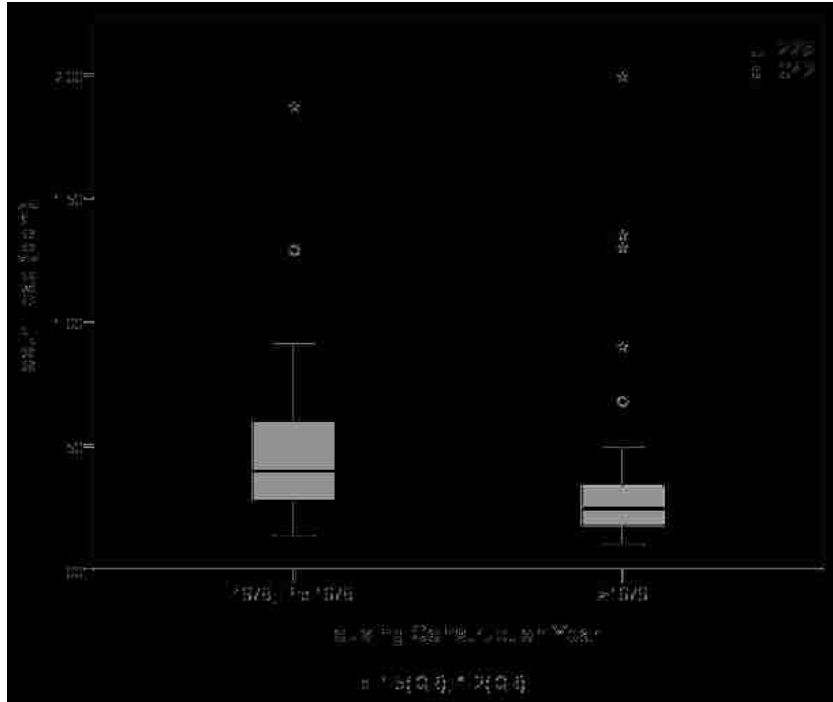


Figure 10. Tooth Lead (ppm) for Housing Age of Current Residence of Participants

Participants with Outlier Tooth Lead Concentrations

The lead concentration of the teeth ranged from 0.1 ppm lead to 1.99 ppm lead, with a mean (\pm SD) of 0.46 ± 0.41 (mdn= 0.31) ppm lead. Nine participants had tooth lead concentrations considered to be outliers, which are defined as points that extend greater than 1.5 box-lengths from box edges. See Figure 11. All patients with tooth lead concentrations exceeding 0.9 ppm were older children and five of the six patients were Hispanic. Also, target zip codes and pre-1978 housing are represented by these participants. See Table 9 for demographic information, tooth condition, tooth lead concentration, zip code and housing construction year for participants with tooth lead concentrations exceeding 0.9 ppm.

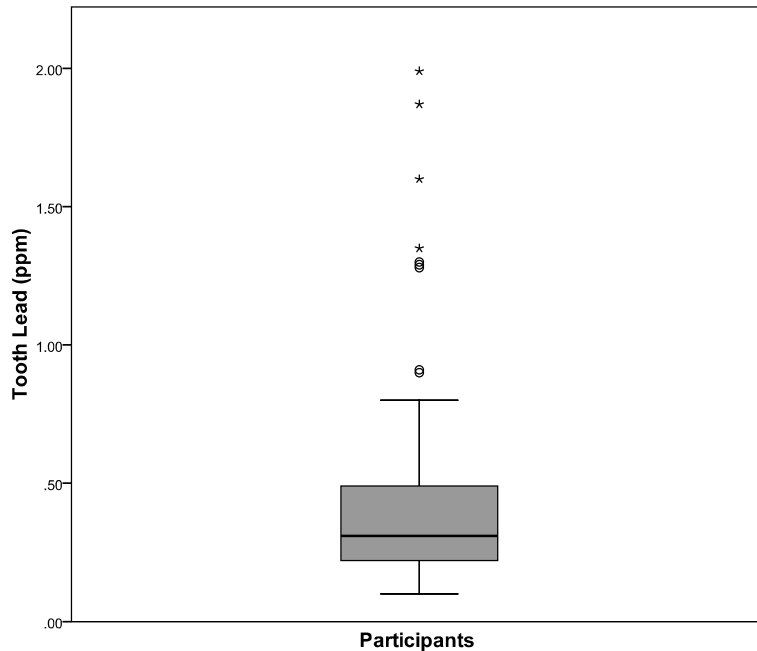


Figure 11. Tooth Lead Concentrations of Participants, Including Outliers

Table 9. Characteristics of Outliers (Tooth Lead Concentrations ≥ 0.90 ppm Lead)

Participant	Lead (ppm)	Root	Age (yrs)	Sex	Zip Code	Race	Housing Year
1002-3362 8071	0.90	Intact	8	F	89115	Hispanic	1979
1002-3362 4077	0.91	Partial	8	F	89115	Hispanic	1963
1002-3362 9863	1.29	Intact	11	M	89115	Hispanic	1971
1002-3362 7390B	1.28	Partial	13	M	89030	Hispanic	Unknown
1002-3362 9826A	1.30	Partial	5	M	89106	Hispanic	1998
1002-3362 8717	1.35	No Root	8	M	89119	Hispanic	1981
1002-3362 9556	1.87	Partial	8	M	89030	Hispanic	1969
1002-3362 2887	1.97	Intact	10	F	89119	Hispanic	Unknown
1002-3362 6482a	1.99	Partial	7	M	89115	Hispanic	1996

CHAPTER 6

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

Discussion of Results

Recruitment for this study was successful with a very high participation rate (96%) and reached high risk groups in Clark County. The population reached by this study included young children, the uninsured, low income, minority groups, and families living in older housing (pre1978). The high participation percentage can be attributed to the low risk nature of the project and the short consent process that occurred while the dental appointments were taking place. In other words, participation in the study did not affect patient care or increase the time of the child's dental appointment. For parents or legal guardians that didn't speak English, a translator was always available and willing to translate the parent permission and intake forms. The pediatric dental clinic was an ideal location for recruiting participants from 28 zip codes, some of which also belong to the population most likely to benefit from lead testing. During the 5 month recruitment period, only 4 patients requiring extractions were approached in duplication for participation in the study. This is consistent with the high new patient rate at the pediatric dental clinic reported by Dr. Karl Kingsley.

Additionally, children are generally not allowed to take the teeth home following an extraction at the pediatric clinic due to the poor condition and bacterial contamination of the teeth. This occurred often, with 49% (n=52) of the teeth collected contained a crown that was greater than 10% decayed. The pediatric dental patients were satisfied with a certificate for the "tooth fairy," and did not request to take their teeth home.

Tooth characteristics were not a significant source of variability in this study, but may contribute to variability of tooth lead concentrations. A majority of the deciduous teeth were extracted for two reasons: 1) severe decay or damage and 2) to allow adult teeth to move into position. Tooth decay was normally limited to the crown, with some decay extending deep into the center of tooth. Extractions of healthy teeth were performed to create space, which resulted in samples free of caries, but with partial root resorption or no root. In contrast to the findings of Bercovitz and Laufer (1990), there was no difference in tooth lead concentrations between tooth types or percent of tooth decay. The only significant difference in tooth lead concentration was found between teeth with partial root resorption and those with no root. A significant difference between teeth with an intact root and teeth with no root was expected, but not indicated by statistical analysis. Physiologic resorption of deciduous teeth includes periods of active resorption followed by periods of inactivity and repair. During the repair process, new calcific structures may form and result in reattachment of the tooth (Harokopakis-Hajishengallis, 2007). During this time, periods of active resorption could potentially decrease lead concentrations, but then increase during periods of repair and reattachment. This may explain the inconsistent findings in regard to tooth resorption.

Discussion of Research Questions

Demographic and environmental factors relevant to Clark County, Nevada and previously associated with childhood lead poisoning were tested in the form of four hypotheses to determine their impact on childhood lead exposure. These results present evidence that tooth lead concentrations collected from Clark County children are affected

by housing age, race, and income. Participants living in housing constructed in 1978 or prior to 1978, Hispanic participants, and participants living in the target zip codes were found to have significantly higher mean tooth lead concentrations compared to the other participants. A limitation to testing the relationship between housing construction year and tooth lead concentrations was the lack of timeline for length of residence in the current home. Although these factors were independently analyzed, health disparities in childhood poisoning, such as low- income, minority groups and older housing, are interrelated and well documented.

The first environmental factor tested was potential lead exposure from lead based paint found in housing built in 1978 or prior to 1978. These findings are comparable to current high risk groups for childhood lead poisoning, such as minority children and children belonging to low income households. Although Nevada's housing stock is young, with most housing built after 1980, 27% of homes in Clark County were built prior to 1979 (Rothweiler et al., 2007). Income can be considered a demographic factor and indirect environmental factor that contributes to lead exposure. Overall, a majority of participants were currently residing in one of the target zip codes. The target zip codes were used to assess the impact of income on childhood lead exposure. The relationship between income and childhood lead poisoning is well established. The analysis of NHANES III data from 1989 to 1994 by Bernard and McGeehin (2003) revealed that 12.5% of children at or below the poverty income ratio (PIR) had BLLs of 10ug/dL or greater, compared to 3.3% of children living above the PIR. As an environmental factor, lower income is associated with an increased risk of lead exposure from older housing and poor housing conditions (Rothweiler et al., 2007). Since housing built prior to 1979

are generally occupied by lower income families (Rothweiler et al., 2007), these children are at risk for environmental lead exposure via lead-based paint hazards.

A good gender and age distribution was achieved, with a nearly equal distribution of male and female participants. Even though participants 6 years of age and younger are considered to be at higher risk (Rothweiler et al., 2007), there was no significant difference in tooth lead concentrations between young children (0 to 6 years) and older children (7 years of age or older). Furthermore, the mean tooth lead concentration was actually greater for older children. Lead storage in the bones and teeth is cumulative throughout life. However, root resorption prior to natural tooth shedding may alter lead concentrations (Fergusson and Purchase, 1987). This may be a limitation to the use of tooth lead concentrations as a biomarker for lead exposure for older children, whose teeth are currently undergoing periods of root and bone resorption.

In this study, children of Hispanic race were the most well represented minority group, which are considered high risk for lead exposure. Hispanic children were unintentionally oversampled. This is most likely a reflection of the population served by the pediatric clinic at the UNLV School of Dental Medicine and is not generalizable to Clark County or Nevada. Hispanic participants in this study had a significantly increased mean tooth lead concentration compared to non-Hispanic children. This is consistent with NHANES data from 1999-2002 that minorities, non-Hispanic blacks and Hispanics, had higher BLLs than White children (CDC, 2005). For statistical analysis, non-Hispanic black, white, and American Indian children had to be combined. Hispanic children are at greater risk for both traditional and non-traditional sources of lead and recent poverty rates are greatly increasing among Hispanic children (Rothweiler et al., 2007).

Nine participants were found to have tooth lead concentrations that exceeded 0.90 ppm lead and considered outliers. These children belonged to Hispanic race, were 7 years of age or older, and currently reside in one of the target zip codes. The elevated concentrations could be attributed to a combination of risk factors for lead exposure and age group. However, participants were not asked whether they were U.S. born or foreign born. Even after immigration, foreign born children are more likely to have EBLs than U.S. born children (Levin et al., 2008)

The environmental and demographic risk factors identified by this study are consistent with those recognized by the SNHD, such as immigration, poverty, Medicaid enrollment, homes containing lead-based paint, and atypical sources (e.g. imported candies and glazed pottery). Additionally, the SNHD aims to improve primary prevention, conduct surveillance, and provide community outreach (SNHD, 2011). These goals are also achievable through the establishment of a dental screening program for childhood lead exposure through the UNLV School of Dental Medicine and other Clark County Clinics.

Conclusion and Recommendations

The number of children screened by blood lead testing in Clark County, Nevada has greatly improved, but would benefit from additional screening methods. Measuring lead in teeth is a convenient and effective alternative method of testing that reaches communities at risk and would increase the number of children screened for childhood lead exposure. This study successfully recruited children belonging to high risk groups and assessed tooth lead concentrations as a potential screening alternative for Nevada.

Important advantages to measuring tooth lead concentrations as indicated by this study are the high participation percentage of parents, high new patient rate at the pediatric dental clinic, on-site sample collection, no additional invasive sample collection, and relatively simple sample processing. High risk families utilize the services at the UNLV School of Dental Medicine and do not have to be sought out in the community. In the Childhood Lead Poisoning Prevention Program 2008-2009 Annual Report, the SNHD expressed the lack of zip code data, with zip codes reported for only 31% of children screened (SNHD, 2011). The overlay of the selected target zip codes for this study and the frequency of detectable blood lead levels (Figure 9), indicate that tooth lead concentrations can accurately be used to identify communities at risk by geography and economic characteristics. A longer recruitment period would increase the number of represented zip codes and allow education efforts to be directed at specific communities within target Clark County zip codes. An overall small sample size hinders the ability to generalize these results for all children or all children at risk for lead poisoning in Clark County, NV.

The extraction of a tooth is generally painful and traumatic and only performed if dentally necessary. Sample collection for measuring tooth lead concentrations occurs after the extraction and does not require any additional procedures. This is in contrast to capillary collection by finger stick or venipuncture for blood lead testing. Sample analysis was performed at a reference laboratory, and required minimal sample preparation prior to shipping for analysis. Tooth lead concentrations were obtained within 10 days of sending samples to the reference laboratory. The cost of ICPMS analysis by Exova (Santa Fe Springs, CA, USA), when 75 samples or more were sent at

once, was \$65.00/sample. In comparison, capillary testing performed at the SNHD using the Lead Care II[®] costs approximately \$12.00/sample (G. Gholson, personal communication, April 5 2011). Similar to capillary testing, a confirmatory BLL test by venipuncture collection would be required if a child was found to have an elevated tooth lead concentration. Although screening by tooth lead concentrations is not as cost effective, both screening methods would decrease the future economic impact of childhood lead poisoning over time. Childhood lead poisoning prevention efforts have a considerable impact on averting future economic losses. Using the NHANES 2003-2006, Gould (2009) calculated lifetime earnings lost to range from \$165 to 233 billion for children 6 years of age or younger (2006 cohort). Other losses include costs associated with continuing healthcare for the treatment of lead poisoning, special education, and criminal activity (Gould, 2009).

Future Research

The analysis of lead in deciduous teeth has been investigated and well documented as an indicator of cumulative lead exposure; however, the methodology has not been standardized. This study used opportunistic sampling, which provided samples that varied by type and condition. Questions not answered by this study include: 1) How to prepare, assess, and analyze samples with metal filling or caps? 2) What is the maximum allowable percentage of root decay or root resorption that will insure comparable samples? and 3) What concentration of lead in teeth indicates a case of childhood lead poisoning? A gap in current studies is the lack of direct correlation between tooth lead concentrations and BLLs (Bergdahl and Skerfving, 2008). An additional consideration is

the clinical significance of tooth quality on tooth lead concentrations as a diagnostic tool for assessing lead exposure.

A targeted screening protocol needs to be developed in order to focus screening efforts on children that would benefit the most from tooth lead screening. The SNHD focuses on the blood lead testing of children 6 years of age or younger and recommends that all children should be tested at 1 to 2 years of age (UNLV & SNHD, 2011). Even though older children were found to have greater mean tooth lead concentrations than younger children, children under the age of 6 should remain the target age group. Age is a well established risk factor. The frequency of teeth with partial root resorption or no root increased with age; the collection of teeth undergoing resorption peaked at approximately 10 years (no root) and 11 years (partial root resorption). Based on these data and established risk groups for increased lead exposure, it is recommended that tooth lead concentrations are measured for children 6 years old or younger.

Although, a dental screening program is a viable option for Clark County, Nevada, this method of screening should be used to increase childhood lead poisoning awareness and screening concurrently with blood lead testing (capillary and venipuncture collections). Future efforts regarding the advancement of using tooth lead concentrations as a biomarker for childhood lead poisoning and lead exposure should focus on the standardization of methodology, protocols to handle sample interferences (e.g. caps, fillings, and root resorption), and create a model for deducing estimated BBLs from tooth lead concentrations. Until this research is conducted and methodology validated, a dental screening program should be established to aid the lead poisoning prevention efforts of the SNHD and continue collecting samples to determine a baseline level for children 6

years of age and younger living in Clark County, Nevada. Even though measured tooth lead concentrations may not specifically indicate recent exposure, tooth lead concentrations are a unique biomarker for lead exposure in that fetal stages and childhood exposure is represented. Blood lead testing could be recommended for children having higher than expected tooth lead concentrations compared to an established baseline. In regard to the modulation of lead concentrations in the body, a combination of biomarkers indicating recent exposure (blood) and past exposure (deciduous teeth) could help identify a timeline. Therefore, assisting in the identification of potential sources of lead exposure during a specific period.

For children not tested by blood lead testing, a tooth lead concentration would be an improved alternative to no screening for lead exposure. Continued sampling could be utilized as an opportunity to distribute childhood lead poisoning prevention materials created by the CCCLPPP and SNHD. Considering the close physical proximity of the SNHD clinic to the UNLV School of Dental Medicine (approximately 0.5 miles), this could help increase screening rates at the SNHD clinic.

Study Contributions

This study did not increase the number of children screened for BLLs for 2010 or 2011, but parents were given the opportunity to ask questions about childhood lead poisoning and at a minimum, it may been an indirect reminder to have their child's BLL tested. Pediatric residents at the UNLV School of Dental Medicine were also informally educated about the effects of childhood lead poisoning, toxicokinetics of lead, and importance of blood lead testing. Pediatric residents will now be able to answer basic questions about childhood lead poisoning and to recommend parents visit the SNHD

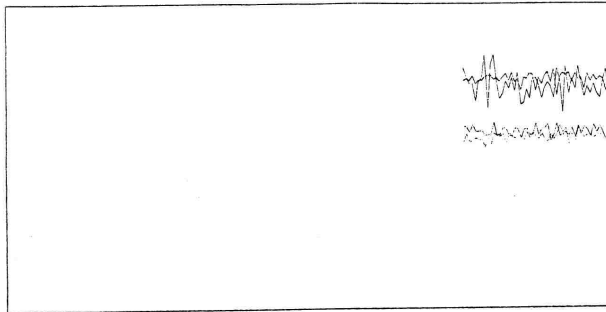
clinic near the UNLV School of Dental Medicine. The findings of this study will be given to the SNHD to help direct childhood lead poisoning prevention efforts in Clark County, Nevada.

APPENDIX 1

EXOVA ICPMS INSTRUMENT PARAMETERS

Sensitivity

Tune File : he.u
 Instrument Name : G3272A JP51201756



Integration Time: 0.1000 sec
 Sampling Period: 1.4300 sec
 n: 50

m/z	Range	Count	Mean	RSD%
7	500	371.0	364.7	5.90
59	10,000	5806.0	5768.3	2.68
89	5,000	2875.0	2798.3	2.61
205	20,000	14586.0	14977.1	2.41
156/140	1	0.358%	0.415%	16.88
70/140	5	1.967%	1.964%	9.00
51/59	1	0.362%	0.426%	19.26
27	20	2.0	2.1	77.11
11	20	4.0	5.5	40.31
114	20	0.0	0.7	119.87
202	20	0.0	2.5	67.59
208	20	15.0	12.4	32.75
118	50	5.0	14.8	25.85

ICPMS #11

Reviewed by:

Date: 03/10/11

Tuning Parameters

```

===Plasma Condition===
RF Power : 1500 W
RF Matching : 1.75 V
Smpl Depth : 7.4 mm
Torch-H : -0.2 mm
Torch-V : 0.2 mm
Carrier Gas : 1.12 L/min
Makeup Gas : 0 L/min
Optional Gas : 0 %
Nebulizer Pump : 0.1 rps
Sample Pump : --- rps
S/C Temp : 2 degC

===Ion Lenses===
Extract 1 : 2.5 V
Extract 2 : -120 V
Omega Bias-ce : -20 V
Omega Lens-ce : -1 V
Cell Entrance : -40 V
QP Focus : -12 V
Cell Exit : -50 V

===Q-Pole Parameters===
AMU Gain : 135
AMU Offset : 128
Axis Gain : 1.0007
Axis Offset : -0.07
QP Bias : -16 V

===Detector Parameters===
Discriminator : 8 mV
Analog HV : 1810 V
Pulse HV : 1310 V

===Octopole Parameters===
OctP RF : 150 V
OctP Bias : -18 V

===Reaction Cell===
Reaction Mode : ON
H2 Gas : 0 mL/min
He Gas : 5.2 mL/min
Optional Gas : --- %
  
```

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APPENDIX 2

INSTITUTIONAL REVIEW BOARD APPROVAL



Biomedical IRB – Full Board Review Approval Notice

NOTICE TO ALL RESEARCHERS:

Please be aware that a protocol violation (e.g., failure to submit a modification for any change) of an IRB approved protocol may result in mandatory remedial education, additional audits, re-consenting subjects, researcher probation suspension of any research protocol at issue, suspension of additional existing research protocols, invalidation of all research conducted under the research protocol at issue, and further appropriate consequences as determined by the IRB and the Institutional Officer.

DATE: March 25, 2010
TO: **Dr. Karl Kingsley**, School of Dental Medicine
FROM: Office of Research Integrity – Human Subjects
RE: Notification of IRB Action *CB*
Protocol Title: **Evaluation of Metal Levels (Specifically Targeting Lead) In
Extracted Primary Teeth Among Pediatric Patients**
Protocol #: 1002-3362

This memorandum is notification that the project referenced above has been reviewed by the UNLV Biomedical Institutional Review Board (IRB) as indicated in Federal regulatory statutes 45CFR46. The protocol has been reviewed and approved.

The protocol is approved for a period of one year from the date of IRB approval. The expiration date of this protocol is March 22, 2011. Work on the project may begin as soon as you receive written notification from the Office of Research Integrity – Human Subjects.

PLEASE NOTE:

Attached to this approval notice is the **official Informed Consent/Assent (IC/A) Form** for this study. The IC/A contains an official approval stamp. Only copies of this official IC/A form may be used when obtaining consent. Please keep the original for your records.

Should there be *any* change to the protocol, it will be necessary to submit a **Modification Form** through ORI – Human Subjects. No changes may be made to the existing protocol until modifications have been approved by the IRB.

Should the use of human subjects described in this protocol continue beyond March 22, 2011, it would be necessary to submit a **Continuing Review Request Form** *60 days* before the expiration date.

If you have questions or require any assistance, please contact the Office of Research Integrity – Human Subjects at IRB@unlv.edu or call 895-2794.

Office of Research Integrity – Human Subjects
4505 Maryland Parkway • Box 451047 • Las Vegas, Nevada 89154-1047

APPENDIX 3

PARENT PERMISSION FORM

RECEIVED

MAR 18 2010



PARENT PERMISSION

UNLV Pediatric Dental Residency Program

TITLE OF STUDY: Evaluation of Metal Levels (Specifically Targeting Lead) in Extracted Primary Teeth Among Pediatric Patients

INVESTIGATOR(S): Kristin Murphy DMD Jeanne Hibler DDS Karl Kingsley, PhD Shawn Gerstenberger, PhD

CONTACT PHONE NUMBER: (702) 774-2415 Dr. Murphy and (702) 774-2623 Dr. Kingsley

Purpose of the Study

Your child has been invited to participate in a research study. The purpose of this study is to evaluate metal levels (specifically targeting lead) in baby teeth extracted in pediatric patients. Following the extraction of the tooth, it will be analyzed for lead and metal levels using a thermo scientific niton X-ray fluorescence analyser in the UNLV lab. This study is designed to assess any possible exposure of lead and other metals to children in our community.

Participants

Your child is being asked to participate in the study because your child's medical treatment plan indicates it is medically necessary to have a baby tooth extracted at the UNLV Pediatric Dental Residency Clinic. Please understand that there is no change in the treatment of your child if you decide to participate in this study.

Procedures

By volunteering to participate in this study, your child will be asked to do the following: Donate one (1) of your child's extracted tooth/teeth to the UNLV Pediatric Dental Clinic for further study.

Benefits of Participation

There *may not* be direct benefits to your child as a participant in this study. However, we hope to learn more about children's exposure to lead and other metals by measuring their levels present in baby teeth.

Risks of Participation

There are risks involved in all research studies. This study may include only minimal risks. *There is no anticipated risk by your child's participation in this study.* We will not divulge any private or personal information about you or your child's medical record nor will we allow any party other than the primary investigators to study the samples collected. Your child's extracted tooth/teeth will be studied for academic purposes only. Please understand that there is no change in the treatment of your child if you decide to participate in this study.

Participant Initials _____

RECEIVED

MAR 18 2010



TITLE OF STUDY: Evaluation of Metal Levels (Specifically Targeting Lead) in Extracted Primary Teeth Among Pediatric Patients

Cost /Compensation

There *will not* be any additional financial cost to you or your child if you choose to participate in this study. The study will take 0 (no) additional *minutes/hours/days* of your time, but will take place after your child's tooth has been extracted. There is no financial compensation for your child's participation.

Contact Information

If you have any questions or concerns about the study, you may contact **Dr. Kristin Murphy at (702) 774-2415 or Dr. Karl Kingsley at (702) 774-2623**. For questions regarding the rights of research subjects, any complaints or comments regarding the manner in which the study is being conducted you may contact **the UNLV Office for the Protection of Research Subjects at 702-895-2794**.

Voluntary Participation

Your child's participation in this study is voluntary. You may refuse to participate in this study or in any part of this study. You may withdraw at any time without prejudice to your relations with the university. You are encouraged to ask questions about this study at the beginning or any time during the research study. Please understand that there is no change in the treatment of your child if you decide to participate in this study.

Confidentiality

All information gathered in this study will be kept completely confidential. No reference will be made in written or oral materials that could link your child to this study. All records will be stored in a locked facility at UNLV for ten (10) years after completion of the study. After ten year the files will be deleted and printed materials shredded and destroyed.

Note: Subjects are patients at the UNLV Pediatric Dental Residency Program, and their confidentiality is protected within HIPAA regulations. Subjects' teeth samples will not be shared with any other institution or unrelated persons to dental academia within the school.

Participant Consent:

I have read the above information and agree to participate in this study. I am at least 18 years of age. A copy of this form has been given to me.

Signature of Participant

Date

Participant Name (Please Print)

Participant Note: Please do not sign this document if the Approval Stamp is missing or is expired.

Participant Initials _____

APPENDIX 4

ASSENT TO PARTICIPATE IN RESEARCH FORM

RECEIVED
MAR 18 2010



ASSENT TO PARTICIPATE IN RESEARCH

Evaluation of Metal Levels (Specifically Targeting Lead) in Extracted Primary Teeth Among Pediatric Patients

1. My name is Dr. Kristin.
2. We are asking you to take part in a research study because we are trying to learn more about lead and other metals that might be in your baby teeth.
3. If you agree to be in this study, we will donate your extracted baby tooth to the UNLV Pediatric Dental Clinic for further study. In our lab, we will measure lead and other metal levels in your baby teeth with a special instrument.
4. Research studies can have risks. This study may have very small or minimal risks. Your participating in this study will not hurt you. We will not tell anyone your name, where you live or anything about your background. Only the people working on this research will study your extracted baby teeth.
5. There may not be a direct benefit to you by participating in our study. However, we hope to learn more about lead and other metal levels in kid's teeth.
6. Please talk this over with your parents before you decide whether or not to participate. We will also ask your parents to give their permission for you to take part in this study. But even if your parents say "yes" you can still decide not to do this.
7. If you don't want to be in this study, you don't have to participate. Remember, being in this study is up to you and no one will be upset if you don't want to participate or even if you change your mind later and want to stop.
8. You can ask any questions that you have about the study. If you have a question later that you didn't think of now, you can call me at (702) 774-2415 or ask me next time. You may call me at any time to ask questions. If I have not answered your questions or you do not feel comfortable talking to me about your question, you or your parent can call the UNLV Office for the Protection of Research Subjects at 702-895-2794.
9. Signing your name at the bottom means that you agree to be in this study. You and your parents will be given a copy of this form after you have signed it.

Print your name

Date

Sign your name

APPENDIX 5
INTAKE FORM

1002-3362 Pediatric Lead surveillance study: Intake Collection Form			
Place label here....	(check one) SEX <input type="checkbox"/> F <input type="checkbox"/> M RACE: <input type="checkbox"/> White <input type="checkbox"/> Black <input type="checkbox"/> Hispanic	AGE: CODE:	ZIP _____ _____
STREET ADDRESS:			
Place label here....	(check one) SEX <input type="checkbox"/> F <input type="checkbox"/> M RACE: <input type="checkbox"/> White <input type="checkbox"/> Black <input type="checkbox"/> Hispanic	AGE: CODE:	ZIP _____ _____
STREET ADDRESS:			
Place label here....	(check one) SEX <input type="checkbox"/> F <input type="checkbox"/> M RACE: <input type="checkbox"/> White <input type="checkbox"/> Black <input type="checkbox"/> Hispanic	AGE: CODE:	ZIP _____ _____
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Place label here....	(check one) SEX <input type="checkbox"/> F <input type="checkbox"/> M RACE: <input type="checkbox"/> White <input type="checkbox"/> Black <input type="checkbox"/> Hispanic	AGE: CODE:	ZIP _____ _____
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Place label here....	(check one) SEX <input type="checkbox"/> F <input type="checkbox"/> M RACE: <input type="checkbox"/> White <input type="checkbox"/> Black <input type="checkbox"/> Hispanic	AGE: CODE:	ZIP _____ _____
STREET ADDRESS:			
Place label here....	(check one) SEX <input type="checkbox"/> F <input type="checkbox"/> M RACE: <input type="checkbox"/> White <input type="checkbox"/> Black <input type="checkbox"/> Hispanic	AGE: CODE:	ZIP _____ _____
STREET ADDRESS:			
Place label here....	(check one) SEX <input type="checkbox"/> F <input type="checkbox"/> M RACE: <input type="checkbox"/> White <input type="checkbox"/> Black <input type="checkbox"/> Hispanic	AGE: CODE:	ZIP _____ _____
STREET ADDRESS:			
Place label here....	(check one) SEX <input type="checkbox"/> F <input type="checkbox"/> M RACE: <input type="checkbox"/> White <input type="checkbox"/> Black <input type="checkbox"/> Hispanic	AGE: CODE:	ZIP _____ _____
STREET ADDRESS:			

APPENDIX 6

LABELS FOR FORMS AND SAMPLES

1002-3362 (1 of 5) 9894 Tooth ID:	1002-3362 (1 of 5) 8077 Tooth ID:	1002-3362 (1 of 5) 9556 Tooth ID:
1002-3362 (2 of 5) 9894 Saliva collection	1002-3362 (2 of 5) 8077 Saliva collection	1002-3362 (2 of 5) 9556 Saliva collection
1002-3362 (3 of 5) 9894 Informed Consent label	1002-3362 (3 of 5) 8077 Informed Consent label	1002-3362 (3 of 5) 9556 Informed Consent label
1002-3362 (4 of 5) 9894 Assent label	1002-3362 (4 of 5) 8077 Assent label	1002-3362 (4 of 5) 9556 Assent label
1002-3362 (5 of 5) 9894 Intake form label	1002-3362 (5 of 5) 8077 Intake form label	1002-3362 (5 of 5) 9556 Intake form label
1002-3362 (1 of 5) 8118 Tooth ID:	1002-3362 (1 of 5) 9222 Tooth ID:	1002-3362 (1 of 5) 8090 Tooth ID:
1002-3362 (2 of 5) 8118 Saliva collection	1002-3362 (2 of 5) 9222 Saliva collection	1002-3362 (2 of 5) 8090 Saliva collection
1002-3362 (3 of 5) 8118 Informed Consent label	1002-3362 (3 of 5) 9222 Informed Consent label	1002-3362 (3 of 5) 8090 Informed Consent label
1002-3362 (4 of 5) 8118 Assent label	1002-3362 (4 of 5) 9222 Assent label	1002-3362 (4 of 5) 8090 Assent label

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Peden-Adams, M.M., Stuckey, J.E., Gawarecki, K., **Berger-Ritchie, J.**, Bryant, K., Jodice, P.G., Scott, T., Boone, J.S., Ferrario, J.B., Guan, B., Vigo, C., McGuinn, W.C., DeWitt, J.C., and Keil, D.E. 2009. Developmental toxicity of perfluorooctane sulfonate (PFOS) in white leghorn chickens following in ovo exposure. *Reproductive Toxicology*, 27:207-318. Impact Factor: 2.957

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Poster Presentations:

Nevada Public Health Association, September 2010, An Assessment of Lead Concentrations in Imported Hot Sauces Purchased in Clark County, Nevada

Thesis Title: Lead Concentrations in Extracted Primary Teeth Among Clark County Pediatric Patients

Thesis Examination Committee:

Chairperson, Dr. Shawn Gerstenberger, Ph.D.
Committee Member, Dr. Chad Cross, Ph.D.
Committee Member, Dr. Timothy Bungum, Ph.D.
Graduate Faculty Representative, Dr. Deborah Keil, Ph.D.